OSONG PUBLIC HEALTH
AND RESEARCH PERSPECTIVES
https://ophrp.org

phrp OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

Vol. 16, No. 4, August 2025

Pages 309-422

Vol. 16, No. 4, August 2025

phrp
OSONG PUBLIC HEALTH
AND RESEARCH PERSPECTIVES

Vol. 16, No. 4, August 2025

pISSN 2210-9099
eISSN 2233-6052

Vol. 16, No. 4, August 2025

Korea Disease Control and
Prevention Agency

https://ophrp.org

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

https://ophrp.org

pISSN 2210-9099
eISSN 2233-6052

Vol. 16, No. 4, August 2025

Published on 31 August 2025

Aims & Scope

Osong Public Health and Research Perspectives (PHRP) is the international bimonthly (published at the end of February, April, June, August, October, and December) journal founded in 2010 by the Korea Disease Control and Prevention Agency (KDCA). With the mission of the KDCA, to create a disease-free world, PHRP encourages sharing medical information and knowledge in the areas of public health.
PHRP publishes original articles, review articles, guidelines, data profiles (including cohort profiles), special articles, short communications, viewpoints, editorials, commentaries, and correspondence, and book reviews, with a focus on the following areas of expertise: emerging infectious diseases, vaccinology, zoonotic diseases, non-communicable diseases, intractable and rare diseases, and human genomics.

Abstracting and Indexing Services

A part of articles, metadata, or full text is available from CrossRef metadata (2010–), PubMed Central (2010–), PubMed (2010–), Scopus (2010–), Embase (2010–), EBSCO Host (2010–), Directory of Open Access Journals (2018–), Korea Citation Index (2011–), and Emerging Sources Citation Index (2020–).

Open Access

PHRP is an open access journal. All contents of the journal are available immediately upon publication without embargo period (https://ophrp.org). All articles are distributed under the terms of the Creative Commons Attribution Non-Commercial License (https://creativecommons.org/licenses/by-nc/4.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher: The Korea Disease Control and Prevention Agency

Editor-in-Chief: Jong-Koo Lee, MD, PhD

Editorial Office:

- Korea Disease Control and Prevention Agency
  National Center for Medical Information and Knowledge, 202 Osongsengmyung 2nd street, Osong-eup, Heungdeok-gu, Cheongju 28159, Republic of Korea. E-mail: ophrp@korea.kr

- Infolumi
  #245, 10 Jangmi-ro 48beon-gil, Bundang-gu, Seongnam 13496, Republic of Korea
  Tel: +82-31-709-0918, E-mail: osong.editorialoffice@gmail.com

© 2025 Korea Disease Control and Prevention Agency
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
♾ This paper meets the requirements of KS X ISO 9706, ISO 9706-1994 & ANSI/NISO Z39.48-1992 (Permanence of Paper).

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

https://ophrp.org
pISSN 2210-9099 eISSN 2233-6052

Editorial Board

Editor-in-Chief

Jong-Koo Lee, MD, PhD
National Academy of Medicine of Korea, Seoul, Korea

Editors

Curie Ahn, MD, PhD National Medical Center, Korea
Shilpa Buch, PhD University of Nebraska, USA
Young June Choe, MD, PhD Korea University, Korea
Thomas Grischott, MD University of Zurich, Switzerland
Sun Huh, MD, PhD Hallym University, Korea
Sun Ha Jee, PhD Yonsei University, Korea
Yong Seok Jeong, PhD Kyung Hee University, Korea
Joseph Kamgno, MD, PhD CRFilMT, Cameroon
Jae-Heon Kang, MD, PhD Sungkyunkwan University, Korea
Dong-Hyun Kim, MD, PhD Hallym University, Korea
Dong-Su Kim, PhD Ajou University, Korea
Jinhyun Kim, PhD Seoul National University, Korea
Kyehun Kim, MD, PhD Chonnam National University, Korea
Yongkuk Kim, PhD Kyungpook National University, Korea
Moo-Song Lee, MD, PhD University of Ulsan, Korea
Martin Meltzer, PhD CDC, USA
Hiroshi Nishiura, MD, PhD Kyoto University, Japan
Hyun Joo Pai, MD, PhD Hanyang University, Korea
Kholoud Porter, PhD Medical Research Council, UK
Tomoya Saito, MD, PhD National Institute of Infectious Diseases, Japan
Gabriel Sanfélix-Gimeno, PharmD Foundation for the Promotion of Health and Biomedical Research of Valencia Region (FISABIO), Spain
James Sneyd, PhD The University of Auckland, New Zealand
Aeree Sohn, PhD Sahmyook University, Korea
Byoung-Joon Song, PhD National Institutes of Health, USA
Kiyosu Taniguchi, MD, PhD National Institute of Infectious Diseases, Japan

Statistical Editors

Eunok Jung, PhD Konkuk University, Korea
Ho Kim, PhD Seoul National University, Korea
Nam-Kyoo Lim, PhD Korea Disease Control and Prevention Agency, Korea

Journal Management Team

Director

Hee Youl Chai, PhD Korea Disease Control and Prevention Agency, Korea

Managing Editors

Se Hee Hwang, MS Korea Disease Control and Prevention Agency, Korea
Chaeshin Chu, PhD Korea Disease Control and Prevention Agency, Korea

Editorial Coordinator

Hye-Min Cho, MA Infolumi, Korea
Jisoo Yoon, MS Infolumi, Korea

Manuscript Editors

Sue Yeon Chung Infolumi, Korea
Yoon Joo Seo, ELS Infolumi, Korea

© 2025 Korea Disease Control and Prevention Agency

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

https://ophrp.org
pISSN 2210-9099 eISSN 2233-6052

Vol. 16, No. 4, August 2025

Editorial

309 Artificial intelligence and biohealth: the Republic of Korea's emerging priorities in health care R&D
Jong-Koo Lee

Original Articles

311 Immunoinformatics study of CD40 ligand-targeting vaccine constructs: a novel immunotherapeutic approach
Seyed Amir Sadeghi, Mahroo Mohamadi, Hadi Bamehr, Fatemeh Heidarnejad, Azam Bolhassani

333 Deep learning-based prognosis of major adverse cardiac events in patients with acute myocardial infarction: a retrospective observational study in the Republic of Korea
Vungsovanreach Kong, Kyung Ah Kim, Ho Sun Shon

348 Genetic diversity and spatiotemporal population structure of *Anopheles sinensis* in the Republic of Korea based on the mitochondrial cytochrome c oxidase subunit I (*COI*) marker
Haneul Jung, Bo Gyeong Han, Hyun-Il Shin, Myoung-Ro Lee, Jung-Won Ju, Hee-Il Lee

357 Assessment of healthcare workers' knowledge and attitudes toward mpox and acceptance of its vaccine: a health belief model-based analysis in the Gulf region
Mona Gamal Mohamed, Eman Abdelaziz Ahmed Dabou, Fatma M. Ibrahim, Shaimaa Abdelsamad, Jibin Kunjavara, Kamaruddeen Mannethodi, Moayad Ahmad Wishah, Abdullah Y. Shihab, Khaled Mohammed Al-Sayaghi, Hammad Ali Fadlalmola, Mohammed Musaed Al-Jabri

373 Development and evaluation of the Korea Disease Control and Prevention Agency's scenario-based training program for effective response to emerging infectious disease outbreaks
Mi Yu, Eun Kyoung Kim, Kyungnam Kim, Jin Lee, Jin Hwan Jeon, Soyeon Kim, Sangwon Lee, Yunhyung Kwon

381 Impact of direct prehospital transport on mortality in patients with severe trauma based on the injury severity score: a nationwide observational study in the Republic of Korea
Sun Ju Kim, KeunKyun Kim, Oh Hyun Kim, Chan Yong Park

392 Prevalence and determinants of medication adherence in Moroccan patients with type 2 diabetes: a multicenter cross-sectional study
Maryem Arraji, Nadia Al Wachami, Younes Iderdar, Fatima Zahra Bouchachi, Karima Boumendil, Mohamed Chahboune

406 Risk of tuberculosis in individuals with type 2 diabetes mellitus based on the tuberculosis predictive index score: a case-control study in Indonesia
Dea P. Audina, Rachel S. Aritonang, Muhammad I. Mokoagow

415 Drug use intentions among young adults in the Republic of Korea: a cross-sectional study applying the extended theory of planned behavior with emphasis on impulsive behavior and sensation seeking
Aeree Sohn

© 2025 Korea Disease Control and Prevention Agency

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2025.0335
Osong Public Health Res Perspect 2025;16(4):309-310

Editorial

Artificial intelligence and biohealth: the Republic of Korea's emerging priorities in health care R&D

Jong-Koo Lee

National Academy of Medicine of Korea, Seoul, Republic of Korea

Students have resumed their studies, and medical residents have returned to their hospitals, as the healthcare system gradually recovers from a collapse that lasted more than a year and a half. This disruption revealed numerous problems, which are now the subject of ongoing forums and discussions. Within this context, topics such as addressing regional disparities in healthcare and fostering biohealth as a next-generation growth engine are directly connected to our research priorities and deserve close attention [1].

The coronavirus disease 2019 (COVID-19) pandemic left painful lessons. Epidemiological data, vaccination records, and clinical information were not adequately linked, delaying critical decision-making. The lack of real-time access to essential datasets also hindered timely research that could have guided treatment strategies. Challenges related to system governance and privacy protections further complicated collaboration, even in moments of crisis when rapid decision-making was imperative. Fortunately, after overcoming the immediate COVID-19 emergency, the government recognized the central importance of big data and designated artificial intelligence (AI) and biohealth as strategic industries, now seeking concrete application areas.

The first area is disease prediction through health data. The Republic of Korea possesses extensive health data resources, including those from the National Health Insurance Service, Health Insurance Review and Assessment Service (HIRA), hospital electronic medical records, and national cohorts. By applying AI, it becomes possible to predict the recurrence of myocardial infarction or estimate the risk of tuberculosis in patients with diabetes. These applications are not only noteworthy academic outcomes, but also practical tools for personalized care and the allocation of community health resources, ultimately contributing to the reduction of regional disparities in healthcare.

Second, vaccines and therapeutics for pandemic countermeasures are a promising area [2]. Traditional clinical trials and candidate discovery processes are time-consuming. Even if we prepare for Disease X through the prototype pathogen approach, achieving a 100-day vaccine target may remain an aspirational slogan. AI, however, can simulate millions of protein–gene combinations to identify high-probability candidates rapidly, and the Republic of Korea already has facilities capable of scaling production. If the Republic of Korea successfully cultivates domestic suppliers of critical new technologies, including those essential for mRNA despite significant patent barriers, its capacity will expand further. Moreover, the International

Received: August 23, 2025
Accepted: August 25, 2025

Corresponding author:
Jong-Koo Lee
National Academy of Medicine of Korea, 51 Seochojungang-ro, Seocho-gu, Seoul 06654, Republic of Korea
E-mail: docmohw@snu.ac.kr

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Korea Disease Control and Prevention Agency and Korea National Institute of Health.

© 2025 Korea Disease Control and Prevention Agency.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Vaccine Institute and the World Health Organization (WHO) Global Training Hub for Biomanufacturing can create strong international synergies.

The third area is health system reform and regulatory rationalization. AI cannot be integrated into clinical practice through technology alone. The approval pathway for software as a medical device at the Ministry of Food and Drug Safety, health technology assessments at National Evidence-Based Healthcare Collaborating Agency, and reimbursement evaluations by HIRA all prioritize safety and effectiveness. Harmonization across these processes will be essential. For AI to be applied in practice, regulatory frameworks, clinical evidence, and reimbursement strategies must be incorporated into research design from the outset.

Finally, frontline applications of AI warrant attention. In infectious disease control, simulation-based training and digital dashboards for epidemiologists can accelerate outbreak response [3]. In emergency and trauma care, AI-assisted triage can support appropriate hospital selection, advancing the principle of choosing the right hospital over the nearest one. In remote monitoring and digital therapeutics, AI integration can strengthen chronic disease management, reduce regional disparities, and enable performance-based payment models as well as health system performance assessment [4].

From this perspective, the current issue highlights significant contributions. Iranian researchers designed a CD40L-based multi-epitope vaccine using immunoinformatics, which elicited stronger immune responses than full-length constructs. A Gulf-region study confirmed that mpox vaccine acceptance was linked to self-efficacy and prior COVID-19 vaccination, emphasizing the dual challenge of technological innovation and social acceptance.

Korean researchers developed an AI model based on myocardial infarction cohorts that predicted major adverse cardiac events with 88% to 92% accuracy, aligning with international momentum. Studies from Morocco and Indonesia addressed adherence to diabetes medication and proposed a tuberculosis progression index, offering practical tools for managing the dual burden of noncommunicable and infectious diseases in low- and middle-income countries.

In system-level research, the Republic of Korea's scenario-based training improved outbreak investigation competencies, genetic analysis of Anopheles sinensis revealed cross-border vector linkages, and a national study demonstrated that direct transport of trauma patients to higher-level centers reduced mortality, confirming the importance of structural improvements.

Finally, a Korean study on youth drug use identified impulsivity and sensation-seeking as risk factors, while self-efficacy acted as a protective factor, underscoring the importance of behavioral science approaches beyond legal regulation.

In summary, the 9 papers in this issue illustrate how global research trends—vaccine innovation, disease prediction and cohort-based research, health security through preparedness, and behavioral studies—are being realized through diverse regional case studies. Among these, vaccine and cohort research stand out as top international priorities. The results generated by researchers in the Republic of Korea and partner countries provide both globally relevant evidence and regionally grounded insights for advancing biohealth and health system performance.

Notes

Ethics Approval
Not applicable.

Conflicts of Interest
Jong-Koo Lee has been the editor-in-chief of *Osong Public Health and Research Perspectives* since October 2021.

Funding
None.

References

1. Ministry of Health and Welfare (MOHW) (KR). Ministry of Health and Welfare, Health Care R & D [Internet]. MOHW; 2025 Jun 20 [cited 2025 Aug 23]. Available from: https://www.mohw.go.kr/menu.es?mid=a10707020100. Korean.
2. Krammer F. SARS-CoV-2 vaccines in development. Nature 2020; 586:516–27.
3. World Health Organization (WHO) (CH). WHO simulation exercise manual: a practical guide and tool for planning, conducting and evaluating simulation exercises for outbreaks and public health emergency preparedness and response [Internet]. WHO; 2017 Feb [cited 2025 Aug 23]. Available from: https://iris.who.int/handle/10665/254741.
4. World Health Organization (WHO) (CH). Health system performance assessment [Internet]. WHO; 2022 Apr 13 [cited 2025 Aug 23]. Available from: https://www.who.int/publications/i/item/9789240042476.

https://doi.org/10.24171/j.phrp.2025.0335

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2025.0077
Osong Public Health Res Perspect 2025;16(4):311-332

Original Article

Immunoinformatics study of CD40 ligand-targeting vaccine constructs: a novel immunotherapeutic approach

Seyed Amir Sadeghi[1], Mahroo Mohamadi[2], Hadi Bamehr[3], Fatemeh Heidarnejad[1], Azam Bolhassani[1]

[1]Department of Hepatitis, AIDS and Blood-borne Diseases, Pasteur Institute of Iran, Tehran, Iran
[2]Department of Clinical Biochemistry, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Department of Medical Biotechnology, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran

ABSTRACT

Objectives: Incorporating CD40 ligand (CD40L) into vaccine strategies has shown considerable potential for enhancing immune responses. In this study, we designed and formulated a CD40L-based multi-epitope vaccine construct using immunoinformatics approaches, and compared it to a full-length CD40L-based vaccine construct.

Methods: The study commenced with the identification and screening of potential T-cell and B-cell epitopes derived from the CD40L protein, followed by the construction of a multi-epitope vaccine from these selected epitopes. We analyzed and validated the physicochemical and structural properties of the vaccine constructs. Further, we predicted disulfide bonds, performed protein-protein docking, and conducted molecular dynamics simulations to evaluate the constructs. Comparative analyses of the ligand-binding site localization were conducted using LigPlot. Additionally, simulation trajectories were analyzed using multiple descriptors, including root mean square deviations, radius of gyration, and root mean square fluctuations.

Results: Our findings indicated that the CD40L multi-epitope vaccine construct possessed favorable physicochemical properties and a validated structural profile. Immune simulation studies showed a stronger affinity of the multi-epitope construct for the CD40 receptor compared to the full-length CD40L construct.

Conclusion: Overall, the CD40L multi-epitope vaccine construct demonstrated greater potency in eliciting an effective immune response than the full-length CD40L construct. These results highlight a promising approach to vaccine design for the prevention or treatment of infections and cancers.

Keywords: CD40 ligand; CD40 receptor; Molecular docking simulation; Molecular dynamics simulation; Therapeutic vaccine

Received: March 9, 2025
Revised: May 29, 2025
Accepted: June 15, 2025

Corresponding author:
Fatemeh Heidarnejad
Department of Hepatitis, AIDS and Blood-borne Diseases, Pasteur Institute of Iran, NO. 69, Pasteur Ave., Tehran, Iran
E-mail: heidarnejadfatemeh@gmail.com

Co-Corresponding author:
Azam Bolhassani
Department of Hepatitis, AIDS and Blood-borne Diseases, Pasteur Institute of Iran, NO. 69, Pasteur Ave., Tehran, Iran
E-mail: A_bolhasani@pasteur.ac.ir

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Korea Disease Control and Prevention Agency and Korea National Institute of Health.

© 2025 Korea Disease Control and Prevention Agency.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

CD40 ligand (CD40L), also known as CD154, is a key member of the tumor necrosis factor superfamily, playing a central role in immune regulation. CD40L is essential for coordinating immune cell interactions and serves as a primary mediator in diverse immune processes [1]. Predominantly expressed on activated T cells, CD40L binds to its receptor CD40 on antigen-presenting cells (APCs), initiating signaling cascades that influence both innate and adaptive immunity [2]. Its functions include B cell activation, class-switching, and the formation of memory responses [3]. Beyond these roles, CD40L is now extensively studied for its therapeutic potential in immunotherapy and vaccine development [4–6]. Epitope vaccines, also known as subunit vaccines, represent a precise approach to vaccination [7]. Compared to whole-sequence vaccines, multi-epitope vaccines provide greater precision and safety by targeting specific antigenic regions, reducing adverse reactions and focusing the immune response [8]. They also allow formulation customization to include multiple epitopes, broadening effectiveness, and their synthetic production simplifies manufacturing [8]. Furthermore, multi-epitope vaccines can target conserved regions shared across pathogen strains, addressing the challenge of pathogen variability [9]. These benefits—improved safety, tailored responses, and broader protection—make epitope vaccines an attractive option [10,11].

Bioinformatics has revolutionized vaccine design by accelerating candidate identification through computational tools [12,13]. Several multi-epitope vaccines developed using immunoinformatics have reached clinical trials or commercialization, such as Russia's EpiVacCorona (using synthetic SARS-CoV-2 peptides) and the R21/Matrix-M malaria vaccine, which targets conserved Plasmodium falciparum epitopes [14,15]. Similarly, multi-epitope vaccines against rotavirus have been developed by coupling selected epitopes with adjuvants and linkers, which showed favorable physicochemical properties and strong receptor binding in computational analyses [16]. In veterinary medicine, multi-epitope vaccines targeting Newcastle disease virus were designed by combining conserved epitopes from viral glycoproteins, demonstrating significant immune receptor interactions and the potential to induce protective immunity [17]. These cases highlight the increasing success of immunoinformatics-driven multi-epitope vaccine design, paving the way for innovative immunotherapeutic strategies in both human and animal health. Hence, this article presents a comprehensive comparative analysis of CD40L whole sequence and CD40L multi-epitope constructs, evaluating them through molecular docking, immune simulation, molecular dynamics (MD), and *in silico* cloning. By comparing these constructs, we aim to provide new insights into CD40L's potential as a vaccine candidate and inform future therapeutic development.

HIGHLIGHTS

- Our study demonstrates that a novel multi-epitope vaccine based on CD40 ligand (CD40L) significantly boosts immune responses compared to traditional full-length CD40L vaccines.
- We utilized advanced *in silico* methods to design the multi-epitope construct, illustrating the potential of this approach to improve vaccine efficacy.
- This study included comprehensive evaluations of physicochemical properties, structural validation, and molecular dynamics simulations to ensure robust findings.
- Immune simulations revealed that the multi-epitope construct may exhibit a stronger affinity for the CD40 receptor, indicating improved interactions with the immune system.
- These findings suggest that our innovative multi-epitope vaccine could offer a promising strategy for combating infections and cancers, laying the groundwork for future vaccine development.

Materials and Methods

Retrieval of Protein Sequence

The CD40L protein sequence in FASTA format was retrieved from the National Center for Biotechnology Information (NCBI) database (accession NP_035746.2).

Epitope Prediction Analyses

We utilized the NetMHCpan 4.1 server (https://www.cbs.dtu.dk/services/NetMHCpan/) to predict cytotoxic T lymphocyte (CTL) epitopes from selected linear peptides, applying a threshold of 0.5 and default parameters. Peptide binding affinity was assessed against human leukocyte antigen (HLA)-I supertypes and common global HLA-I alleles [18]. For major histocompatibility complex (MHC)-II-restricted $CD4^+$ helper T lymphocyte (HTL) epitopes, NetMHCIIpan (http://www.cbs.dtu.dk/services/NetMHCpan/) was used, setting epitope length to 15, with thresholds of 1 and 5 for high- and low-affinity binders, respectively [19]. MHC-I antigen processing—including proteasomal cleavage,

https://doi.org/10.24171/j.phrp.2025.0077

TAP transport, and MHC-I binding—was analyzed using NetCTLpan 1.1 (https://services.healthtech.dtu.dk/services/NetCTLpan-1.1/), which calculates weighted prediction scores based on C-terminal cleavage, binding affinities, and TAP transport efficiency [20]. Linear B-cell epitopes (LBL) were identified using ElliPro (http://tools.iedb.org/ellipro/) with default settings. Molecular docking between selected CD40L epitopes and MHC alleles was performed using GalaxyPepDock (http://galaxy.seoklab.org/cgi-bin/submit.cgi?type=PEPDOCK), with relevant HLA structures sourced from RCSB Protein Data Bank (PDB, https://www.rcsb.org), as listed in Table 1. Antigenicity was determined with VaxiJen v2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html), allergenicity with AllerTOP 2.0 (https://www.ddg-pharmfac.net/AllerTOP/), and toxicity with ToxinPred (http://crdd.osdd.net/raghava/toxinpred/) [21]. The HTL epitopes' ability to induce interferon (IFN)-γ, interleukin (IL)-4, and IL-10 was predicted using IFNepitope (http://crdd.osdd.net/raghava/ifnepitope/), IL4pred (http://crdd.osdd.net/raghava/il4pred/), and IL10pred (http://crdd.osdd.net/raghava/IL-10pred/) [22,23]. Finally, population coverage for selected CTL and HTL epitopes and their associated HLA alleles (MHC-I and MHC-II) was analyzed using the IEDB population coverage tool (http://tools.iedb.org/population/) [24].

Design and Physicochemical Features of Vaccine Constructs

Dominant T and B cell epitopes were linked with AYY linkers to design multi-epitope vaccines using SnapGene 3.2.1 (SnapGene). Antigenicity and allergenicity of both multi-epitope and whole-sequence CD40L constructs were evaluated using VaxiJen 2.0 (https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) and Aller-TOP 2.0 [25].

Table 1. MHC alleles used for peptide-protein docking

MHC-I		MHC-II	
Allele	PDB code	Allele	PDB code
HLA-A01:01	4NQV	DRB1:0101	4AH2
HLA-A02:01	4UQ3	DRB1:0301	2Q6W
HLA-A03:01	3RL2	DRB1:0401	5LAX
HLA-A11:01	1×7Q	DRB1:1101	6CPL
HLA-A24:02	5HGA	DRB1:1501	5V4M
HLA-B07:02	5EO1	DRB5:0101	1FV
HLA-B08:01	3SPV		
HLA-B27:05	1OGT		
HLA-B35:01	3LKN		
HLA-B51:01	1E+27		

MHC, major histocompatibility complex; PDB, Protein Data Bank; HLA, human leukocyte antigen.

Construct solubility was predicted using the Protein-Sol (https://protein-sol.manchester.ac.uk/) server [26]. The physicochemical characteristics of the vaccine constructs were evaluated using ExPASy ProtParam (https://web.expasy.org/protparam/) server [27].

Secondary Structure Prediction

Secondary structural elements (α-helices, β-sheets, β-turns, coils) were predicted using the self-optimized prediction method with alignment (SOPMA) method (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html) [28].

Tertiary Structure Prediction, Refinement, and Validation

Three-dimensional (3D) models of constructs were generated with Robetta (https://robetta.bakerlab.org/) [29], refined, and validated using GalaxyRefine (http://galaxy.seoklab.org/refine) [30] and the PROCHECK server (https://saves.mbi.ucla.edu/) for Ramachandran plots, Verify3D modules and ERRAT score plots [31,32].

Discontinuous B-Cell Epitope Prediction

Discontinuous B-cell epitopes were predicted with ElliPro (http://tools.iedb.org/ellipro/), which uses protein 3D structure to identify accessible residues and cluster them as conformational epitopes. This computational approach helps identify B cell epitopes for further experimental validation or vaccine development [33].

Disulfide Bond Prediction of the Protein Structures

We used the DIpro Scratch server (http://scratch.proteomics.ics.uci.edu/) to predict disulfide bonds, including their number, cysteine bonding state, and paired connections, with 85% accuracy and 90% recall [34].

Molecular Docking Analysis of the CD40 Receptor and Vaccine Constructs

CD40 receptor structures were obtained from RCSB PDB. Refined 3D vaccine constructs (multi-epitope and whole sequence) were docked using ClusPro 2.0 (https://cluspro.bu.edu/login.php) and HDOCK (http://hdock.phys.hust.edu.cn). The best complex was selected based on lowest energy-weighted score and docking efficiency. Both servers use FFT-based global docking, widely applied in protein-protein docking studies [35,36].

Protein-Ligand Interactions

LigPlot was used to visualize protein–ligand interactions, producing 2-dimensional diagrams showing hydrogen

bonds (dashed lines) and hydrophobic contacts (arcs), with participating protein residues indicated by spokes [37].

In Silico Immune Simulation

An immune simulation study was performed to investigate the immunogenicity and immune response profile using the C-ImmSim web tool (http://150.146.2.1/CIMMSIM/index.php), which incorporates machine learning and real-life-like immune interactions [38]. Default parameters were used, with time steps at 1, 84, and 170 (representing 3 injections at 4-week intervals, as in commercial vaccine regimens) [39,40].

Molecular Dynamics Simulation

Vaccine constructs docked with the CD40 receptor were subjected to 50 ns MD simulations using Gromacs v2021.5 (GROMACS Development Team) [41]. Gromacs is designed for efficient, parallel biomolecular simulations [42]. The CHARMM-36 force field was used for topology generation [43]. Each complex was placed in a simulation box with 1 nm distance from the box edges and solvated using the TIP3P water model. Sodium and chloride ions were added for charge neutralization. Energy minimization was performed via the steep descent algorithm. Equilibration was run for 1 ns at 298 K and 1 bar in the normal pressure and temperature (NPT) ensemble, using a Berendsen thermostat and barostat. For the production run, each system was simulated under constant normal volume and temperature at 300 K, using a modified Berendsen thermostat [43]. Afterwards, systems were further simulated under constant pressure (NPT) using a Berendsen barostat at 1 atm [44]. Long-range electrostatic interactions were calculated with the Particle Mesh Ewald method, applying a cutoff distance of 1.0 nm [45]. Periodic boundary conditions were removed before analysis. Simulation trajectories were analyzed for root mean square deviation (RMSD), root mean square fluctuation (RMSF), and radius of gyration (Rg) to assess conformational stability of the complexes.

In Silico Cloning

Codon optimization for *Escherichia coli* expression was performed with JCat (http://www.jcat.de/CAICalculation.jsp), using the codon adaptation index (CAI) to assess synonymous codon bias [46]. SnapGene 3.2.1 was used to identify restriction sites, simulate cloning, validate sequences, and insert optimized constructs into pET24a(+) for expression.

Results

Epitope Prediction Analyses

The CD40L reference sequence was obtained from NCBI in FASTA format for all subsequent analyses. Using NetMHCpan 4.1, we predicted CTL epitopes of CD40L, identifying 6 overlapping sequences with high binding affinities for multiple HLA-I alleles as top CTL epitopes. For HTL epitopes, 5 high-scoring candidates for MHC-II were selected based on strict criteria, all demonstrating strong proteasomal cleavage and TAP transport efficiency (Table 2).

B-cell linear epitopes were predicted using the ElliPro server to improve accuracy. The resulting CD40L epitopes were non-allergenic, non-toxic, and antigenic (Table 3).

CD40L protein was screened for 2 linear B cell epitopes, 5 T cell epitopes and 6 CTL epitopes. Notably, amino acids 1 to 33 overlapped in both B-cell and T-cell epitopes and were the most dominant. The MHC alleles for peptide-protein docking are listed in Table 1. Top models with the highest interaction similarity between CTL/HTL epitopes and their respective HLA class I and II alleles are presented in Tables 4 and 5. Epitopes with support vector machine (SVM) scores above threshold were classified as IL-10 and IL-4 inducers, while positive SVM scores indicated IFN-γ induction. All selected CD40L epitopes induced IFN-γ. Specifically, $CD40L_{200–219}$ induced both IL-4 and IL-10; $CD40L_{135–154}$ and

Table 2. MHC-I processing prediction and immunogenicity scores of CD40L CTL epitopes

Protein name	Position	Epitope sequence	TAP transport efficiency score[a]	Proteasomal C-terminal cleavage score[b]	Epitope identification score[c]	Immunogenicity scores[d]
CD40L	1–13	MIETYSQPSPRSV	1.547	0.585	0.125	−0.44126
	4–17	TYSQPSPRSVATGL	0.722	0.976	0.225	−0.33407
	17–27	LPASMKIFMYL	2.331	0.905	0.442	−0.44533
	34–45	TQMIGSVLFAVY	2.787	0.869	0.422	0.14556
	158–170	KQLTVKREGLYYV	2.968	0.796	0.35	0.03084
	84–95	SQRPFIVGLWLK	1.277	0.974	0.5	0.49246

MHC, major histocompatibility complex; CD40L, CD40 ligand; CTL, cytotoxic T lymphocyte.
[a]A higher score indicates a better quality of TAP transport efficiency. [b]A higher score indicates a better quality of proteasomal cleavage. [c]Higher rates indicate a better quality of epitope identification. [d]A higher score indicates a greater probability of eliciting an immune response.

https://doi.org/10.24171/j.phrp.2025.0077

Table 3. Prediction of dominant T and B-cell epitopes of the CD40L

Epitopes	Methods	Location	Sequence	Average rank scores[a)]	Toxicity	Antigenicity[b)]	Allergenicity
B cell	ElliPro	1–33	MIETYSQPSPRSVATGLPASMKIFMYLL TVFLI	0.862	No	0.6137	Non
		178–187	SNREPSSQRP	0.71	No	1.1488	Non
HTL	NetMHCIIpan	90–108	QFEDLVKDITLNKEEKKENS	2.684	No	0.562	Non
		135–154	SVLQWAKKGYYTMKSNLVML	1.333	No	0.9495	Non
		139–157	WAKKGYYTMKSNLVMLENG	1.401	No	0.9215	Non
		141–160	KKGYYTMKSNLVMLENGKQL	1.610	No	0.7886	Non
		228–249	FELQAGASVFVNVTEASQVIHR	2.758	No	0.6329	Non
CTL	NetCTLpan	1–13	MIETYSQPSPRSV	0.905	No	0.7404	Non
		4–17	TYSQPSPRSVATGL	0.897	No	1.1382	Non
		17–27	LPASMKIFMYL	0.982	No	0.6488	Non
		34–45	TQMIGSVLFAVY	0.965	No	0.6284	Non
		158–176	KQLTVKREGLYYV	0.989	No	0.7354	Non
		84–95	SQRPFIVGLWLK	0.908	No	0.8084	Non

CD40L, CD40 ligand; HTL, helper T lymphocyte; CTL, cytotoxic T lymphocyte.
[a)]Lower rates show better binding affinity. [b)]Higher rate shows high degree of peptide antigenicity.

Table 4. Binding affinities and interaction between selected CTL epitopes and HLA alleles

Epitope	HLA A0101	HLA A0201	HLA A0301	HLA A2402	HLA A1101	HLA B0702	HLA B0801	HLA B2705	HLA B3501	HLA B5101	Average of interaction similarity score[a)]
CD40L (1–13)	162	214	187	216	171	187	211	192	189	207	193.6
CD40L (4–17)	176	203	183	214	180	195	183	179	209	247	196.9
CD40L (17–27)	192	242	206	229	206	215	221	233	251	272	226.7
CD40L (34–45)	192	249	226	222	207	208	216	210	245	235	221
CD40L (84–95)	237	266	252	249	254	253	241	247	268	289	255.6
CD40L (158–176)	190	227	207	202	211	229	220	218	257	241	220.2

CTL, cytotoxic T lymphocyte; HLA, human leukocyte antigen; CD40L, CD40 ligand.
[a)]Higher rate shows better quality of peptide-major histocompatibility complex interactions.

Table 5. Binding affinities and interaction between selected HTL epitopes and HLA alleles

Epitope	DRB1–0101	DRB1–0301	DRB1–0401	DRB1–1101	DRB1–1501	DRB5–0101	Average of Interaction similarity score[a)]
CD40L (90–108)	125	131	117	117	117	117	120.6
CD40L (135–154)	141	150	141	141	141	141	142.5
CD40L (139–157)	141	143	141	141	141	141	141.3
CD40L (141–160)	142	143	135	135	135	135	137.5
CD40L (228–249)	141	131	135	131	151	131	136.6

HTL, helper T lymphocyte; HLA, human leukocyte antigen; CD40L, CD40 ligand.
[a)]Higher rate shows better quality of peptide-major histocompatibility complex interactions.

$CD40L_{141–160}$ induced IL-10; and $CD40L_{90–108}$ and $CD40L_{228–249}$ induced IL-4 (Table 6).

Tables 7 and 8 show the highest-scoring epitopes and their corresponding population coverage percentages. For CTL epitopes, the highest population coverage of the world's population was calculated for $CD40L_{1-13}$ with 95.11%. For helper T-cell epitopes, most of the CD40L epitopes exhibit population coverage exceeding 90%. Overall, the IEDB server indicated that most of the identified epitopes had coverage of over 80%. Finally, the epitopes that met all the specified criteria were chosen for the design of the final multi-epitope construct.

Table 6. Cytokine production of CD40L HTL epitopes

Protein name	Position	Epitope sequence	IL-10 production SVM scores	IL-10 induction	IL-4 production SVM scores	IL-4 induction	IFN-γ production SVM scores	IFN-γ induction
CD40L	90–108	QFEDLVKDITLNKEEKKENS	−0.211	Non- inducer	1.15	Inducer	1.02	Inducer
	135–154	SVLQWAKKGYYTMKSNLVML	0.462	Inducer	−0.03	Non-inducer	1.82	Inducer
	139–157	WAKKGYYTMKSNLVMLENG	0.290	Non- inducer	0.10	Non-inducer	1.22	Inducer
	141–160	KKGYYTMKSNLVMLENGKQL	0.708	Inducer	−1.02	Non-inducer	1.39	Inducer
	228–249	FELQAGASVFVNVTEASQVIHR	0.115	Non- inducer	0.55	Inducer	2.19	Inducer

CD40L, CD40 ligand; HTL, helper T lymphocyte; IL, interleukin; IFN, interferon; SVM, support vector machine.

Table 7. Population coverage of CD40L CTL epitopes

Area	$CD40L_{1-13}$ (%)	$CD40L_{4-17}$ (%)	$CD40L_{17-27}$ (%)	$CD40L_{34-45}$ (%)	$CD40L_{84-95}$ (%)	$CD40L_{158-176}$ (%)
Central Africa	63.96	54.25	51.08	45.85	63.10	49.82
Central America	4.14	2.77	2.77	3.37	4.72	2.77
East Africa	71.38	63.91	61.98	49.84	69.40	60.77
East Asia	94.86	87.36	91.01	89.00	96.00	74.39
Europe	98.34	90.79	92.53	85.42	97.48	92.83
Iran	90.81	77.40	78.49	68.71	89.44	84.13
North Africa	86.43	80.22	73.43	67.68	83.24	70.69
North America	95.01	86.84	87.43	83.24	94.17	83.49
Northeast Asia	87.92	64.89	70.15	70.85	90.66	77.57
Oceania	94.15	81.84	81.62	80.42	93.31	72.26
South Africa	76.68	64.05	61.71	40.21	69.61	60.43
South America	81.50	61.96	61.03	59.58	77.20	48.40
South Asia	89.65	67.93	69.37	59.33	83.59	73.90
Southeast Asia	88.86	72.57	73.43	82.36	92.95	69.56
Southwest Asia	89.00	76.98	73.94	65.88	85.66	72.77
West Africa	75.35	68.99	69.21	59.32	70.65	66.92
West Indies	92.08	82.56	81.61	79.79	91.79	76.55
World	95.11	84.26	85.92	80.13	94.01	85.20

CD40L, CD40 ligand; CTL, cytotoxic T lymphocyte.

Physicochemical Features of Vaccine Construct

The SnapGene 3.2.1 tool was used to design the vaccine construct by joining LBL, CTL, and HTL epitopes with an AAY linker (Figure 1). Properties of epitope-based and whole-protein constructs were compared. ProtParam analysis showed the multi-epitope construct had a molecular weight (MW) of 28.70 kDa (<100 kDa), pI of 9.56, mammalian half-life >20 hours, and *E. coli* half-life >10 hours. The instability index was 35.73 (<40), indicating stability. Protein-Sol analysis gave a solubility score of 0.713 (<45), indicating high hydrophilicity. VaxiJen 2.0 confirmed antigenicity (0.567 >0.4), and Aller-TOP 2.0 predicted non-allergenicity. Compared to the whole sequence, the multi-epitope construct showed superior solubility (0.713 vs. 0.361), stability (46.29 vs. 35.73), and antigenicity (0.567 vs. 0.504). Full comparisons are in Table 9.

Secondary Structure Prediction

SOPMA analysis of both constructs (Table 10) revealed the multi-epitope construct contained significantly more alpha helices, beta sheets, and beta turns than the whole sequence (Figure 2).

Tertiary Structure Prediction, Refinement, and Validation

3D models were generated using Robetta and refined with Galaxy Refine 2. Validation by ERRAT, PROCHECK, and Verify3D showed ERRAT scores of 91.25 for the multi-epitope and 87.288 for the whole sequence. Ramachandran plots showed 92% (multi-epitope) and 90.7% (whole sequence) of residues in favored regions. Verify3D analysis confirmed >80% of residues scored above 0.1 for both constructs. Figures 3 and 4 present the 3D models and validation results.

https://doi.org/10.24171/j.phrp.2025.0077

Table 8. Population coverage of CD40L HTL epitopes

Area	CD40L$_{90-108}$ (%)	CD40L$_{135-154}$ (%)	CD40L$_{139-157}$ (%)	CD40L$_{141-160}$ (%)	CD40L$_{228-249}$ (%)
Central Africa	88.56	99.98	99.96	99.96	94.99
Central America	99.86	100.00	100.00	100.00	99.85
East Africa	94.81	99.98	99.97	99.97	96.86
East Asia	87.83	99.58	99.22	99.22	93.57
Europe	89.85	100.00	100.00	100.00	93.28
Iran	83.40	95.78	96.15	96.15	90.49
North Africa	86.55	99.22	98.32	98.32	93.73
North America	97.76	100.00	100.00	100.00	97.99
Northeast Asia	92.29	99.74	99.72	99.72	95.75
Oceania	95.40	99.97	99.90	99.90	97.85
South Africa	45.98	7.65	7.65	7.65	7.65
South America	99.23	100.00	100.00	100.00	98.61
South Asia	87.87	99.97	99.96	99.96	86.76
Southeast Asia	84.89	98.00	98.03	98.03	90.61
Southwest Asia	81.09	99.30	98.51	98.51	89.04
West Africa	98.14	99.97	99.97	99.97	98.12
West Indies	75.91	95.15	95.60	95.60	94.42
World	93.18	99.91	99.91	99.91	94.10

CD40L, CD40 ligand; HTL, helper T lymphocyte.

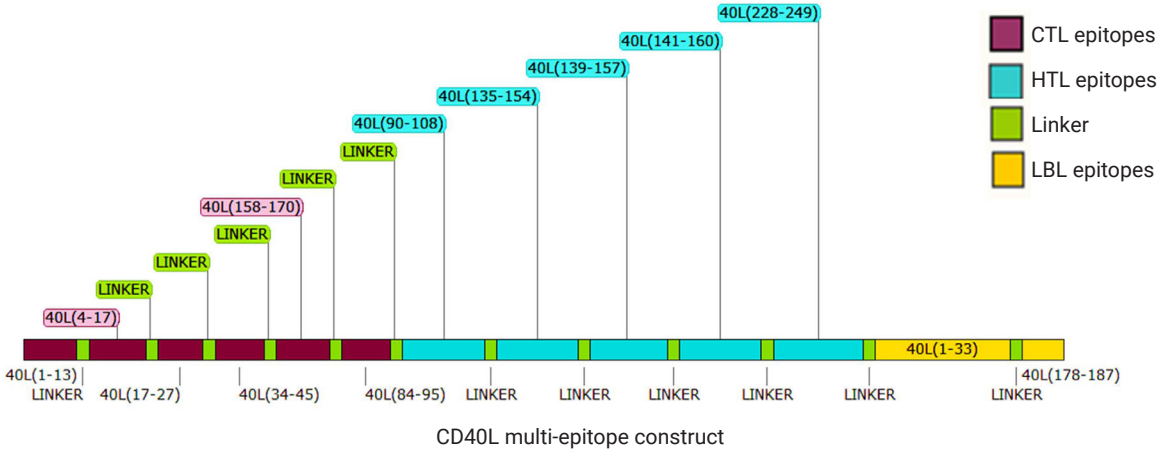


Figure 1. Final CD40 ligand (CD40L) multi-epitope construct. Dominant linear B-cell (LBL), cytotoxic T lymphocyte (CTL), and helper T lymphocyte (HTL) epitopes were linked using an AYY linker to design the multi-epitope construct.

Table 9. Physicochemical properties of CD40L-based vaccine constructs

Construct	Molecular weight (kDa)	Theoretical pI	Half-life (h)	Stability	Solubility >0.45	Allergenicity	Antigenicity >0.4
CD40L whole sequence	29.37	8.26	>20 (mammalian), >10 (*Escherichia coli*)	46.29 (unstable)	In soluble (0.361)	Non-allergen	0.504
CD40L multi-epitopes	28.70	9.56	>20 (mammalian), >10 (*E. coli*)	35.73 (stable)	Soluble (0.713)	Non-allergen	0.567

CD40L, CD40 ligand; pI, isoelectric point.

Table 10. Secondary structure analysis of the CD40L-based vaccine constructs

Construct	Alpha helix (%)	Beta sheet (%)	Beta turn (%)	Random coil (%)
CD40L whole sequence	32.00	21.92	5.77	39.69
CD40L multi-epitope	40.38	21.96	6.27	31.37

CD40L, CD40 ligand.

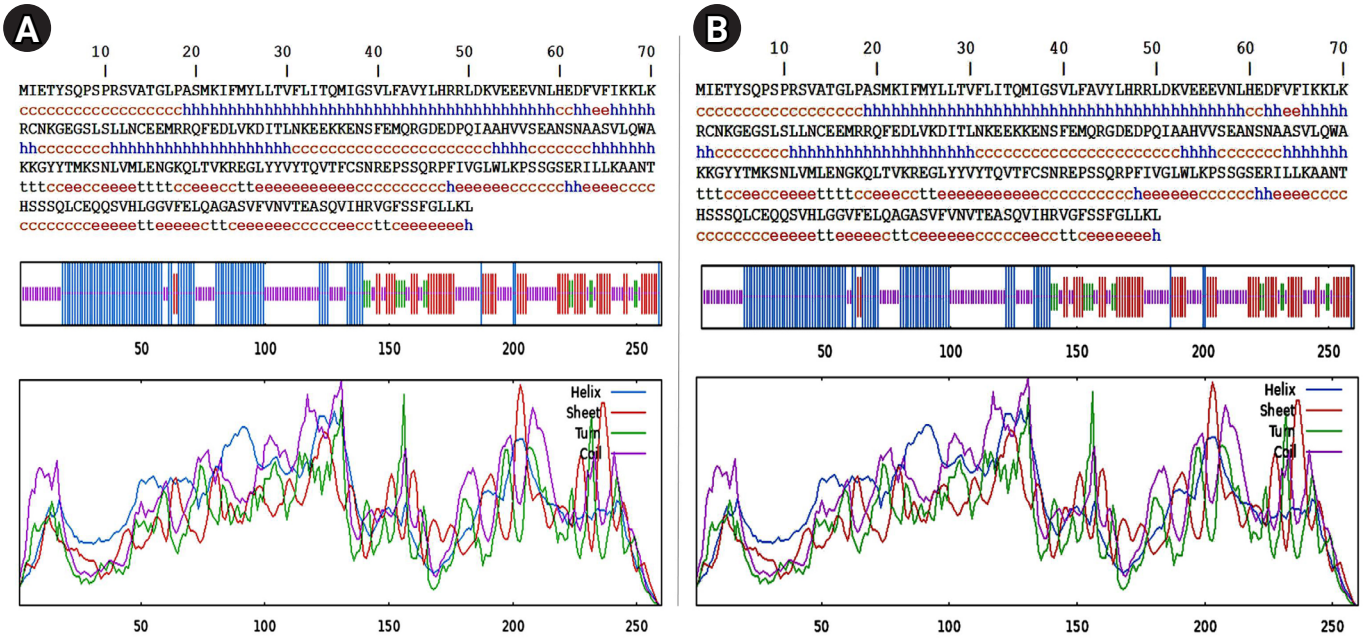


Figure 2. Secondary structure analysis of vaccine constructs. (A) CD40 ligand (CD40L) whole sequence secondary structure; (B) CD40L multi-epitope secondary structure. The multi-epitope construct showed a notable increase in alpha helices, beta sheets, and beta turns compared to the whole sequence.

Discontinuous B-Cell Epitope Prediction

ElliPro analysis on the refined 3D CD40L models identified 5 potential discontinuous B-cell epitopes in the multi-epitope construct. The number and scores of these predicted epitopes suggest strong potential to trigger a humoral immune response (Table 11).

Disulfide Bond Prediction

The DIpro Scratch server predicted disulfide bond connectivity in both the CD40L whole sequence and multi-epitope constructs. The whole sequence had 4 cysteines (positions 72, 84, 177, and 217), forming 2 disulfide bonds. The multi-epitope construct also contained 4 cysteines (positions 6, 20, 155, and 213), predicted to form 2 disulfide bonds.

Molecular Docking Analysis of the CD40 Receptor and Vaccine Constructs

Molecular docking was performed using ClusPro 2.0 and HDOCK web servers. There is an inverse correlation between energy values and binding affinity. ClusPro showed highly negative energies for CD40L multi-epitope and whole sequence constructs docked to CD40 receptor (–1,330.3 kcal/mol and –1,117.3 kcal/mol, respectively). HDOCK docking scores were –226.16 for the multi-epitope and –197.79 for the whole sequence construct. Both tools produced consistent results, indicating that the multi-epitope construct had lower binding energy and a stronger docking score with CD40. Protein-protein docking results are illustrated in Figure 5.

Protein–Ligand Interactions

LigPlot analysis revealed that both CD40L-based constructs interact with the CD40 receptor through hydrophobic and hydrogen bonds, with hydrophobic interactions predominating. The multi-epitope construct had more amino acids involved in hydrogen bonding than the whole sequence. In the multi-epitope structure, aspartic acid 69 bonded with threonine, whereas in the full-length sequence, it bonded with tyrosine. Additionally, glutamic acid 107 in the multi-epitope construct formed a hydrogen bond with tyrosine, while in the whole sequence, it bonded with

https://doi.org/10.24171/j.phrp.2025.0077

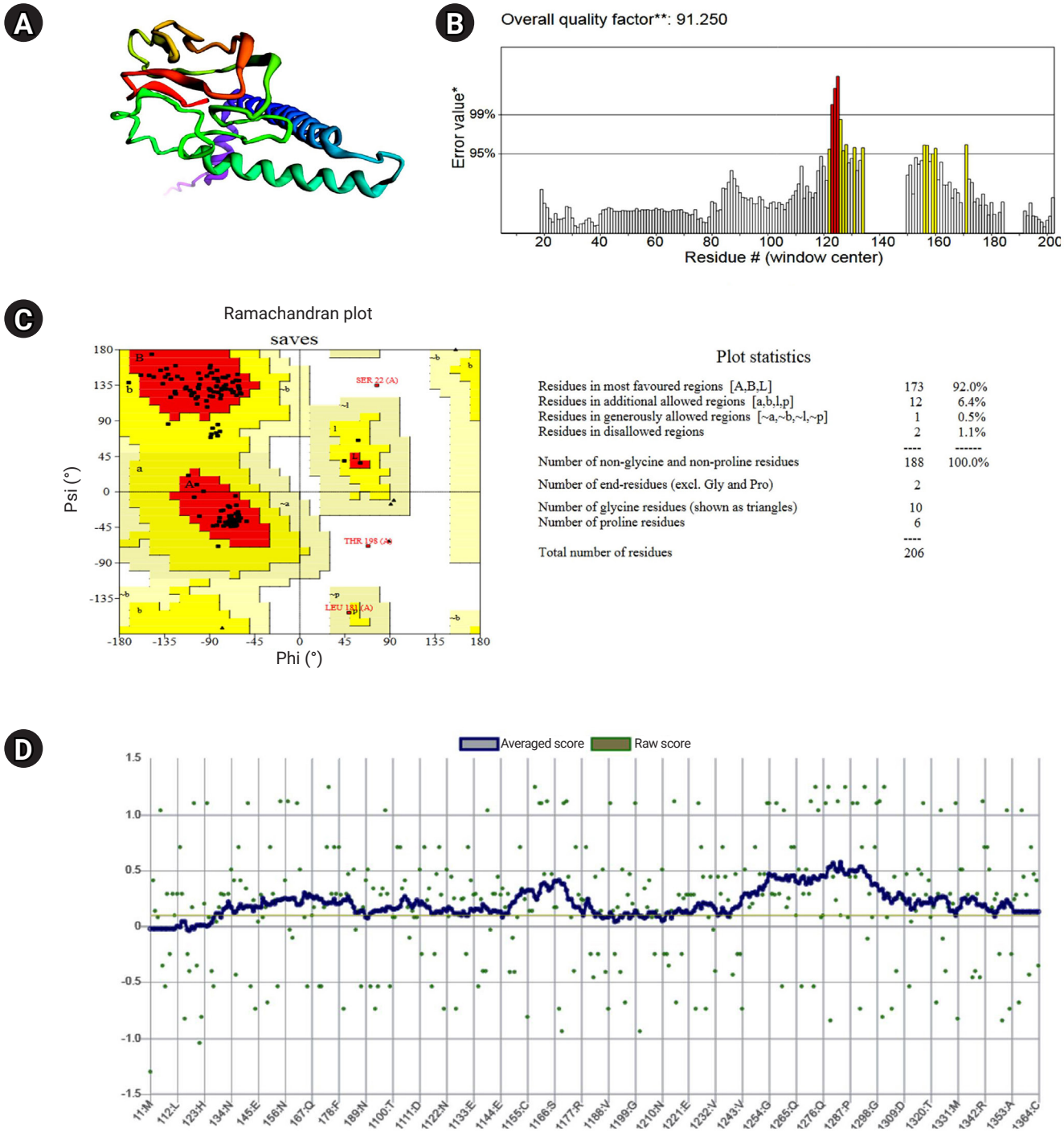


Figure 3. Structural validation of the CD40 ligand (CD40L) multi-epitope construct. (A) Three-dimensional (3D) structure generated by the Robetta server and refined by GalaxyRefine; (B) ERRAT error values, with a quality factor of 91.25; (C) Ramachandran plot analysis by PROCHECK, showing 92% of residues in favored regions; (D) Verify3D plot, with 82.71% of residues scoring ≥0.1, meeting Verify3D's acceptance criteria.

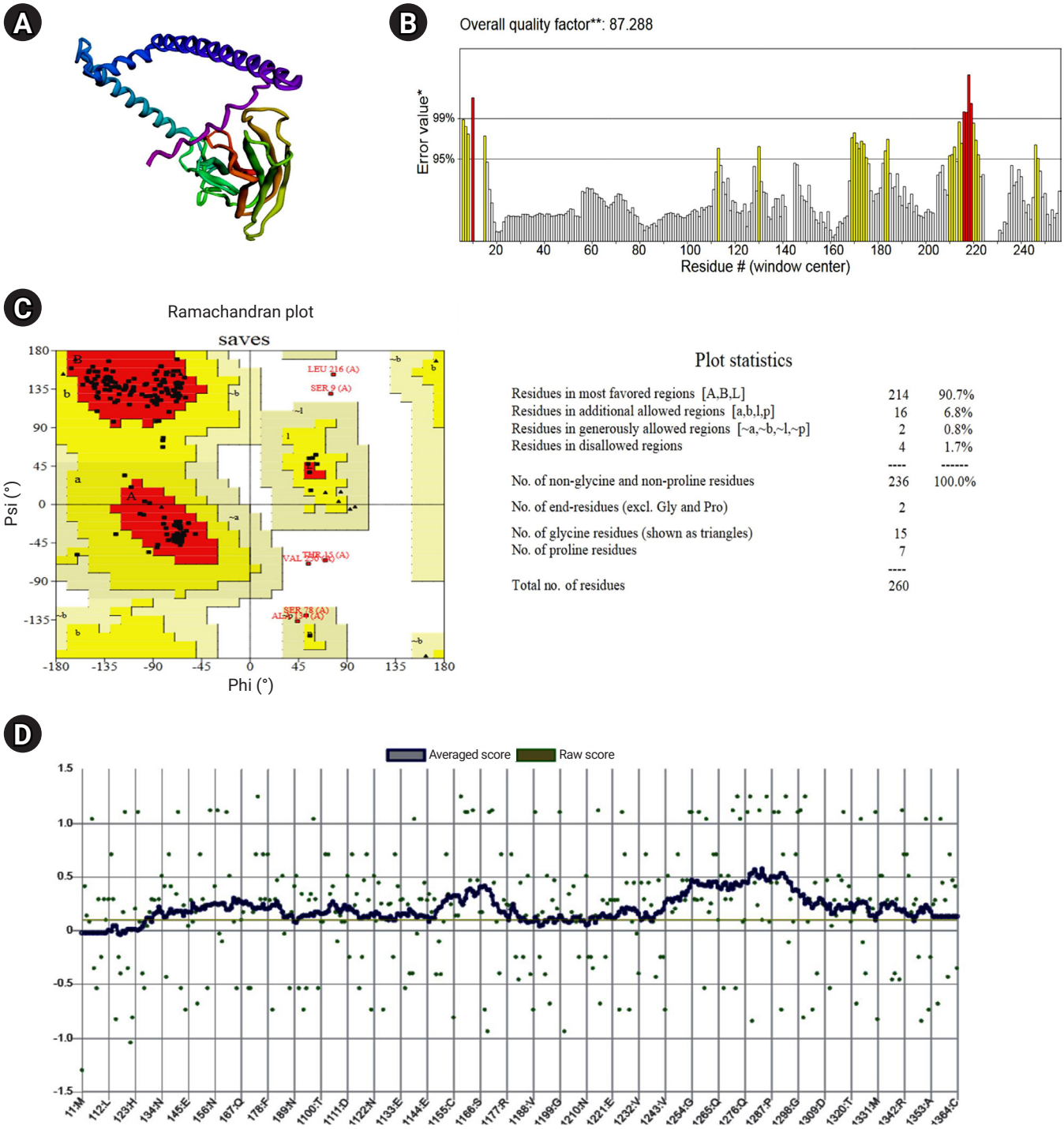


Figure 4. Structural validation of the CD40 ligand (CD40L) whole sequence construct. (A) Three-dimensional (3D) structure generated by the Robetta Baker server and refined by GalaxyRefine; (B) ERRAT error values, with a quality factor of 87.288; (C) Ramachandran plot analysis by PROCHECK, showing 90.7% of residues in favored regions; (D) Verify3D plot, with 80.22% of residues scoring ≥0.1, within acceptable limits.

https://doi.org/10.24171/j.phrp.2025.0077

Table 11. Predicted discontinuous epitopes of CD40 ligand construct

No.	Residues	No. of residues	Score
1	A:M1, A:I2, A:E3, A:T4, A:Y5	5	0.988
2	A:S6, A:Q7, A:P8, A:S9, A:P10, A:R11, A:S12, A:V13, A:A14, A:T15, A:G16, A:L17, A:P18, A:A19, A:S20, A:M21, A:K22, A:I23, A:F24, A:M25, A:Y26, A:L27, A:L28, A:T29, A:V30, A:F31, A:L32, A:I33, A:M36, A:I37, A:V40	31	0.811
3	A:E54, A:E55, A:E56, A:V57, A:N58, A:L59, A:H60, A:E61, A:D62, A:F63, A:V64, A:F65, A:I66, A:K67, A:K68, A:L69, A:K70, A:R71, A:C72, A:N73, A:K74, A:G75, A:E76, A:G77, A:S78, A:L79, A:S80, A:L81, A:L82, A:N83, A:C84, A:E85, A:E86, A:M87, A:R88, A:R89, A:Q90, A:F91, A:E92, A:D93, A:L94, A:V95, A:K96, A:D97, A:I98, A:T99, A:L100, A:N101, A:E104	49	0.79
4	A:S178, A:N179, A:E181, A:P182, A:S183, A:S184, A:Q185, A:R186, A:P187, A:T210, A:H211, A:S212, A:S213, A:S214, A:Q215, A:L216, A:C217	17	0.677
5	A:F110, A:E111, A:R114, A:G115	4	0.569

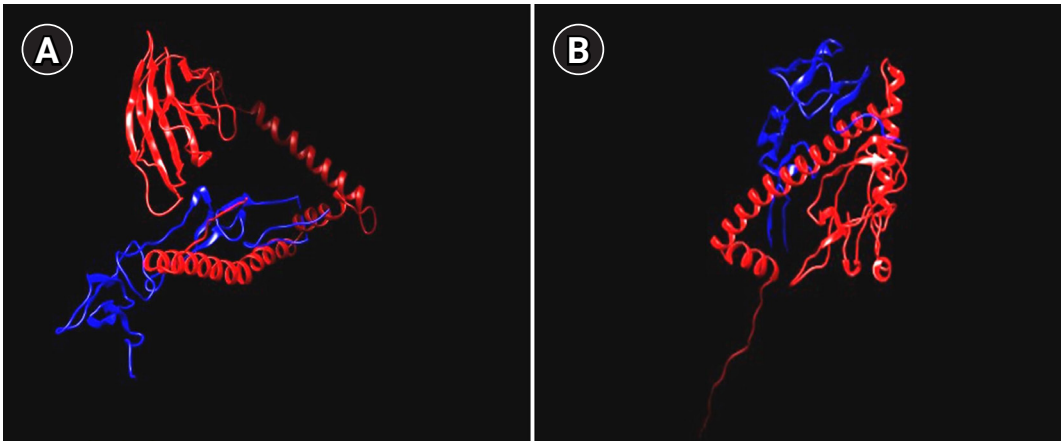


Figure 5. Molecular docking of vaccine constructs with the CD40 receptor. (A) Protein-protein docking of the CD40 ligand (CD40L) whole sequence construct and CD40 receptor; (B) Docking of the CD40L multi-epitope construct and CD40 receptor. Blue indicates the vaccine construct; red indicates the receptor. The multi-epitope construct displayed lower energy and a stronger docking score with CD40 compared to the whole sequence construct.

Table 12. Interacting amino acids of CD40L whole sequence, CD40L multi-epitopes and CD40 receptor complexes by LigPlot software

Complexes	Interacting amino acids of ligand	Interacting amino acids of receptor	No. of H-bonds
CD40L whole sequence-CD40	Arg 249, Gln 219, Arg 49, Gln 35, Glu 218, His 47, Try 26	Glu 117, Glu 107, Glu 117, Cys 116, Lys 81, Ala 115, Gln 79, Asp 69, Thr 70	12
CD40L multi-epitopes-CD40	Try 59, Gln 49, Try 47, Try 73, Try 62, Glu 70, Arg 69, Trh 66, Try 78	Gln 93, Thr 136, Glu 107, Glu 74, Glu 98, Lys 29, Asp 69, Glu 21	10

CD40L, CD40 ligand.

arginine (Table 12, Figure 6).

In Silico Immune Simulation

Immune simulation assessed adaptive responses for both vaccine constructs. After each injection, both primary and secondary immune responses increased, as shown by rising levels of active B-cells, immunoglobulin (Ig) G1+IgG2, IgM, and IgG+IgM (Figures 7, 8A, B, H), as well as helper and cytotoxic T cells (Figures 7, 8C–F). These results suggest strong secondary responses, improved antigen clearance, and robust immune memory generation. Both constructs also stimulated notable IFN-γ and IL-2 cytokine secretion (Figures 7, 8G).

Molecular Dynamics Simulation

Complex stability was analyzed in Gromacs 2021.5. The CD40L multi-epitope construct reached equilibrium after approximately 5 ns, while the whole sequence required

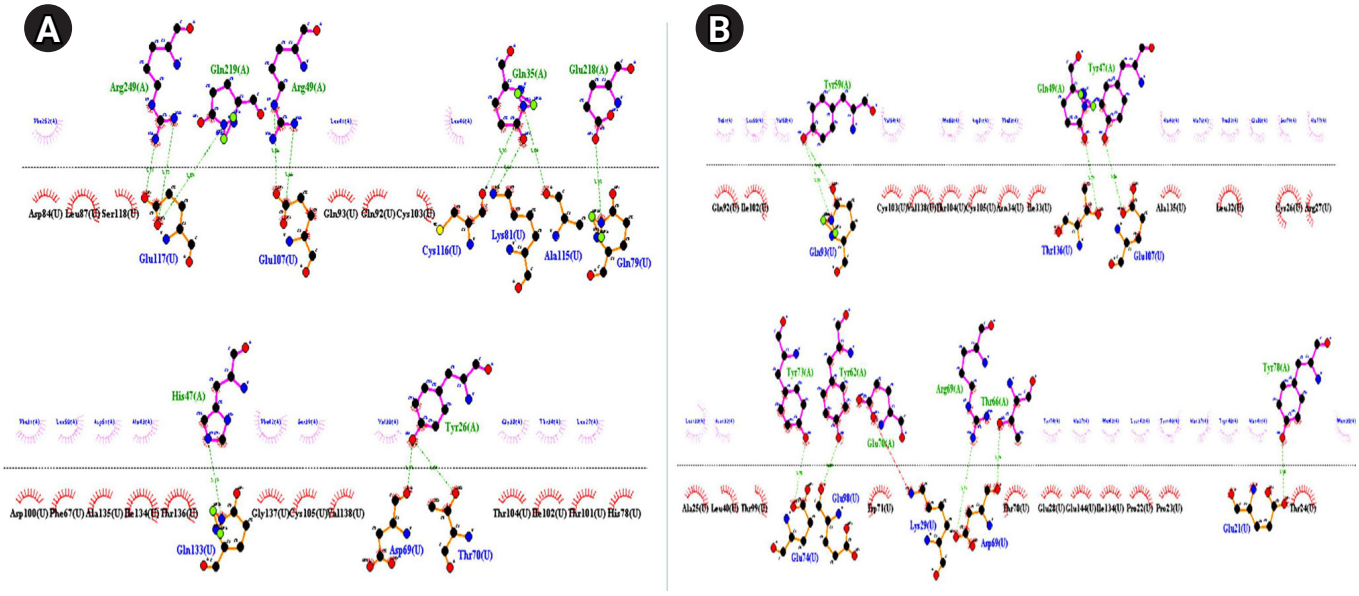


Figure 6. Protein–ligand interactions analyzed with LigPlot. (A) CD40 ligand (CD40L) whole sequence construct and CD40 receptor; (B) CD40L multi-epitope construct and CD40 receptor. Ligands and protein side chains are depicted in ball-and-stick format, with ligand bonds in purple. Hydrogen bonds are shown as green dashed lines, and spoked arcs represent non-bonded contacts. Both constructs interact with CD40 via hydrophobic and hydrogen bonds, but the multi-epitope construct forms more hydrogen bonds, potentially enhancing CD40/CD40L signaling and immune activation.

approximately 15 ns, indicating consistent stability for both. Average RMSD values were 1.11 nm (range, 0.5–1.7 nm) for the multi-epitope and 1.75 nm (range, 1.3–2.2 nm) for the whole sequence, remaining below 2 nm. RMSD graphs showed similar stability, with minimal variation across replicates (Figure 9A). RMSF analysis indicated higher local flexibility in amino acids 20 to 60 of the multi-epitope construct, likely due to proline content. Other regions with notable fluctuations in the whole sequence included residues 8, 10, 18, 119, and 180. Average RMSF was 1.4 (0.5–2.3) for the multi-epitope and 1.65 (0.6–2.7) for the whole sequence (Figure 9B). The Rg showed the multi-epitope construct was more stable (mean Rg 1.76 vs. 1.50), with Rg fluctuations less than 2 Å for both systems (Figure 9C).

In Silico Cloning

CAI and GC content were determined using the JCat server. The CD40L whole sequence construct had a CAI of 0.97 and GC content of 47.31%. The multi-epitope construct had a CAI of 1.0 and GC content of 46.60%, suggesting higher expression potential. Adapted sequences were inserted into the pET24a(+) vector using SnapGene 3.2.1 (Figure 10).

Discussion

Conventional vaccine development often relies on whole organisms, which can lead to unwanted antigen exposure and potential allergic reactions. In contrast, truncated multi-epitope vaccines have shown promise in generating targeted and robust immune responses while reducing the risk of allergic events [9]. Developing vaccines traditionally involves complex and costly *in vivo* and *in vitro* procedures to ensure efficacy [9]. However, advances in computational biology and immunoinformatics have lessened the dependence on *in vitro* experiments and have enabled the design of effective *in silico* vaccines. For instance, vaccinomics has facilitated the creation of multi-epitope-based vaccines against a range of infections, multiple viruses, and cancers, with efficacy validated *in vitro* [47–50]. Epitope-based vaccine design allows for more precise and efficient induction of either humoral or cellular immune responses [51]. Despite the proliferation of web servers for predicting peptide immunogenicity, accurately forecasting immune responses to antigens in living organisms remains a significant challenge [52,53].

CD40 stimulation via CD40L has been explored experimentally as a molecular adjuvant in vaccine research, where it enhances the activation of both $CD4^+$ and $CD8^+$ T cells. The use of CD40L in epitope vaccines has shown potential to advance vaccine technology [53]. The interaction between CD40L on T cells and CD40 on APCs upregulates co-stimulatory molecules, promotes cytokine production, and boosts antigen presentation, thereby amplifying the immune response [54]. CD40L has been widely studied for its effectiveness in

https://doi.org/10.24171/j.phrp.2025.0077

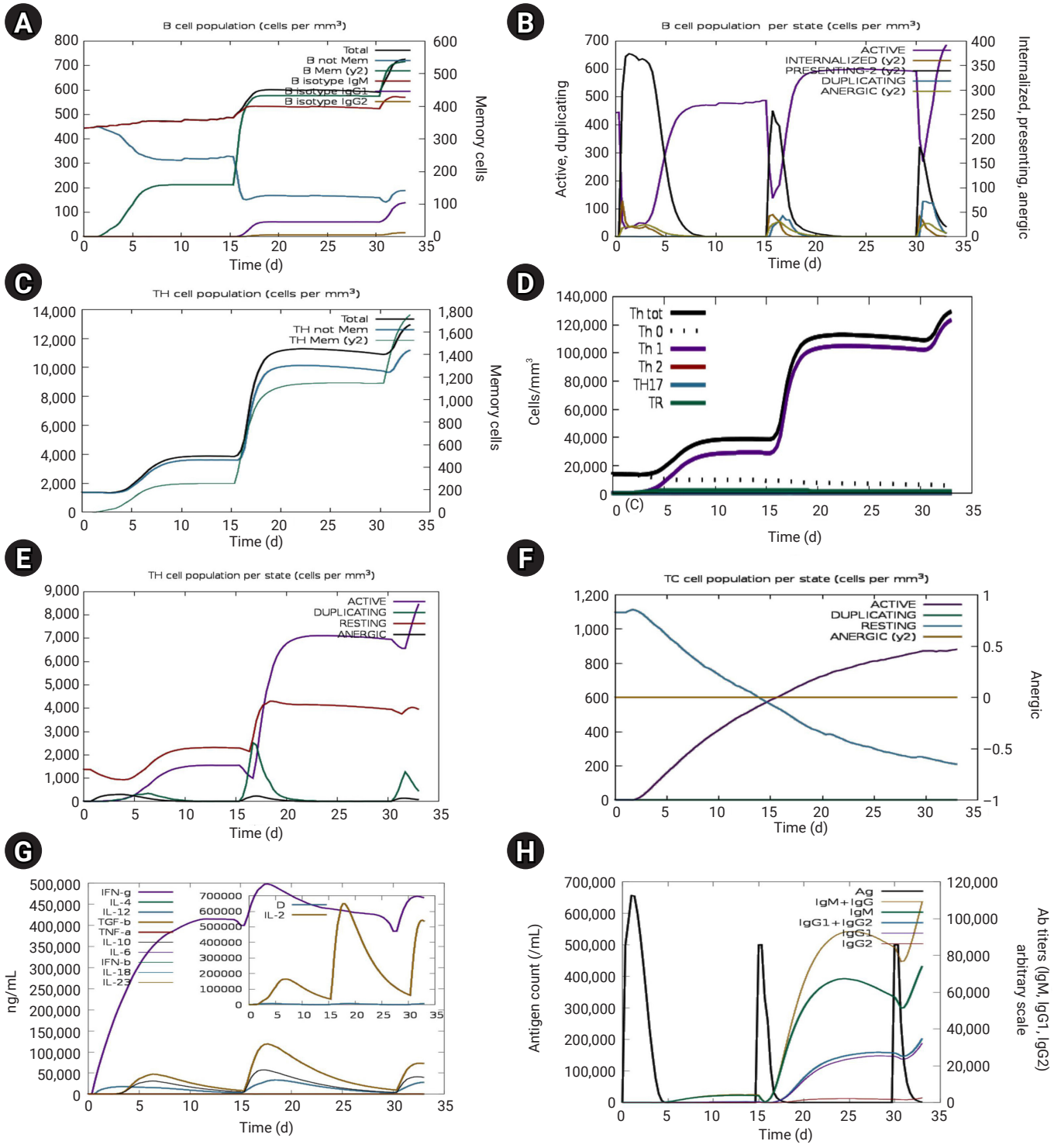


Figure 7. Immune simulation for the CD40 ligand (CD40L) whole sequence construct. (A) Evolution of B-cell populations after 3 injections; (B) active B-cell populations post-vaccination; (C) T helper (Th) cell populations; (D) generation of T cells; (E) active Th cell population; (F) cytotoxic T cells after vaccination. RESTING indicates cells not exposed to antigen, ANERGIC indicates antigen tolerance; (G) cytokine profile, showing increased interferon (IFN)-γ and interleukin (IL)-2; (H) immunoglobulin (Ig) production, indicating robust immune response. Each injection triggered strong secondary responses, enhanced antigen clearance, and promoted immune memory.

TGF, transforming growth factor; TNF, tumor necrosis factor.

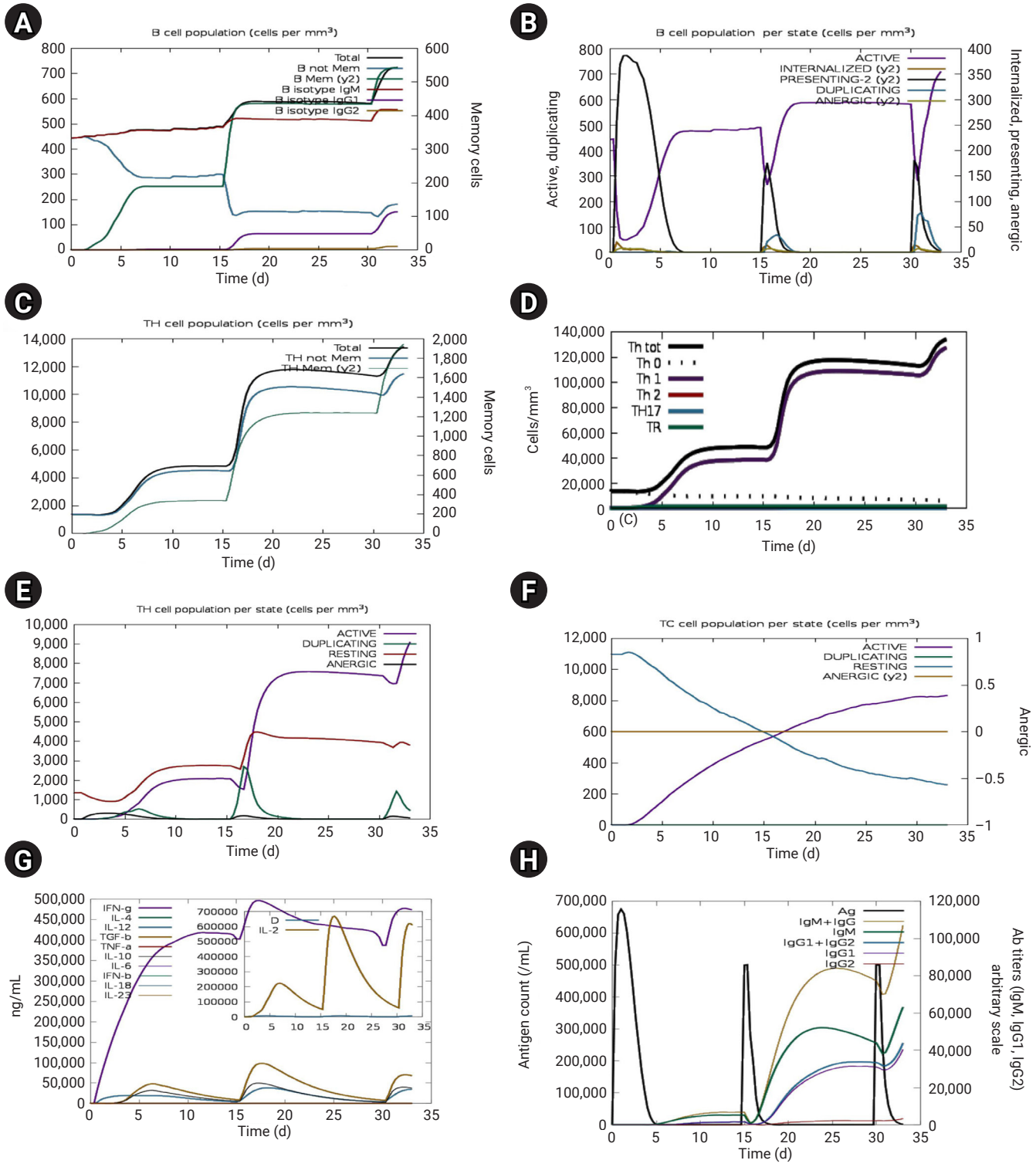


Figure 8. Immune simulation for the CD40 ligand (CD40L) multi-epitope construct. (A) Evolution of B-cell populations after 3 injections; (B) active B-cell populations; (C) T helper (Th) cell populations; (D) generation of T cells; (E) active Th cell population; (F) cytotoxic T cells post-vaccination. RESTING indicates unexposed cells; ANERGIC indicates tolerance. (G) Cytokine profile, with increased interferon (IFN)-γ and interleukin (IL)-2; (H) immunoglobulin (Ig) production. Each injection elicited potent secondary immune responses, improved antigen clearance, and strengthened immune memory.
TGF, transforming growth factor; TNF, tumor necrosis factor.

https://doi.org/10.24171/j.phrp.2025.0077

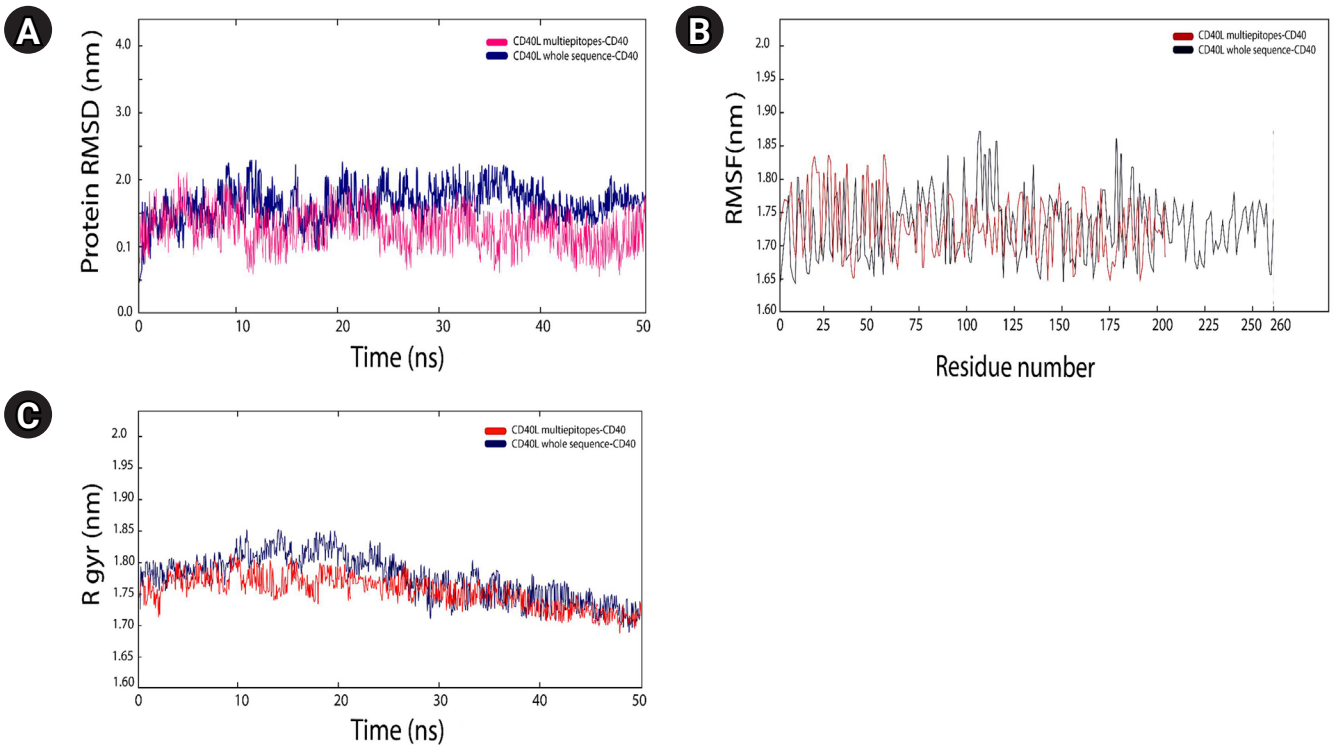


Figure 9. Molecular dynamics simulation of the vaccine construct-CD40 complex: (A) Root mean square deviations (RMSD) plots, showing that the proteins maintained comparable stability. Each protein's replicates exhibited only slight variations in their RMSD values, (B) root mean square fluctuation (RMSF) plot; A comparison of fluctuations between the CD40 ligand (CD40L) multi-epitope sequence and the CD40L whole sequence highlights that proline residues within amino acids 20 to 60 of the multi-epitope sequence contribute to increased local flexibility, resulting in greater RMSF variations in this region. (C) Radius of gyration (Rg) plotted against simulation time, showing that the CD40L multi-epitope sequence exhibited a larger Rg than the CD40L whole sequence, suggesting that it possesses greater stability.

activating both $CD8^+$ and $CD4^+$ T cell responses, making it a promising vaccine component for diseases such as human immunodeficiency virus and cancer [4,55–57]. Utilizing CD40L in cancer immunotherapy has shown considerable potential to enhance antitumor immune responses and improve therapeutic outcomes by activating dendritic cells [58,59]. Using recombinant modified vaccinia virus Ankara encoding CD40L as a vaccine against solid tumors has demonstrated therapeutic benefits by inducing cytotoxic $CD8^+$ T cells and activating natural killer cells [60]. CD40L also serves as an effective adjuvant to enhance the immune response generated by epitope-based vaccines [61,62]. Thus, in this study, we assessed and compared the ability of the CD40L whole sequence construct and CD40L multi-epitope construct to enhance immune responses against infections and cancers. Both constructs were analyzed using a range of *in silico* tools. Recent research has underscored the value of immunoinformatics pipelines for designing multi-epitope vaccines, highlighting the crucial roles of epitope prediction, structural validation, molecular docking, and immune simulation in predicting vaccine efficacy. These approaches support our methodology and reinforce the rationale for utilizing a multi-epitope strategy in CD40L-targeted vaccine development [63–67].

Initially, we selected the CD40L protein sequence from NCBI to identify its HTL, CTL, and B-cell epitopes, given their roles in host protection against infections [68,69]. Both B-cell and T-cell epitopes were employed to design peptides capable of stimulating humoral and cellular immunity. We conducted a comprehensive screening to identify potential T-cell and B-cell epitopes from CD40L, evaluating them primarily for antigenicity, allergenicity, and toxicity. Subsequent analysis examined T-cell antigen processing for these CD40L epitopes. Higher processing scores indicated more efficient antigen processing [70]. All predicted CD40L epitopes achieved top-tier identification scores,

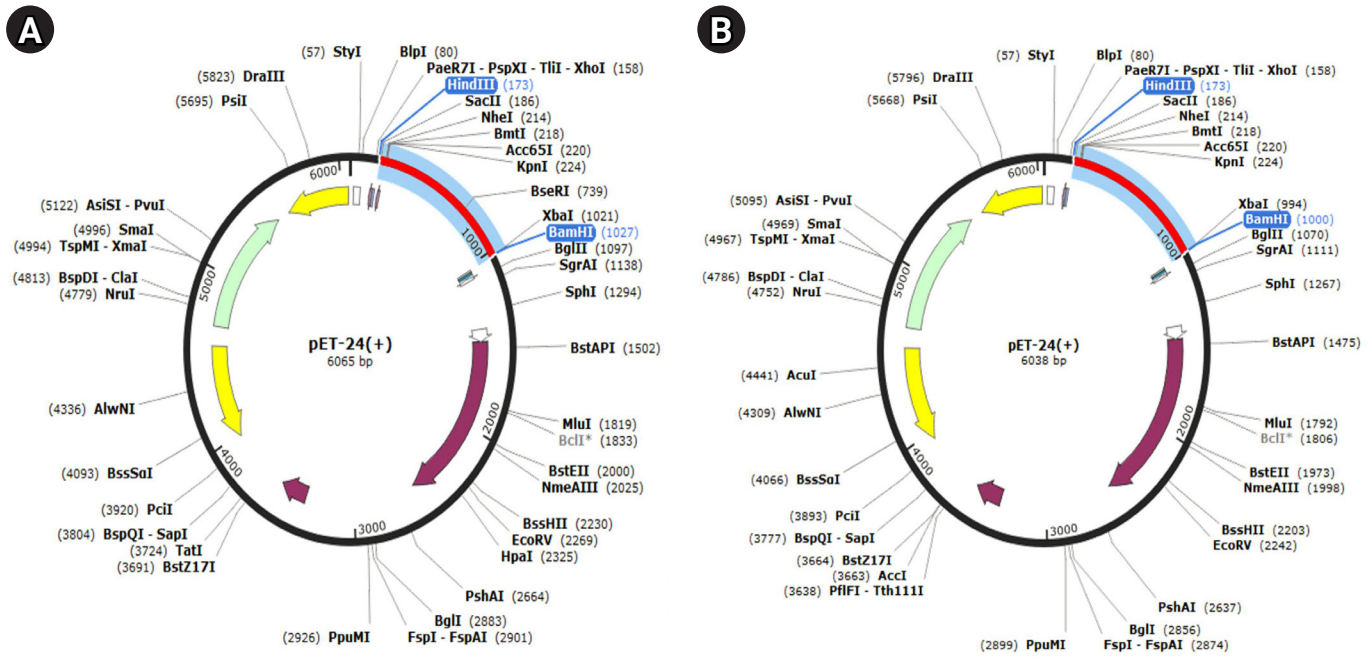


Figure 10. Schematic representation of *in silico* cloning of vaccine candidate constructs within the pET24a(+) prokaryotic expression vector: (A) CD40 ligand (CD40L) whole sequence cloned into pET24a(+) vector by *Bam*HI/*Hind*III restriction enzymes, (B) CD40L multi-epitope sequence cloned into pET24a(+) vector by *Bam*HI/*Hind*III restriction enzymes. For the CD40L whole sequence, we recorded a codon adaptation index (CAI) of 0.97 and a GC content of 47.31%. In comparison, the CD40L multi-epitope sequence showed a CAI of 1.0 and a GC content of 46.60%. These findings indicate that the optimized nucleotide sequence of the CD40L multi-epitope construct is likely to achieve more efficient expression than the CD40L whole sequence construct.

signifying superior proteasomal cleavage and efficient TAP transport. Molecular docking of protein-peptide interactions demonstrated high interaction similarity scores for all predicted CTL and HTL epitopes. Among the CTL epitopes, CD40L residues 4–17 and 84–95 displayed particularly strong binding affinity to the HLA-B5101 allele, as shown by high average docking scores. For HTL epitopes, CD40L segments 135–154 and 139–157 exhibited robust interactions with the HLA-DRB10301 allele, also achieving the highest docking scores. These findings highlight the strong HLA binding potential of these epitopes, indicating promise for effective immune recognition [71].

Since IFN-γ, IL-10, and IL-4 are pivotal cytokines in both innate and adaptive immunity, particularly for reducing viral load [72], we assessed the ability of MHC-II epitopes to induce these cytokines. Notably, most predicted CD40L HTL epitopes were found to induce IL-4. Published data indicate that IL-4 promotes viral replication and progression, as well as T cell expansion and antibody production [73]. In contrast, most CD40L epitopes did not induce IL-10. IL-10 can both inhibit and stimulate IFN-γ and IL-4 production, respectively; elevated IL-10 levels may suggest immune dysfunction [74–77]. Thus, these constructs may mitigate the deleterious effects of IL-10 during infection. Crucially, all predicted CD40L epitopes were found to induce IFN-γ, which is associated with reduced viral load in infected hosts [78]. Collectively, these results suggest that the selected HTL epitopes can induce both T helper (Th) 1 and Th2 immune responses *in vivo*. Our vaccine construct demonstrated a population coverage of 87.92%, supporting its global applicability. We further identified 6 candidate CD40L epitopes for MHC-I, 5 immunodominant epitopes for MHC-II, and 2 B-cell epitopes. Among the predicted CTL epitopes, $CD40L_{4–17}$ and $CD40L_{1–13}$, as well as HTL epitopes $CD40L_{135–154}$ and $CD40L_{139–157}$, showed superior MHC binding ranks.

The selected epitopes were used to construct a multi-epitope vaccine, incorporating AAY linkers to preserve the functional integrity of each epitope and regulate flexibility and rigidity [79]. Linkers offer important advantages, such as reducing junctional antigen formation and improving antigen processing and presentation [80]. AAY linkers in particular act as proteasome cleavage sites and help minimize junctional immunogenicity [81–83]. When attached to epitopes, these linkers facilitate the recognition and separation of each epitope [39,84,85]. Consequently, the designed vaccine was found to be highly antigenic

https://doi.org/10.24171/j.phrp.2025.0077

and non-allergenic, based on evaluations by multiple prediction servers. In comparison to the CD40L whole sequence construct, this finding highlights the CD40L multi-epitope construct's ability to provoke robust immune responses without causing undesired allergic reactions. Additionally, the average MW of the CD40L multi-epitope and whole sequence constructs was 28.70 kDa and 29.37 kDa, respectively. This difference supports the enhanced antigenicity observed in the multi-epitope construct [86]. Proteins with MW below 110 kDa are considered good vaccine candidates [87]. The theoretical isoelectric point (pI) for the multi-epitope and whole sequence constructs was 9.56 and 8.26, respectively, suggesting stable interactions within the human body. The short half-life of peptides is a notable limitation in therapeutic protein development [88]. Nevertheless, both vaccine constructs demonstrated half-lives of over 10 and 30 hours in *E. coli* and mammalian cells, respectively, which is considered satisfactory [89]. The CD40L multi-epitope construct exhibited an instability index of 35.73, whereas the whole sequence construct's predicted index was 46.29, indicating greater stability for the multi-epitope construct in biological environments. Compounds with instability indices below 40 are classified as stable [90]. The solubility score indicated that the vaccine is hydrophilic, facilitating formulation and purification [91,92]. Protein solubility in *E. coli* is also crucial for functional and biochemical analyses [93]. The CD40L multi-epitope construct was found to be soluble (0.713), while the whole sequence construct's solubility score was 0.361. This further demonstrates that the multi-epitope construct is more amenable to post-production processing, as highly soluble proteins are easier to purify during downstream applications [94].

In this study, the SOPMA technique was employed to analyze protein secondary structure. Secondary structure analysis determines whether amino acids are located in alpha helices or beta sheets, both of which are essential for protein structure and function. The alpha helix is especially beneficial for proteins requiring strength and stability [95]. The results for the CD40L multi-epitope construct indicated higher proportions of amino acids in alpha helices, beta sheets, and beta turns compared to the CD40L whole sequence construct. Notably, the multi-epitope protein exhibited a pronounced helical structure, suggesting a more condensed and tightly bound configuration, including its transmembrane segment [96].

After predicting and refining the 3D structure of the vaccine model using Robetta and Galaxy Refine, the resulting models were validated by SAVES v6.0 ERRAT, PROCHECK, and Verify3D. Evaluating the tertiary structure quality is crucial, as it influences peptide presentation for immune activation [97]. The refined CD40L multi-epitope model had 92% of residues in the most favored Ramachandran plot zones, compared to 90.7% for the whole sequence construct, indicating the high quality of the multi-epitope model. Modeling quality metrics and Verify3D results further showed that the multi-epitope vaccine model was at least as acceptable as the whole sequence construct. Using ElliPro, we identified numerous linear and discontinuous B-cell epitopes within the multi-epitope construct, underscoring its strong potential to stimulate a robust humoral immune response [98].

Two disulfide bonds were predicted in both structures, which are critical for protein folding and stability. Disulfide bond formation limits conformational diversity, enhancing thermal stability and reducing entropy [93]. To assess the interaction between vaccine constructs and the CD40 receptor, molecular docking was performed with ClusPro and HDOCK. Previous studies showed that CD40 receptor engagement with CD40L increases cytokine production and co-stimulatory molecule expression, linking adaptive and innate immunity and contributing to protection against infection and cancer [99–102]. Docking revealed negative binding energy values, indicating strong affinity between the vaccine constructs and the CD40 receptor. The multi-epitope constructs showed stronger docking with CD40 compared to the whole sequence, suggesting enhanced potential to elicit a protective immune response.

LigPlot analysis revealed that more amino acids participate in the interaction between the CD40 receptor and the CD40L multi-epitope construct than with the whole sequence, indicating a stronger interaction. An increased number of hydrogen bonds between 2 proteins correlates with greater interaction strength [103]. In the CD40L multi-epitope structure, a hydrogen bond formed between glutamic acid 107 and tyrosine, whereas in the whole sequence, glutamic acid 107 bonded to arginine. Studies suggest the Tyr-Glu bond is stronger than the Arg-Glu bond, with machine learning analyses indicating that the Tyr/Glu pair most often forms strong hydrogen bonds in proteins [104]. At position 69, the CD40 receptor forms a hydrogen bond with threonine in the multi-epitope construct, whereas tyrosine replaces threonine in the whole sequence. Threonine is crucial for maintaining protein stability [105]. Thus, LigPlot results confirmed the higher affinity between the CD40 receptor and the multi-epitope structure, supporting the docking findings.

To be effective, a vaccine construct must induce a robust immune response [106]. Immune simulation analysis showed that administration of both vaccine constructs

activated both primary and secondary immune responses. Elevated concentrations of memory B-cells, helper T-cells, and cytotoxic T-cells were observed after vaccination, indicating enhanced immune activation. High levels of IgM, IgG2, and IgG1 antibodies were produced during both responses. Additionally, IFN-γ and IL-2 levels increased with repeated exposures, while IL-10 remained low, a pattern linked to higher Th1 cell frequency and improved viral immunity [107].

Increased IgM and IgG reflect adaptive immune system activation. IgM is produced first and rapidly neutralizes pathogens while activating complement. IgG antibodies arise later, providing lasting protection through neutralization, opsonization, and complement activation. Together, these antibodies establish protective immunity, reducing disease severity upon future exposures. Cytokines coordinate the immune response: IFN-γ promotes pathogen elimination and immune cell activation; IL-2 supports T cell proliferation and memory; IL-10 regulates and restrains the immune response, preventing excessive inflammation. This interplay ensures a strong, lasting immunity [108].

In this study, MD simulations indicated improved stability and favorable binding of the CD40L multi-epitope/CD40 complex compared to the whole sequence/CD40 complex. The RMSD, Rg, and RMSF values for the multi-epitope complex showed less fluctuation and more consistent patterns. In this study, cloning the vaccine construct into the pET24a(+) vector using SnapGene 3.2.1 was expected to increase codon expression. CAI values confirmed that the multi-epitope construct had higher compatibility and expression levels than the whole sequence construct.

Immunoinformatics approaches, such as those used here to design a CD40L-based multi-epitope vaccine, provide valuable insights into epitope prediction, structural stability, and receptor binding affinity. However, these findings must be interpreted cautiously, as computational models cannot fully replicate the complexity of biological systems. Experimental validation is essential to confirm immunogenicity, safety, and efficacy. Only through *in vitro* and *in vivo* testing can the true potential of the multi-epitope vaccine be determined, and discrepancies between predictions and actual responses identified. Computational methods often simplify immune complexity, overlooking diverse cell interactions and microenvironmental factors. Epitope prediction tools rely on existing datasets, which may not capture full antigenic diversity or population-specific HLA variation, potentially causing false positives or negatives. Molecular docking and dynamics simulations, while informative, may not precisely mirror physiological conditions. For example, greater *in silico* affinity does not guarantee superior immunogenicity *in vivo*, where factors like tolerance, adjuvant effects, and genetic variation matter. Likewise, immune simulations are based on theoretical frameworks that cannot fully recapitulate complex *in vivo* antigen processing, presentation, and regulation.

Conclusion

Using an immunoinformatics approach, we constructed a safe and effective multi-epitope vaccine candidate. The amino acid sequence of CD40L was analyzed, and B-cell, T-cell, and CTL epitopes were predicted and screened. These epitopes were combined to create the multi-epitope vaccine, using AAY linkers for optimal assembly. The safety and stability of the vaccine were assessed by comparing its biochemical properties and structure to the CD40L whole sequence. Results showed that the CD40L multi-epitope vaccine had high antigenicity, safety, and stability. Molecular docking and dynamics studies revealed strong binding affinity and stability between the multi-epitope construct and the CD40 receptor. Normal mode analysis and immune simulations provided satisfactory results. *In silico* cloning in the pET24a(+) vector indicated greater expression potential for the multi-epitope construct compared to the whole sequence vaccine. Overall, these findings highlight promising strategies for vaccine development against infections and cancers. However, further experimental studies are needed to verify the efficacy, effectiveness, and safety of this vaccine candidate.

Notes

Ethics Approval
Not applicable.

Conflicts of Interest
The authors have no conflicts of interest to declare.

Funding
None.

Availability of Data
All data generated or analyzed during this study are included in this published article.

Authors' Contributions
Conceptualization: FH, AB; Investigation: FH, MM; Software: SAS, HB, FH; Supervision: FH, AB; Writing original draft: SAS, FH; Writing–review & editing: all authors. All authors read and approved the final manuscript.

References

1. Karnell JL, Rieder SA, Ettinger R, et al. Targeting the CD40-CD40L pathway in autoimmune diseases: hgumoral immunity and beyond. Adv Drug Deliv Rev 2019;141:92–103.
2. Fadul CE, Mao-Draayer Y, Ryan KA, et al. Safety and immune effects

https://doi.org/10.24171/j.phrp.2025.0077

of blocking CD40 ligand in multiple sclerosis. Neurol Neuroimmunol Neuroinflamm 2021;8:e1096.
3. Lacy M, Burger C, Shami A, et al. Cell-specific and divergent roles of the CD40L-CD40 axis in atherosclerotic vascular disease. Nat Commun 2021;12:3754.
4. Daoussis D, Andonopoulos AP, Liossis SN. Targeting CD40L: a promising therapeutic approach. Clin Diagn Lab Immunol 2004; 11:635–41.
5. Zhang B, Wu T, Chen M, et al. The CD40/CD40L system: a new therapeutic target for disease. Immunol Lett 2013;153:58–61.
6. Zhang S, Breidenbach JD, Russell BH, et al. CD40/CD40L signaling as a promising therapeutic target for the treatment of renal disease. J Clin Med 2020;9:3653.
7. Yurina V, Adianingsih OR. Predicting epitopes for vaccine development using bioinformatics tools. Ther Adv Vaccines Immunother 2022 May 21 [Epub]. https://doi.org/10.1177/25151355221100218.
8. De Groot AS, Moise L, McMurry JA, et al. Epitope-based immunome-derived vaccines: a strategy for improved design and safety. In: Falus A, editor. Clinical applications of immunomics. Springer; 2009. p. 39–69.
9. Malonis RJ, Lai JR, Vergnolle O. Peptide-based vaccines: current progress and future challenges. Chem Rev 2020;120:3210–29.
10. Oscherwitz J. The promise and challenge of epitope-focused vaccines. Hum Vaccin Immunother 2016;12:2113–6.
11. Palma M. Epitopes and mimotopes identification using phage display for vaccine development against infectious pathogens. Vaccines (Basel) 2023;11:1176.
12. Arya H, Bhatt TK. Role of bioinformatics in subunit vaccine design. In: Coumar MS, editor. Molecular docking for computer-aided drug design. Elsevier; 2021. p. 425–39.
13. Nandy A, Basak SC. Bioinformatics in design of antiviral vaccines. Encycl Biomed Eng 2019;280–90.
14. Collins KA, Snaith R, Cottingham MG, et al. Enhancing protective immunity to malaria with a highly immunogenic virus-like particle vaccine. Sci Rep 2017;7:46621.
15. Jain R, Jain A, Verma SK. Prediction of epitope based peptides for vaccine development from complete proteome of novel corona virus (SARS-COV-2) using immunoinformatics. Int J Pept Res Ther 2021; 27:1729–40.
16. Sharma AD, Magdaleno JS, Singh H, et al. Immunoinformatics-driven design of a multi-epitope vaccine targeting neonatal rotavirus with focus on outer capsid proteins VP4 and VP7 and non structural proteins NSP2 and NSP5. Sci Rep 2025;15:11879.
17. Randriamamisolonirina NT, Razafindrafara MS, Maminiaina OF. Design of a multi-epitope vaccine against the glycoproteins of Newcastle disease virus by using an immunoinformatics approach. ACS Omega 2025;10:4007–18.
18. Larsen MV, Lundegaard C, Lamberth K, et al. Large-scale validation of methods for cytotoxic T-lymphocyte epitope prediction. BMC Bioinformatic 2007;8:424.
19. Andreatta M, Nielsen M. Bioinformatics tools for the prediction of T-cell epitopes. Methods Mol Biol 2018;1785:269–81.
20. Stranzl T, Larsen MV, Lundegaard C, et al. NetCTLpan: pan-specific MHC class I pathway epitope predictions. Immunogenetics 2010;62: 357–68.
21. Gupta S, Kapoor P, Chaudhary K, et al. In silico approach for predicting toxicity of peptides and proteins. PLoS One 2013;8:e73957.
22. Dhanda SK, Gupta S, Vir P, et al. Prediction of IL4 inducing peptides. Clin Dev Immunol 2013;2013:263952.
23. Nagpal G, Usmani SS, Dhanda SK, et al. Computer-aided designing of immunosuppressive peptides based on IL-10 inducing potential. Sci Rep 2017;7:42851.
24. Bui HH, Sidney J, Dinh K, et al. Predicting population coverage of T-cell epitope-based diagnostics and vaccines. BMC Bioinformatics 2006;7:153.
25. Dimitrov I, Bangov I, Flower DR, et al. AllerTOP v.2: a server for in silico prediction of allergens. J Mol Model 2014;20:2278.
26. Hebditch M, Carballo-Amador MA, Charonis S, et al. Protein-Sol: a web tool for predicting protein solubility from sequence. Bioinformatics 2017;33:3098–100.
27. Garg VK, Avashthi H, Tiwari A, et al. MFPPI: multi FASTA ProtParam Interface. Bioinformation 2016;12:74–7.
28. Geourjon C, Deleage G. SOPMA: significant improvements in protein secondary structure prediction by consensus prediction from multiple alignments. Comput Appl Biosci 1995;11:681–4.
29. Kim DE, Chivian D, Baker D. Protein structure prediction and analysis using the Robetta server. Nucleic Acids Res 2004;32(Web Server issue):W526–31.
30. Seok C, Baek M, Steinegger M, et al. Accurate protein structure prediction: what comes next. Biodesign 2021;9:47–50.
31. Colovos C, Yeates TO. Verification of protein structures: patterns of nonbonded atomic interactions. Protein Sci 1993;2:1511–9.
32. Laskowski R, MacArthur M, Thornton J. PROCHECK: validation of protein-structure coordinates. Wiley; 2006.
33. Mukonyora M. A review of important discontinuous B-cell epitope prediction tools. J Clin Cell Immunol 2015;6:358.
34. Cheng J, Randall AZ, Sweredoski MJ, et al. SCRATCH: a protein structure and structural feature prediction server. Nucleic Acids Res 2005;33(Web Server issue):W72–6.
35. Kozakov D, Hall DR, Xia B, et al. The ClusPro web server for protein-protein docking. Nat Protoc 2017;12:255–78.
36. Yan Y, Zhang D, Zhou P, et al. HDOCK: a web server for protein-protein and protein-DNA/RNA docking based on a hybrid strategy. Nucleic Acids Res 2017;45(W1):W365–73.
37. Laskowski RA, Swindells MB. LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. J Chem Inf Model 2011;51: 2778–86.
38. Rapin N, Lund O, Bernaschi M, et al. Computational immunology meets bioinformatics: the use of prediction tools for molecular binding in the simulation of the immune system. PLoS One 2010;

5:e9862.
39. Sami SA, Marma KK, Mahmud S, et al. Designing of a multi-epitope vaccine against the structural proteins of Marburg virus exploiting the immunoinformatics approach. ACS Omega 2021;6:32043–71.
40. Lopez-Blanco JR, Aliaga JI, Quintana-Orti ES, et al. iMODS: internal coordinates normal mode analysis server. Nucleic Acids Res 2014;42(Web Server issue):W271–6.
41. Lopez-Blanco JR, Garzon JI, Chacon P. iMod: multipurpose normal mode analysis in internal coordinates. Bioinformatics 2011;27:2843–50.
42. Ghahremanian S, Rashidi MM, Raeisi K, et al. Molecular dynamics simulation approach for discovering potential inhibitors against SARS-CoV-2: a structural review. J Mol Liq 2022;354:118901.
43. Best RB, Zhu X, Shim J, et al. Optimization of the additive CHARMM all-atom protein force field targeting improved sampling of the backbone φ, ψ and side-chain χ(1) and χ(2) dihedral angles. J Chem Theory Comput 2012;8:3257–73.
44. Berendsen HJ, Postma Jv, Van Gunsteren WF, et al. Molecular dynamics with coupling to an external bath. J Chem Phys 1984;81:3684–90.
45. Petersen HG. Accuracy and efficiency of the particle mesh Ewald method. J Chem Phys 1995;103:3668–79.
46. Grote A, Hiller K, Scheer M, et al. JCat: a novel tool to adapt codon usage of a target gene to its potential expression host. Nucleic Acids Res 2005;33(Web Server issue):W526–31.
47. Kar T, Narsaria U, Basak S, et al. A candidate multi-epitope vaccine against SARS-CoV-2. Sci Rep 2020;10:10895.
48. Mugunthan SP, Harish MC. Multi-epitope-based vaccine designed by targeting cytoadherence proteins of Mycoplasma gallisepticum. ACS Omega 2021;6:13742–55.
49. Nezafat N, Ghasemi Y, Javadi G, et al. A novel multi-epitope peptide vaccine against cancer: an in silico approach. J Theor Biol 2014;349:121–34.
50. Tahir Ul Qamar M, Rehman A, Tusleem K, et al. Designing of a next generation multiepitope based vaccine (MEV) against SARS-COV-2: immunoinformatics and in silico approaches. PLoS One 2020;15:e0244176.
51. Li M, Zhu Y, Niu C, et al. Design of a multi-epitope vaccine candidate against Brucella melitensis. Sci Rep 2022;12:10146.
52. Aleksandrov A, Myllykallio H. Advances and challenges in drug design against tuberculosis: application of in silico approaches. Expert Opin Drug Discov 2019;14:35–46.
53. Chakraborty AK, Dustin ML, Shaw AS. In silico models for cellular and molecular immunology: successes, promises and challenges. Nat Immunol 2003;4:933–6.
54. Auten MW, Huang W, Dai G, et al. CD40 ligand enhances immunogenicity of vector-based vaccines in immunocompetent and $CD4^+$ T cell deficient individuals. Vaccine 2012;30:2768–77.
55. Bullock TNJ. CD40 stimulation as a molecular adjuvant for cancer vaccines and other immunotherapies. Cell Mol Immunol 2022;19:14–22.
56. Godot V, Tcherakian C, Gil L, et al. TLR-9 agonist and CD40-targeting vaccination induces HIV-1 envelope-specific B cells with a diversified immunoglobulin repertoire in humanized mice. PLoS Pathog 2020;16:e1009025.
57. Heidarnejad F, Namvar A, Sadat SM, et al. In silico designing of novel epitope-based peptide vaccines against HIV-1. Biotechnol Lett 2024;46:315–54.
58. Tang YC, Thoman M, Linton PJ, et al. Use of CD40L immunoconjugates to overcome the defective immune response to vaccines for infections and cancer in the aged. Cancer Immunol Immunother 2009;58:1949–57.
59. Zhang L, Tang Y, Akbulut H, et al. An adenoviral vector cancer vaccine that delivers a tumor-associated antigen/CD40-ligand fusion protein to dendritic cells. Proc Natl Acad Sci U S A 2003;100:15101–6.
60. Medina-Echeverz J, Hinterberger M, Testori M, et al. Synergistic cancer immunotherapy combines MVA-CD40L induced innate and adaptive immunity with tumor targeting antibodies. Nat Commun 2019;10:5041.
61. Lookian PP, Zhao D, Medina R, et al. Mannan-BAM, TLR ligands, anti-CD40 antibody (MBTA) vaccine immunotherapy: a review of current evidence and applications in glioblastoma. Int J Mol Sci 2021;22:3455.
62. Stone GW, Barzee S, Snarsky V, et al. Multimeric soluble CD40 ligand and GITR ligand as adjuvants for human immunodeficiency virus DNA vaccines. J Virol 2006;80:1762–72.
63. Khan N, Rehman B, Almanaa TN, et al. A novel therapeutic approach to prevent Helicobacter pylori induced gastric cancer using networking biology, molecular docking, and simulation approaches. J Biomol Struct Dyn 2024;42:13876–89.
64. Ahmad S, Almanaa TN, Khan S, et al. Identification of potential drug molecules against fibroblast growth factor receptor 3 (FGFR3) by multi-stage computational-biophysics correlate. J Biomol Struct Dyn 2025;43:1240–8.
65. Momajadi L, Khanahmad H, Mahnam K. Designing a multi-epitope influenza vaccine: an immunoinformatics approach. Sci Rep 2024;14:25382.
66. Jaan S, Shah M, Ullah N, et al. Multi-epitope chimeric vaccine designing and novel drug targets prioritization against multi-drug resistant Staphylococcus pseudintermedius. Front Microbiol 2022;13:971263.
67. Sarfraz A, Wara TU, et al. Structural informatics approach for designing an epitope-based vaccine against the brain-eating Naegleria fowleri. Front Immunol 2023;14:1284621.
68. Jain N, Shankar U, Majee P, et al. Scrutinizing the SARS-CoV-2 protein information for designing an effective vaccine encompassing both the T-cell and B-cell epitopes. Infect Genet Evol 2021;87:104648.
69. Lam JH, Smith FL, Baumgarth N. B cell activation and response regulation during viral infections. Viral Immunol 2020;33:294–306.
70. Fleri W, Paul S, Dhanda SK, et al. The immune epitope database and analysis resource in epitope discovery and synthetic vaccine design. Front Immunol 2017;8:278.

https://doi.org/10.24171/j.phrp.2025.0077

71. Barazesh M, Abbasi M, Mohammadi M, et al. Bioinformatics analysis to design a multi-epitope mRNA vaccine against S. agalactiae exploiting pathogenic proteins. Sci Rep 2024;14:28294.
72. Hosseini Khorami SH, Nejatollahi F, Davarpanah MA. Serum levels of interleukin-4, interleukin-10 and interferon-γ in patients with chronic hepatitis B infection. Hepat Mon 2018;48:e60377.
73. Valentin A, Lu W, Rosati M, et al. Dual effect of interleukin 4 on HIV-1 expression: implications for viral phenotypic switch and disease progression. Proc Natl Acad Sci U S A 1998;95:8886–91.
74. Browne EP. The role of toll-like receptors in retroviral infection. Microorganisms 2020;8:1787.
75. Csiszar A, Nagy G, Gergely P, et al. Increased interferon-gamma (IFN-gamma), IL-10 and decreased IL-4 mRNA expression in peripheral blood mononuclear cells (PBMC) from patients with systemic lupus erythematosus (SLE). Clin Exp Immunol 2000;122:464–70.
76. Osuji FN, Onyenekwe CC, Ahaneku JE, et al. The effects of highly active antiretroviral therapy on the serum levels of pro-inflammatory and anti-inflammatory cytokines in HIV infected subjects. J Biomed Sci 2018;25:88.
77. Stylianou E, Aukrust P, Kvale D, et al. IL-10 in HIV infection: increasing serum IL-10 levels with disease progression: down-regulatory effect of potent anti-retroviral therapy. Clin Exp Immunol 1999;116:115–20.
78. Yuan S, Jiang SC, Zhang ZW, et al. Quantification of cytokine storms during virus infections. Front Immunol 2021;12:659419.
79. Lovell SC, Davis IW, Arendall WB 3rd, et al. Structure validation by Calpha geometry: phi,psi and Cbeta deviation. Proteins 2003;50:437–50.
80. Athanasiou E, Agallou M, Tastsoglou S, et al. A Poly(Lactic-co-Glycolic) acid nanovaccine based on chimeric peptides from different Leishmania infantum proteins induces dendritic cells maturation and promotes peptide-specific IFNγ-producing $CD8^+$ T cells essential for the protection against experimental visceral leishmaniasis. Front Immunol 2017;8:684.
81. Dolenc I, Seemuller E, Baumeister W. Decelerated degradation of short peptides by the 20S proteasome. FEBS Lett 1998;434:357–61.
82. Jahantigh HR, Stufano A, Lovreglio P, et al. In silico identification of epitope-based vaccine candidates against HTLV-1. J Biomol Struct Dyn 2022;40:6737–54.
83. Yang Y, Sun W, Guo J, et al. In silico design of a DNA-based HIV-1 multi-epitope vaccine for Chinese populations. Hum Vaccin Immunother 2015;11:795–805.
84. Hossan MI, Chowdhury AS, Hossain MU, et al. Immunoinformatics aided-design of novel multi-epitope based peptide vaccine against Hendra henipavirus through proteome exploration. Inform Med Unlocked 2021;25:100678.
85. Velders MP, Weijzen S, Eiben GL, et al. Defined flanking spacers and enhanced proteolysis is essential for eradication of established tumors by an epitope string DNA vaccine. J Immunol 2001;166:5366–73.
86. Khatoon N, Pandey RK, Prajapati VK. Exploring Leishmania secretory proteins to design B and T cell multi-epitope subunit vaccine using immunoinformatics approach. Sci Rep 2017;7:8285.
87. Naz A, Awan FM, Obaid A, et al. Identification of putative vaccine candidates against Helicobacter pylori exploiting exoproteome and secretome: a reverse vaccinology based approach. Infect Genet Evol 2015;32:280–91.
88. Mathur D, Prakash S, Anand P, et al. PEPlife: a repository of the half-life of peptides. Sci Rep 2016;6:36617.
89. Pandey RK, Bhatt TK, Prajapati VK. Novel immunoinformatics approaches to design multi-epitope subunit vaccine for Malaria by investigating anopheles salivary protein. Sci Rep 2018;8:1125.
90. Guruprasad K, Reddy BV, Pandit MW. Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting in vivo stability of a protein from its primary sequence. Protein Eng 1990;4:155–61.
91. Chang KY, Yang JR. Analysis and prediction of highly effective antiviral peptides based on random forests. PLoS One 2013;8:e70166.
92. Kyte J, Doolittle RF. A simple method for displaying the hydropathic character of a protein. J Mol Biol 1982;157:105–32.
93. Almofti YA, Abd-Elrahman KA, Eltilib EE. Vaccinomic approach for novel multi epitopes vaccine against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). BMC Immunol 2021;22:22.
94. Magnan CN, Randall A, Baldi P. SOLpro: accurate sequence-based prediction of protein solubility. Bioinformatics 2009;25:2200–7.
95. Kwok SC, Mant CT, Hodges RS. Importance of secondary structural specificity determinants in protein folding: insertion of a native beta-sheet sequence into an alpha-helical coiled-coil. Protein Sci 2002;11:1519–31.
96. Santhoshkumar R, Yusuf A. In silico structural modeling and analysis of physicochemical properties of curcumin synthase (CURS1, CURS2, and CURS3) proteins of Curcuma longa. J Genet Eng Biotechnol 2020;18:24.
97. Meza B, Ascencio F, Sierra-Beltran AP, et al. A novel design of a multi-antigenic, multistage and multi-epitope vaccine against Helicobacter pylori: an in silico approach. Infect Genet Evol 2017;49:309–17.
98. Kuruvilla Thomas R, Balasundaram A, Fathima G, et al. Identification and validation of B-cell epitopes on the VP1 protein of parvovirus B19 through molecular docking and dynamics simulation. ACS Omega 2025;10:3598–609.
99. Ara A, Ahmed KA, Xiang J. Multiple effects of CD40-CD40L axis in immunity against infection and cancer. Immunotargets Ther 2018;7:55–61.
100. Elgueta R, Benson MJ, de Vries VC, et al. Molecular mechanism and function of CD40/CD40L engagement in the immune system. Immunol Rev 2009;229:152–72.
101. Sharma MD, Leite de Moraes M, Zavala F, et al. Induction and inhibition of CD40-CD40 ligand interactions: a new strategy underlying host-virus relationships. J Immunol 1998;161:5357–65.

102. Tang T, Cheng X, Truong B, et al. Molecular basis and therapeutic implications of CD40/CD40L immune checkpoint. Pharmacol Ther 2021;219:107709.
103. Pace CN, Fu H, Lee Fryar K, et al. Contribution of hydrogen bonds to protein stability. Protein Sci 2014;23:652–61.
104. Zhou S, Liu Y, Wang S, et al. Effective prediction of short hydrogen bonds in proteins via machine learning method. Sci Rep 2022; 12:469.
105. Smith SO, Eilers M, Song D, et al. Implications of threonine hydrogen bonding in the glycophorin A transmembrane helix dimer. Biophys J 2002;82:2476–86.
106. Sarkar B, Ullah MA, Araf Y, et al. Immunoinformatics-guided designing and in silico analysis of epitope-based polyvalent vaccines against multiple strains of human coronavirus (HCoV). Expert Rev Vaccines 2022;21:1851–71.
107. Sher H, Sharif H, Zaheer T, et al. Employing computational tools to design a multi-epitope vaccine targeting human immunodeficiency virus-1 (HIV-1). BMC Genomics 2023;24:276.
108. Delves PJ. Innate and adaptive systems of immunity. In: Rose NR, Mackay IR, editors. The autoimmune diseases. 6th ed. Elsevier; 2020. p. 45–61.

https://doi.org/10.24171/j.phrp.2025.0077

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2025.0120
Osong Public Health Res Perspect 2025;16(4):333-347

Original Article

Deep learning-based prognosis of major adverse cardiac events in patients with acute myocardial infarction: a retrospective observational study in the Republic of Korea

Vungsovanreach Kong[1], **Kyung Ah Kim**[2,3], **Ho Sun Shon**[4]

[1]Department of Big Data, Chungbuk National University, Cheongju, Republic of Korea
[2]Biomedical Research Institute, Chungbuk National University Hospital, Cheongju, Republic of Korea
[3]Department of Biomedical Engineering, College of Medicine, Chungbuk National University, Cheongju, Republic of Korea
[4]Medical Research Institute, School of Medicine, Chungbuk National University, Cheongju, Republic of Korea

ABSTRACT

Objectives: This study developed deep neural network (DNN) models capable of accurately classifying major adverse cardiac events (MACE) in patients with acute myocardial infarction (AMI) after hospital discharge, across 3 follow-up intervals: 1, 6, and 12 months.
Methods: DNN models were constructed to predict post-discharge MACE across 4 categories. Multiple traditional machine learning models were implemented as controls to benchmark the performance of our DNN approach. All models were evaluated based on their ability to predict MACE occurrence during the specified follow-up periods.
Results: The DNN models demonstrated superior predictive performance over conventional machine learning methods, achieving high accuracies of 0.922, 0.884, and 0.913 for the 1-month, 6-month, and 12-month follow-up periods, respectively.
Conclusion: The high accuracy of our DNN models highlights their practical advantages for AMI diagnosis and guidance of follow-up treatment. These models can serve as valuable decision support tools, enabling clinicians to optimize the overall management of AMI patients and potentially enhance their hospitalization experience.

Keywords: Cardiovascular; Deep learning; Early diagnosis; Major adverse cardiac events

Received: April 14, 2025
Revised: June 10, 2025
Accepted: June 15, 2025

Corresponding author:
Ho Sun Shon
Medical Research Institute, School of Medicine, Chungbuk National University, 1 Chungdae-ro, Seowon-gu, Cheongju 28644, Republic of Korea
E-mail: shon0621@gmail.com

Co-Corresponding author:
Kyung Ah Kim
Biomedical Research Institute, Chungbuk National University Hospital, and Department of Biomedical Engineering, College of Medicine, Chungbuk National University, 1 Chungdae-ro, Seowon-gu, Cheongju 28644, Republic of Korea
E-mail: kimka@chungbuk.ac.kr

Introduction

The National Health Insurance Service of Korea (NHIS) analyzed trends in cardiovascular disease treatment over the past 5 years (2018–2022), reporting a 19.9% increase in patient

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Korea Disease Control and Prevention Agency and Korea National Institute of Health.

© 2025 Korea Disease Control and Prevention Agency.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

numbers and a 38.5% rise in medical expenses related to heart disease during this period. The proportion of patients with cardiovascular disease increased across all age groups, with a particularly notable rise among individuals aged 30 years and below [1].

Acute myocardial infarction (AMI) occurs when the coronary arteries are blocked by a blood clot, cutting off blood supply to the heart muscle [2]. Approximately one-third of patients die before reaching the hospital immediately after onset. Rapid reopening of the occluded coronary artery is therefore critical to AMI management [3]. If a patient can be transported to a hospital capable of coronary intervention within 2 to 3 hours of symptom onset, balloon dilatation and stent insertion are recommended as standard treatments. Once stabilized and discharged, patients should receive secondary preventive treatments to reduce the risk of recurrence and complications. Secondary prevention includes optimal pharmacologic management for coronary artery disease. For patients with comorbid diabetes, hypertension, or hyperlipidemia, comprehensive self-management—such as smoking cessation, dietary changes, regular exercise, and stress management—should be undertaken alongside medication. However, even after specialized acute care and discharge, AMI patients often face challenges in resuming appropriate physical activity due to tachycardia, frequent arrhythmias, reduced cardiac output, orthostatic hypotension, and exercise-induced hypertension [4].

Numerous studies have applied deep learning algorithms to the study of cardiovascular diseases. For example, fundus photographs of the retina and ocular vasculature have been used in end-to-end deep learning models to directly predict cardiovascular diseases such as stroke and myocardial infarction (MI). In addition, a deep survival learning model was developed to account for the patient follow-up period and cardiovascular disease occurrence using fundus photography data [5]. Another study implemented an artificial intelligence (AI) model for detecting AMI from asynchronous electrocardiogram (ECG) data, comparing its performance with that of commercial ECG analysis software [6]. Lee et al. [7] further demonstrated the utility of deep learning models for predicting cardiovascular mortality and admission among patients with hypertension using the Korean National Health Information Database, showing improved performance relative to traditional statistical approaches. Rim et al. [8] advanced non-invasive risk stratification by developing a deep-learning system to predict coronary artery calcium scores from retinal photographs. In acute care settings, Liu et al. [9] used machine learning (ML) for variable selection and prediction of major adverse cardiac events (MACE) in emergency department patients presenting with chest pain. The rapid progress and potential of advanced analytical techniques in cardiovascular medicine have been summarized in comprehensive reviews, such as Krittanawong et al. [10], which offer practical primers on deep learning applications. Our study builds on this expanding literature by specifically applying deep neural networks (DNNs) to the multiclass prediction of MACE in post-AMI patients, using comprehensive clinical registry data. Recent work has also explored cardiovascular disease prediction via deep learning with feature augmentation [11]. The present study developed deep learning-based models to classify MACE in AMI patients for 3 follow-up periods: 1, 6, and 12 months. The Korea Acute Myocardial Infarction Registry (KAMIR) registry provides a robust data foundation for cardiovascular research, and DNNs are increasingly recognized for their strong predictive capabilities. The unique contribution of our study lies in the specific, multifaceted prognostic challenge addressed: using the KAMIR dataset to develop DNN models for nuanced, multiclass prediction of 4 MACE categories. An innovative feature of this research is the evaluation of predictive performance across 3 distinct and clinically relevant follow-up intervals. Additional novelty stems from our comprehensive comparison of the tailored DNN architecture with 6 traditional ML algorithms, each rigorously tuned for this complex classification task. Importantly, the application of Shapley additive explanations (SHAP) analysis to ensure interpretability of the DNN model's MACE predictions in post-AMI patients marks a significant step toward practical clinical utility, distinguishing our work from more generalized approaches. The remainder of this paper is organized as follows: Section 2 presents the materials and methods, Section 3 details the results of both ML and deep learning models, and Section 4 discusses the findings in depth.

HIGHLIGHTS

This study developed deep neural network models to predict major adverse cardiac events in patients following discharge for acute myocardial infarction (AMI). The models achieved high accuracy rates of 92.2%, 88.4%, and 91.3% for 1-, 6-, and 12-month follow-up periods, respectively, outperforming traditional machine learning approaches. These results validate the practical utility of deep neural networks for AMI diagnosis and post-discharge treatment planning. The models provide medical professionals with valuable tools to optimize patient management and improve the overall hospitalization experience.

https://doi.org/10.24171/j.phrp.2025.0120

Materials and Methods

This section outlines the methodology for developing a DNN to classify MACE in patients. First, we describe the processes of data collection, filtration, and preprocessing. We then detail the feature selection approach, followed by a description of our DNN model architecture. Finally, we explain how the DNN's performance was assessed and evaluated using various metrics. The aim was to classify MACE in AMI patients at 3 specific follow-up intervals: 1, 6, and 12 months, and to categorize MACE at each interval into 1 of 4 categories: coronary artery bypass grafting (CABG), cardiac death (CD), MI, and repeat percutaneous coronary intervention (Re-PCI). Distinct models were trained for each period to optimize classification accuracy. Figure 1 presents the experimental workflow, illustrating the processing of AMI patient data, including preprocessing, feature selection, model training, prediction, and evaluation.

Study Population

This study utilized data from the KAMIR. The dataset was constructed from AMI patients admitted to the hospital, incorporating a variety of patient information collected during hospitalization—including admission assessments, prior medical history, clinical measurements, cardiac interventions, and post-procedure follow-up data. Initially, we obtained 25,195 patient samples, each with 303 features. To ensure data quality, we applied predefined criteria to filter out irrelevant or outlier samples. First, patients who were pronounced dead on arrival were excluded. Next, we removed samples with excessive missing information, defined as lacking more than 70% of features, to avoid negative impacts on model development [12]. Finally, we excluded any cases lacking complete follow-up data for MACE—the primary target variable—at 1, 6, or 12 months. The extraction and filtering process is summarized in Figure 2, which displays the number of excluded samples at each step and the remaining dataset size. Through this multi-step process, 10,091 samples were excluded, resulting in a final cohort of 15,104 patients for analysis. The primary inclusion criterion was patients diagnosed with AMI who were admitted to the hospital. To ensure data quality and suitability for analysis, a multi-step filtration process was implemented (Figure 2). First, 278 patients dead on arrival were removed, followed by 3,438 cases with excessive missing data, and 6,375 lacking complete MACE follow-up for the target periods. This systematic filtering yielded a robust experimental dataset of 15,104 samples for further analysis, including feature selection, preprocessing, and model training.

Baseline clinical and demographic characteristics of the final patient cohort are detailed in Table 1. The table compares characteristics across the entire study population

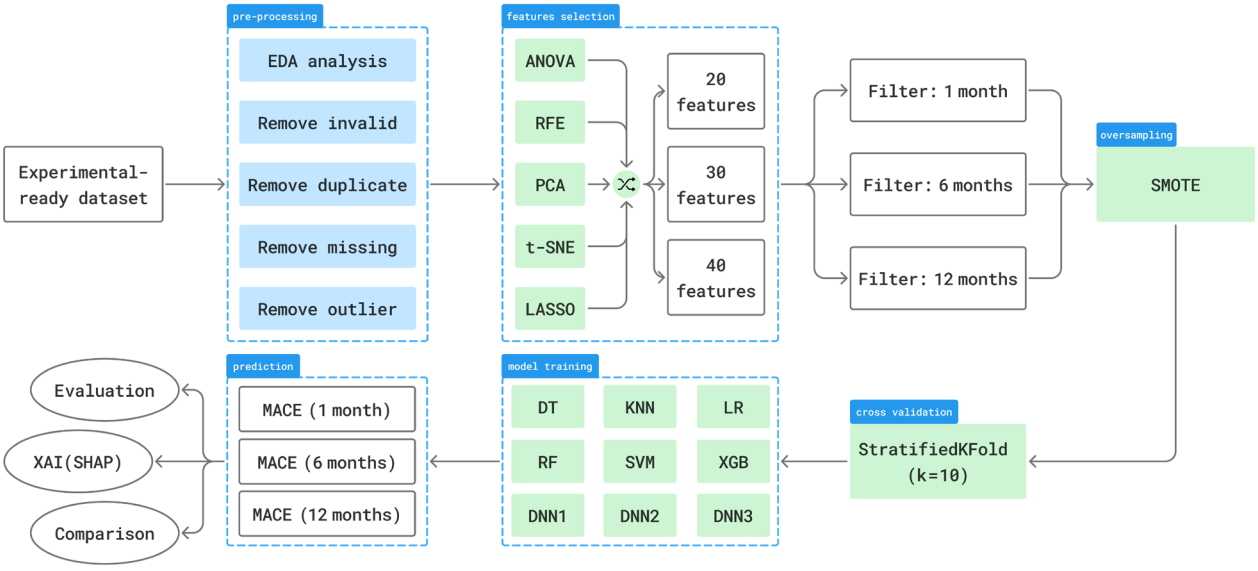


Figure 1. Development of DNN models for classifying MACE in AMI patients.
DNN, deep neural network; MACE, major adverse cardiovascular events; AMI, acute myocardial infarction; EDA, exploratory data analysis; ANOVA, analysis of variance; RFE, recursive feature elimination; PCA, principal component analysis; t-SNE, t-distributed stochastic neighbor embedding; LASSO, least absolute shrinkage and selection operator; SMOTE, synthetic minority over-sampling technique; XAI, explainable artificial intelligence; SHAP, Shapley additive explanations; DT, decision tree; KNN, k-nearest neighbors; LR, logistic regression; RF, random forest; SVM, support vector machine; XGB, extreme gradient boosting; DNN, deep neural network.

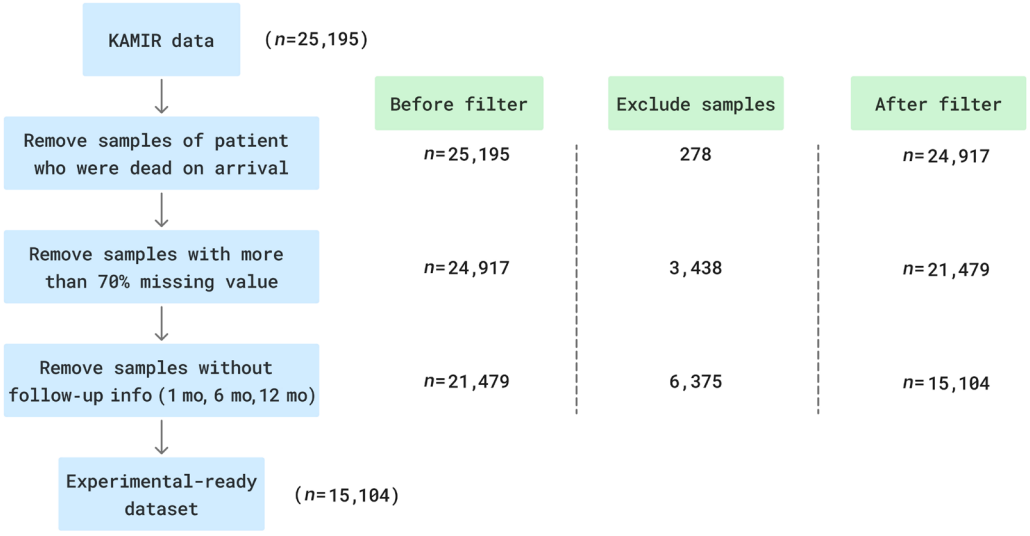


Figure 2. Sample filtration for reducing non-relevant and outlier samples from the dataset. KAMIR, Korea Acute Myocardial Infarction Registry.

and by post-discharge MACE status, including groups with no MACE and those who experienced CABG, CD, MI, or Re-PCI. For example, patients who experienced CD were on average older (75.72 ± 10.20 years) than those without MACE (65.77 ± 12.68 years) and had a lower mean left ventricular ejection fraction (43.48% ± 13.46% vs. 53.53% ± 22.27%). Such differences underscore the clinical heterogeneity of the cohort and highlight variables potentially associated with MACE outcomes.

Data Preprocessing

Comprehensive data preprocessing was performed to enhance dataset quality and ultimately improve model performance. An essential first step was data cleaning, which involved verifying and correcting temporal and structural errors in the dataset [13]. Missing values were imputed using the mean for numerical variables and the mode for categorical variables. Duplicate entries were identified and removed to ensure data uniqueness. Exploratory data analysis (EDA) was also carried out to detect outliers and better understand the overall data structure [14]. Outliers identified during EDA were removed prior to model training. For rigorous and unbiased evaluation, the dataset was randomly divided into training (70%), validation (15%), and testing (15%) subsets. The training set was used for model fitting, enabling the DNN to learn patterns in the data. The validation set supported hyperparameter tuning and overfitting detection, while the independent testing set provided an unbiased assessment of final model performance on unseen data.

Feature Selection

It is common for medical datasets to include a large number of features, such as clinical variables and gene expression data. However, too many features can cause overfitting and unnecessary computational complexity in deep learning models. Therefore, feature selection techniques are essential for improving classification performance by removing duplicate, irrelevant, or noisy features. To identify the most informative variables, we used 5 feature selection methods: analysis of variance (ANOVA), t-distributed stochastic neighbor embedding (t-SNE), least absolute shrinkage and selection operator (LASSO), recursive feature elimination (RFE), and principal component analysis (PCA). ANOVA is effective for identifying variables that differ significantly between groups, making the process more targeted and efficient [15]. The LASSO technique removes less relevant features by shrinking their coefficients to zero [16]. RFE works by sequentially eliminating features that have the least impact on model performance [17]. In contrast, t-SNE reduces dimensionality by grouping similar data points together in a lower-dimensional space [18], while PCA transforms the data into a new set of principal components, also reducing dimensionality [19]. These feature-selection techniques, which were chosen to optimize the variance, are linear combinations of the initial variables. To systematically compare these techniques, we experimented with subsets of 20, 30, and 40 features, evaluating the resulting impact on model accuracy and performance. This process enabled a direct assessment of the efficacy of each feature selection strategy. After extensive testing, RFE with 30 features was

https://doi.org/10.24171/j.phrp.2025.0120

Table 1. Baseline characteristics of KAMIR dataset used for MACE classification

Characteristic	Post-discharge MACE					
	Total	No MACE	CABG	CD	MI	Re-PCI
Total	15,104 (100.0)	14,317 (94.8)	44 (0.3)	263 (1.7)	171 (1.1)	546 (3.6)
Age (y)	65.91±12.71	65.77±12.68	66.93±10.03	75.72±10.20	68.83±13.93	65.75±11.95
BMI (kg/m^2)	24.03±3.22	24.04±3.21	23.90±2.74	22.79±3.35	23.40±3.39	24.26±3.13
Systolic blood pressure (mmHg)	130.09±27.39	130.07±27.29	125.39±25.72	123.35±30.80	129.87±28.64	132.04±27.08
Diastolic blood pressure (mmHg)	79.16±16.11	79.16±16.04	77.64±15.21	75.26±18.35	78.72±15.67	80.41±16.30
PCI stent size (mm)	23.50±7.73	23.47±7.76	22.88±10.86	24.51±6.48	23.39±7.59	24.82±7.20
PCI stent diameter (mm)	3.17±0.47	3.17±0.46	3.27±0.54	3.12±0.58	3.13±0.41	3.11±0.45
CK (U/L)	1,133.97±1,777.96	1,142.49±1,784.77	819.12±877.10	1,167.22±2,130.81	718.67±1,223.75	1,196.75±1,876.80
CK-MB (U/L)	112.83±217.43	111.26±210.17	109.02±148.23	107.91±212.51	91.63±144.59	111.14±213.07
LVEF	53.41±21.94	53.53±22.27	44.10±11.94	43.48±13.46	49.77±13.38	53.72±11.65
CAG pre-TIMI	1.17±1.27	1.17±1.27	1.03±1.28	1.22±1.27	1.07±1.18	1.17±1.24
CAG post-TIMI	2.89±0.45	2.90±0.44	2.43±1.08	2.74±0.72	2.78±0.67	2.92±0.36
HDL cholesterol (mg/dL)	43.65±16.27	43.71±16.18	41.27±8.94	42.96±16.67	44.20±29.78	42.88±13.90
LDL cholesterol (mg/dL)	115.41±40.39	115.54±40.56	113.47±40.06	103.11±39.89	113.57±37.40	114.06±38.57
Sex						
Female	10,897 (72.1)	10,355 (72.3)	33 (75.0)	160 (60.8)	113 (66.1)	395 (72.3)
Male	4,157 (27.5)	3,915 (27.3)	11 (25.0)	103 (39.2)	58 (33.9)	150 (27.5)
Chest pain						
No	11,413 (75.6)	3,303 (23.1)	9 (20.5)	113 (43.0)	47 (27.5)	160 (29.3)
Yes	3,435 (22.7)	10,787 (75.3)	35 (79.5)	135 (51.3)	119 (69.6)	382 (70.0)
Killip class						
I	10,274 (68.0)	9,814 (68.5)	32 (72.7)	94 (35.7)	95 (55.6)	373 (68.3)
II	1,810 (12.0)	1,691 (11.8)	4 (9.1)	32 (12.2)	29 (17.0)	67 (12.3)
III	1,073 (7.1)	989 (6.9)	2 (4.5)	82 (31.2)	25 (14.6)	47 (8.6)
IV	559 (3.7)	519 (3.6)	4 (9.1)	24 (9.1)	10 (5.8)	21 (3.8)
CAG target vessel						
LAD	6,398 (42.4)	6,038 (42.2)	10 (22.7)	90 (34.2)	79 (46.2)	244 (44.7)
LCX	2,352 (15.6)	2,248 (15.7)	11 (25.0)	29 (11.0)	18 (10.5)	100 (18.3)
Left main	262 (1.7)	241 (1.7)	5 (11.4)	15 (5.7)	4 (2.3)	6 (1.1)
RCA	4,448 (29.4)	4,256 (29.7)	12 (27.3)	63 (24.0)	36 (21.1)	174 (31.9)

Data are presented as *n* (%) or mean±standard deviation.
KAMIR, Korea Acute Myocardial Infarction Registry; MACE, major adverse cardiovascular event; CABG, coronary artery bypass grafting; CD, cardiac death; MI, myocardial infarction; Re-PCI, repeat percutaneous coronary intervention; BMI, body mass index; PCI, percutaneous coronary intervention; CK, creatine kinase; CK-MB, creatine kinase MB; LVEF, left ventricular ejection fraction; CAG, coronary angiography; TIMI, thrombolysis in myocardial infarction; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LAD, left anterior descending artery; LCX, left circumflex artery; RCA, right coronary artery.

found to yield the highest predictive accuracy on a dedicated validation set. This combination was thus selected for all subsequent ML and deep learning models presented in this study. Such comprehensive experimentation was crucial for understanding how feature selection methods and the number of features affect model predictive ability.

Synthetic Minority Over-sampling Technique

Synthetic minority over-sampling technique (SMOTE) addresses imbalanced datasets in ML by generating synthetic samples for minority classes, thereby improving class distribution without discarding valuable information [20]. SMOTE operates by selecting samples from the minority class and identifying their nearest neighbors in feature space; new synthetic samples are then created along the lines connecting these points, diversifying the representation of the minority class. This approach helps to reduce model bias toward the majority class and enhances predictive performance for minority classes [21]. By integrating SMOTE into data preprocessing—potentially in combination with undersampling the majority class—a more balanced dataset is achieved. This comprehensive strategy enhances both the

generalizability and accuracy of classification models when facing imbalanced data [22]. As demonstrated in Table 2, the KAMIR dataset exhibits significant imbalance across all MACE classes.

Traditional Machine Learning

A variety of traditional ML algorithms were used as benchmarks to compare with the proposed DNN model. These algorithms included multinomial logistic regression (LR) [23], random forest (RF) [24], decision trees [25], support vector machines (SVM) [26], k-nearest neighbors (KNN) [27], and extreme gradient boosting (XGBoost) [28]. Each of these ML approaches was selected for its unique method of data analysis, ranging from linear models like LR, which estimates associations between variables, to ensemble methods such as RF and XGBoost, which construct multiple decision trees to improve prediction accuracy. SVM classifies data by finding the optimal hyperplane that separates different classes, while KNN assigns class labels based on the closest training samples in the feature space. To ensure the models' generalizability to independent data, cross-validation was employed, thereby mitigating overfitting and supporting the selection of models with the best predictive capability [29]. All ML algorithms were fine-tuned using GridSearch, and Table 3 summarizes the optimal hyperparameters for each algorithm.

Proposed Deep Learning Architecture

Our DNN model was designed for multiclass classification tasks, specifically tailored for tabular clinical datasets. The architecture combines dense layers, batch normalization, rectified linear unit (ReLU) activation, and dropout regularization, allowing the model to efficiently process and learn from high-dimensional data [30]. The network begins with an input layer followed by 3 sequential dense layers, which are essential for capturing the underlying patterns within the dataset. Batch normalization is applied after each dense layer to stabilize and speed up the training process by normalizing outputs [31]. ReLU activation introduces nonlinearity, enabling the model to learn more complex relationships without excessive computational cost [32]. To reduce the risk of overfitting, dropout layers are placed after each ReLU activation, randomly omitting a portion of neuron outputs during training [33]. This encourages the model to develop generalized features that perform well on new, unseen data. Consequently, the model could learn more generalized features that are robust across new, unseen data. The output layer consists of 4 neurons with a SoftMax activation function, facilitating classification into 1 of 4 possible categories based on the highest predicted probability. The Adam optimizer is employed for training due to its adaptive learning rate and established performance in deep learning applications, while categorical cross-entropy serves as the loss function to maximize classification accuracy [34]. The DNN's architecture thus achieves a balance between complexity and efficiency, ensuring strong performance on multiclass classification tasks using tabular data.

Specifically, the architecture starts with an input layer and proceeds through 3 dense layers with 512, 256, and 128 neurons, respectively. This narrowing structure was empirically chosen to capture hierarchical feature representations while controlling for overfitting. Batch normalization and ReLU activation are applied after each dense layer, while a dropout rate of 0.25, selected through preliminary tuning, provides robust regularization for this dataset and model configuration. By integrating these strategies, the model is able to efficiently learn and generalize, as depicted in the block diagram shown in Figure 3.

Model Evaluation

To comprehensively assess classification performance, we used a set of evaluation metrics appropriate for multiclass problems, including accuracy, precision, recall, and area

Table 2. Count of patient samples for each MACE type and each discharge duration

Period	Patient sample (*n*)				
	Total	CABG	CD	MI	Re-PCI
1 mo	370	22	150	97	101
6 mo	468	15	143	46	264
12 mo	398	7	137	36	218
Total	1,236	44	430	179	583

MACE, major adverse cardiovascular event; CABG, coronary artery bypass grafting; CD, cardiac death; MI, myocardial infarction; Re-PCI, repeat percutaneous coronary intervention.

Table 3. Best hyperparameter configuration for each ML algorithm after fine-tuning

ML algorithm	Best hyperparameters
Decision tree	max_depth = 5; min_samples_leaf = 10; criterion = 'gini'
Random forest	n_estimators = 100; max_depth = 4; max_features = 'sqrt'
Support vector machines	C = 10; kernel = 'rbf'; gamma = 'scale'; degree = 3
K-nearest neighbor	n_neighbors = 5; weights = 'uniform'; algorithm = 'auto'
Logistic regression	C = 0.1; penalty = 'l2'; solver = 'liblinear'
XGBoost	n_estimators = 50; learning_rate = 0.1; max_depth = 3; gamma = 0

ML, machine learning; XGBoost, extreme gradient boosting.

https://doi.org/10.24171/j.phrp.2025.0120

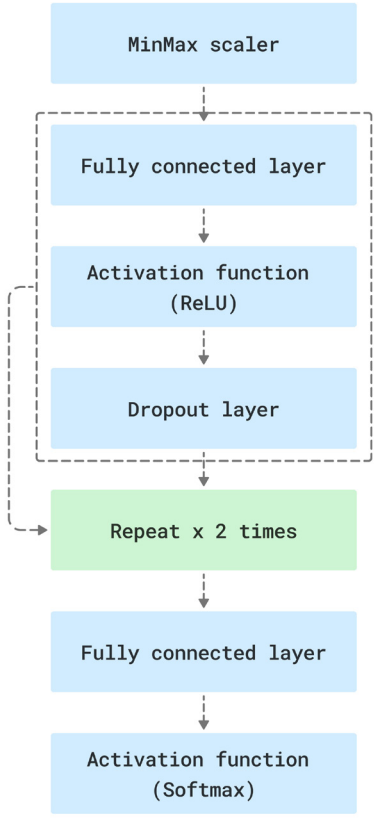


Figure 3. Architecture of proposed deep neural network models using fully connected layers. ReLU, rectified linear unit.

under the receiver operating characteristic curve (AUC) [35]. For greater insight into the decision-making processes of the models, we incorporated explainable AI (XAI) techniques, with a focus on SHAP values [36]. This approach ensures that model predictions are transparent, reliable, and clinically meaningful, providing actionable insights for medical professionals. We conducted a detailed comparison of all models, evaluating predictive performance, interpretability, and computational efficiency to determine the most effective approach for MACE classification. The standard formulas used for each evaluation metric are as follows, where TP denotes true positives, TN true negatives, FP false positives, and FN false negatives:

$$\text{Accuracy} = \frac{TP + TN}{TP + TN + FP + FN}$$

$$\text{Precision} = \frac{TP}{TP + FP}$$

$$\text{Recall} = \frac{TP}{TP + FN}$$

$$\text{F1} = 2 \times \frac{Precision \times Recall}{Precision + Recall}$$

Results

This section presents the results of our experiments using both traditional ML algorithms and the proposed DNN models for classifying patient MACE. All models were evaluated using the metrics described previously. The section is organized as follows: Section 3.1 presents the outcomes of the traditional ML models, while Section 3.2 details the results of the proposed DNN models.

Machine Learning Performance Evaluation

To assess the effectiveness of the proposed DNN models, we conducted a comparative analysis using traditional ML algorithms as baseline controls. Each baseline model was trained with optimal hyperparameters, as summarized in Table 3. The primary objective was to evaluate the ability of each model to accurately classify MACE in patients discharged after AMI. The results of this analysis, which compare the performance of both the DNN and traditional ML models on this classification task, are systematically reported in Tables 4–6. These tables provide a comprehensive overview of model performance, allowing for a clear comparison of their effectiveness in predicting MACE and managing post-discharge outcomes for AMI patients.

As shown in Tables 4–6, the SVM model achieved the highest scores for metrics such as accuracy, recall, and F1-score, whereas the RF model outperformed others in terms of precision and AUC values. The use of SMOTE notably improved overall model performance by effectively addressing data imbalance through over-sampling. However, the remaining ML algorithms did not demonstrate satisfactory efficacy in classifying MACE in this patient population.

Proposed DNN Performance Evaluation

The proposed DNN models exhibited excellent performance in classifying MACE among AMI patients. Performance metrics for each follow-up period are provided in Tables 7–9, with the highest accuracy observed in the 1-month post-discharge classification. These tables present outcomes across various sets of training hyperparameters, highlighting the substantial benefits of hyperparameter optimization. Fine-tuning was found to be critical for identifying the best parameter configuration and maximizing model reliability for MACE classification.

Figure 4 illustrates the loss and accuracy curves for the DNN models on both training and validation datasets. These plots detail the changes in loss and accuracy over training epochs, illustrating that the model underwent substantial improvements during the first 15 epochs, followed by gradual

Table 4. Classification performance of traditional machine learning models (1 month)

ML model	SMOTE	ACC	RE	PR	F1	AUC
SVM	No	0.8254	0.8125	0.8395	0.8258	0.8712
	Yes	0.8423	0.8615	0.8246	0.8425	0.8854
DT	No	0.7915	0.8054	0.7789	0.792	0.8461
	Yes	0.8136	0.8425	0.7856	0.8132	0.8654
RF	No	0.8365	0.8215	0.8521	0.8366	0.8823
	Yes	0.8554	0.8754	0.8356	0.8553	0.9015
LR	No	0.8023	0.7954	0.8095	0.8024	0.8495
	Yes	0.8256	0.8456	0.8054	0.8254	0.8685
KNN	No	0.8512	0.8354	0.8675	0.8514	0.9005
	Yes	0.8725	0.8954	0.8502	0.8723	0.9154
XGB	No	0.7542	0.7754	0.7335	0.754	0.8154
	Yes	0.7756	0.8054	0.7456	0.7754	0.8325

ML, machine learning; SMOTE, synthetic minority over-sampling technique; ACC, accuracy; RE, recall; PR, precision; F1, F1-score; AUC, area under the receiver operating characteristic curve; SVM, support vector machine; DT, decision tree; RF, random forest; LR, logistic regression; KNN, k-nearest neighbors; XGB, extreme gradient boosting.

Table 5. Classification performance of traditional machine learning models (6 months)

ML model	SMOTE	ACC	RE	PR	F1	AUC
SVM	No	0.8235	0.8156	0.8421	0.8287	0.8876
	Yes	0.8362	0.8475	0.8254	0.8363	0.8954
DT	No	0.7952	0.7821	0.8095	0.7957	0.8523
	Yes	0.8105	0.8234	0.7978	0.8105	0.8679
RF	No	0.8176	0.8054	0.8302	0.8177	0.8754
	Yes	0.8254	0.8395	0.8112	0.8252	0.8836
LR	No	0.8025	0.7954	0.8102	0.8028	0.8615
	Yes	0.8195	0.8321	0.8065	0.8193	0.8782
KNN	No	0.8315	0.8254	0.8376	0.8314	0.8905
	Yes	0.8392	0.8435	0.8354	0.8394	0.8985
XGB	No	0.7854	0.7725	0.7985	0.7855	0.8425
	Yes	0.7954	0.8095	0.7815	0.7954	0.8536

ML, machine learning; SMOTE, synthetic minority over-sampling technique; ACC, accuracy; RE, recall; PR, precision; F1, F1-score; AUC, area under the receiver operating characteristic curve; SVM, support vector machine; DT, decision tree; RF, random forest; LR, logistic regression; KNN, k-nearest neighbors; XGB, extreme gradient boosting.

Table 6. Classification performance of traditional machine learning models (12 months)

ML model	SMOTE	ACC	RE	PR	F1	AUC
SVM	No	0.8254	0.8012	0.8423	0.8215	0.8652
	Yes	0.8417	0.8495	0.8352	0.8423	0.8789
DT	No	0.7925	0.7568	0.8211	0.788	0.8365
	Yes	0.8103	0.8054	0.8154	0.8104	0.8492
RF	No	0.8075	0.7895	0.8256	0.8073	0.8521
	Yes	0.8231	0.8412	0.8054	0.823	0.8654
LR	No	0.7854	0.7625	0.8095	0.7858	0.8312
	Yes	0.8015	0.7954	0.8076	0.8015	0.8456
KNN	No	0.8156	0.8025	0.8289	0.8157	0.8585
	Yes	0.8324	0.8456	0.8195	0.8325	0.8712
XGB	No	0.7542	0.7254	0.7854	0.7549	0.8054
	Yes	0.7715	0.7654	0.7776	0.7715	0.8185

ML, machine learning; SMOTE, synthetic minority over-sampling technique; ACC, accuracy; RE, recall; PR, precision; F1, F1-score; AUC, area under the receiver operating characteristic curve; SVM, support vector machine; DT, decision tree; RF, random forest; LR, logistic regression; KNN, k-nearest neighbors; XGB, extreme gradient boosting.

https://doi.org/10.24171/j.phrp.2025.0120

Table 7. Classification performance of DNNs for various hyperparameters (1 month)

Hyperparameter	SMOTE	ACC	RE	PR	F1	AUC
Epoch = 50, batch_size = 64, learning_rate = 1e-5	No	0.9054	0.9154	0.8956	0.9055	0.9425
	Yes	0.9185	0.9354	0.9025	0.9184	0.9554
Epoch = 50, batch_size = 64, learning_rate = 1e-4	No	0.9085	0.9254	0.8925	0.9084	0.9465
	Yes	0.9215	0.9454	0.8975	0.9214	0.9584
Epoch = 50, batch_size = 64, learning_rate = 1e-3	No	0.8955	0.8854	0.9055	0.8955	0.9385
	Yes	0.9125	0.9354	0.8902	0.9124	0.9515

DNN, deep neural network; SMOTE, synthetic minority over-sampling technique; ACC, accuracy; RE, recall; PR, precision; F1, F1-score; AUC, area under the receiver operating characteristic curve.

Table 8. Classification performance of DNNs for various hyperparameters (6 months)

Hyperparameter	SMOTE	ACC	RE	PR	F1	AUC
Epoch = 50, batch_size = 64, learning_rate = 1e-5	No	0.8675	0.8595	0.8754	0.8674	0.9254
	Yes	0.8795	0.8854	0.8735	0.8795	0.9385
Epoch = 50, batch_size = 64, learning_rate = 1e-4	No	0.8725	0.8678	0.8782	0.8727	0.9285
	Yes	0.8842	0.8915	0.8765	0.884	0.9421
Epoch = 50, batch_size = 64, learning_rate = 1e-3	No	0.8654	0.8585	0.8732	0.8656	0.9234
	Yes	0.8812	0.8874	0.8751	0.8813	0.9392

DNN, deep neural network; SMOTE, synthetic minority over-sampling technique; ACC, accuracy; RE, recall; PR, precision; F1, F1-score; AUC, area under the receiver operating characteristic curve.

Table 9. Classification performance of DNNs for various hyperparameters (12 months)

Hyperparameters	SMOTE	ACC	RE	PR	F1	AUC
Epoch = 50, batch_size = 64, learning_rate = 1e-5	No	0.8954	0.8854	0.9056	0.8955	0.9354
	Yes	0.9125	0.9254	0.8995	0.9124	0.9485
Epoch = 50, batch_size = 64, learning_rate = 1e-4	No	0.8954	0.8754	0.9156	0.8953	0.9325
	Yes	0.9035	0.9154	0.8915	0.9032	0.9415
Epoch = 50, batch_size = 64, learning_rate = 1e-3	No	0.8875	0.8654	0.9085	0.8865	0.9254
	Yes	0.8962	0.9054	0.8875	0.8963	0.9345

DNN, deep neural network; SMOTE, synthetic minority over-sampling technique; ACC, accuracy; RE, recall; PR, precision; F1, F1-score; AUC, area under the receiver operating characteristic curve.

stabilization as training continued. This trend suggests an initial phase of rapid learning, with performance plateauing as the model converged toward optimal parameters. Figure 5 presents the confusion matrices of the DNN models, showcasing the top-performing model selected for each follow-up period after hospital discharge.

Discussion

Research Contributions

The primary motivation for this study was to address the critical need for accurate and timely prognostication of diverse MACE in patients following AMI across distinct post-discharge periods. Accurate prediction at 1, 6, and 12 months can significantly aid clinicians in tailoring follow-up intensity, optimizing therapeutic interventions, and improving overall patient outcomes. While previous research has explored ML in cardiovascular disease, this study specifically aimed to develop and validate DNN models capable of distinguishing among 4 MACE categories at these 3 key follow-up intervals. A further distinction of our work is the comprehensive benchmarking of DNN models against a range of traditional ML algorithms on a large-scale registry dataset (KAMIR), coupled with an XAI SHAP approach to provide interpretability of model predictions. This strategy addresses both predictive power and interpretability, offering clinicians valuable insight into the reasoning behind each prediction.

Performance Comparison

We observed a substantial disparity in performance between the ML models and the proposed DNN models. The DNN consistently outperformed all ML algorithms across every evaluation metric discussed in the previous section. Figures

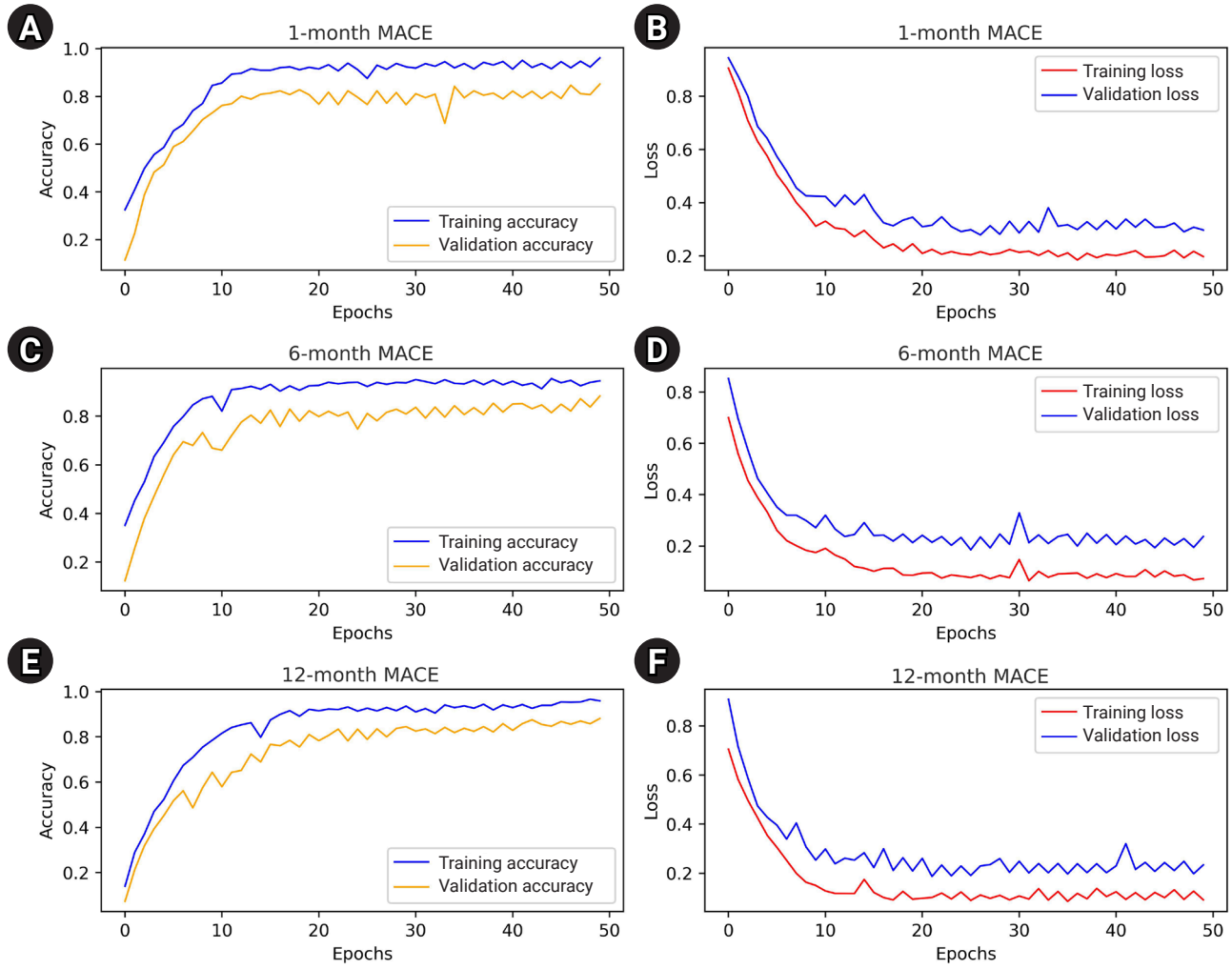


Figure 4. (A–F) Loss and accuracy history of the best deep neural network (DNN) model for training and validation dataset for each discharge period. MACE, major adverse cardiovascular events.

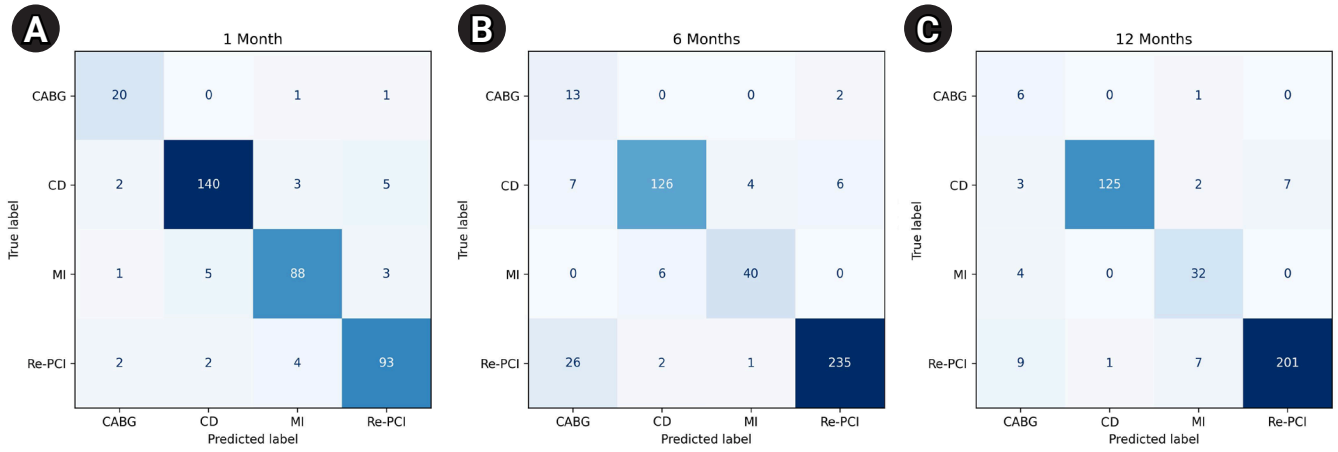


Figure 5. (A–C) Confusion matrices of well-performing DNN models across post-hospitalization periods.
DNN, deep neural network; CABG, coronary artery bypass grafting; CD, cardiac death; MI, myocardial infarction; Re-PCI, repeat percutaneous coronary intervention.

https://doi.org/10.24171/j.phrp.2025.0120

6–8 visually represent the performance of all models in this experiment, specifically for MACE classification at 1, 6, and 12 months. The marked gap in performance underscores the advanced predictive capabilities of deep learning over traditional ML approaches for long-term outcomes in AMI patients.

Analysis of Feature Importance

Further insights were obtained from the deep learning models through the application of the XAI technique known

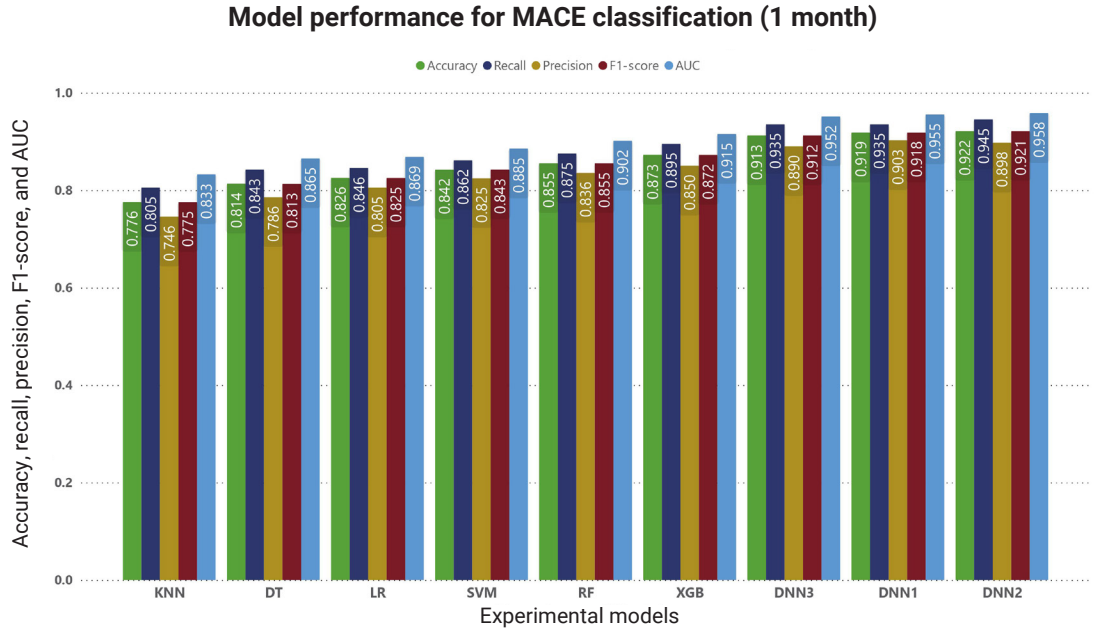


Figure 6. Performance comparison of machine learning and deep learning models (1-month MACE). MACE, major adverse cardiovascular events.

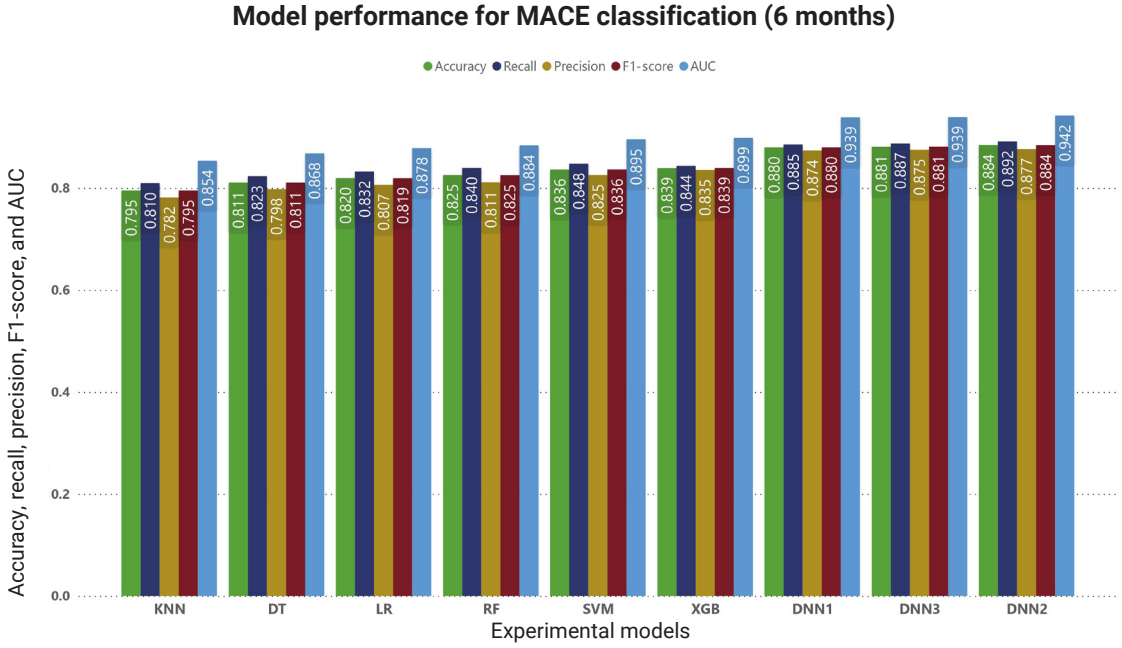


Figure 7. Performance comparison of machine learning and deep learning models (6-month MACE).
MACE, major adverse cardiovascular events; AUC, area under the receiver operating characteristic curve; KNN, k-nearest neighbors; DT, decision tree; LR, logistic regression; RF, random forest; SVM, support vector machine; XGB, extreme gradient boosting; DNN, deep neural network.

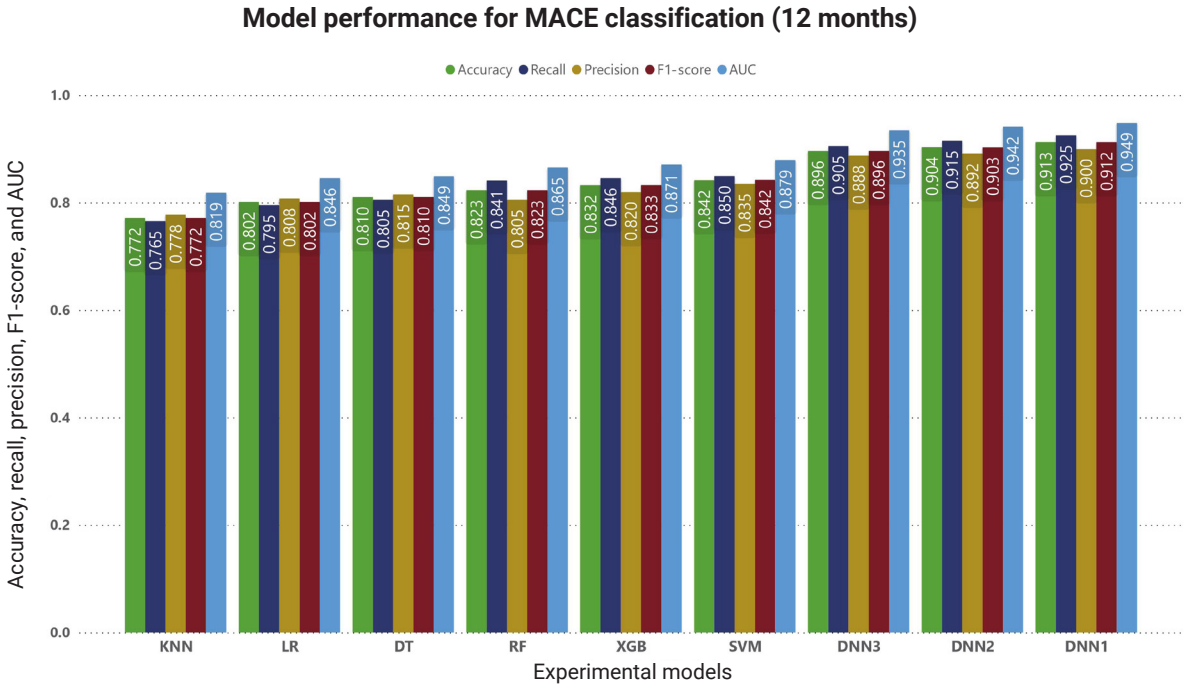


Figure 8. Performance comparison of machine learning and deep learning models (12-month MACE).
MACE, major adverse cardiovascular events; AUC, area under the receiver operating characteristic curve; KNN, k-nearest neighbors; LR, logistic regression; DT, decision tree; RF, random forest; XGB, extreme gradient boosting; SVM, support vector machine; DNN, deep neural network.

as SHAP. Figure 9 illustrates the relative importance of each feature in predicting MACE after discharge in AMI patients. The SHAP summary plots display how each feature contributes to the model's output, which is the probability of MACE at 1, 6, and 12 months. Features are ranked by the average magnitude of their SHAP values, indicating their impact on the model's predictions. For classifying 1-month MACE, the most important features were age, left ventricular ejection fraction, and coronary angiography (CAG) post-thrombolysis in myocardial infarction (TIMI) score. At 6 months, the top features were left ventricular ejection fraction, age, and CAG post-TIMI score. For 12-month MACE, age, Killip class, CAG pre-TIMI score, and left ventricular ejection fraction emerged as the most significant predictors. These findings suggest that these features play a crucial role in determining the probability of MACE in AMI patients. Importantly, while these are the most influential features, all variables included in the model may contribute to the final prediction.

Conclusion

This study proposed a DNN model specifically designed to classify MACE in patients with AMI following discharge. The DNN classified patient MACE into 1 of 4 categories: CABG, CD, MI, and RE-PCI. Separate DNN models were developed to classify MACE at 3 follow-up intervals: 1, 6, and 12 months. The DNN models demonstrated superior classification performance compared to traditional ML approaches, representing a meaningful advancement in diagnostic and follow-up strategies for AMI patients. Cardiac clinicians may leverage these models to proactively assess MACE risk, thereby optimizing patient management and hospitalization. Despite these advances, the proposed DNN model has several limitations. Currently, it processes only clinical data and does not incorporate image data, which may further enhance MACE prediction accuracy. Additionally, the model was trained exclusively on the KAMIR dataset, predominantly sourced from South Korean hospitals. Its generalizability could be improved by including datasets from a wider range of international institutions. In future work, we plan to expand our research to include image-based deep learning models to further improve MACE classification accuracy. We also aim to develop an end-user application that enables healthcare providers without programming expertise to utilize these models in clinical practice. We believe our research represents a significant step toward improving post-discharge monitoring and care for AMI patients, thereby contributing to advancements in cardiac healthcare.

https://doi.org/10.24171/j.phrp.2025.0120

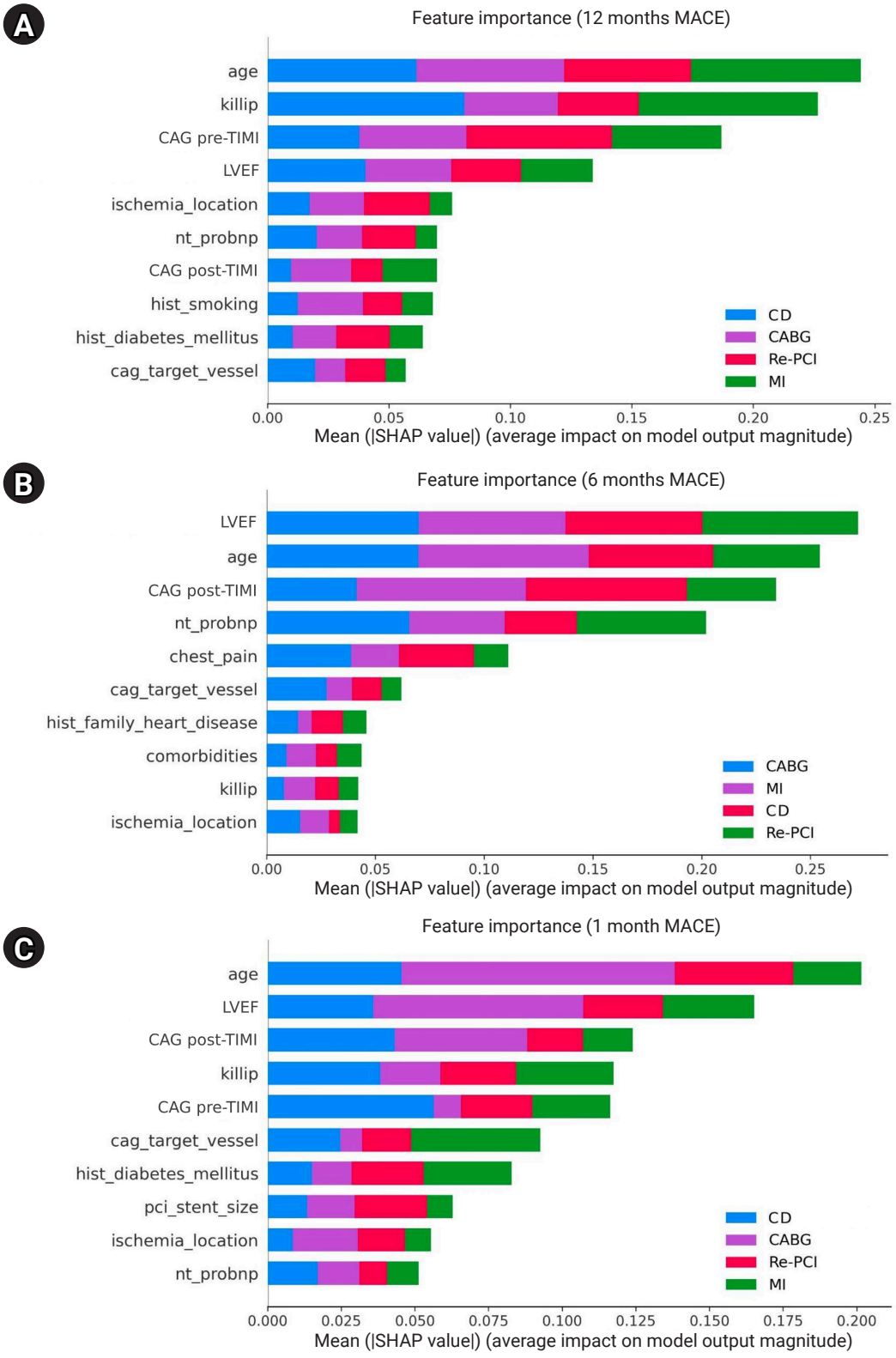


Figure 9. (A–C) Feature importance analysis using SHAP for all discharge periods.
SHAP, Shapley additive explanations; CAG, coronary angiography; TIMI, thrombolysis in myocardial infarction; LVEF, left ventricular ejection fraction; CD, cardiac death; CABG, coronary artery bypass grafting; Re-PCI, repeat percutaneous coronary intervention; MI, myocardial infarction; MACE, major adverse cardiovascular events.

Notes

Ethics Approval
Not applicable.

Conflicts of Interest
The authors have no conflicts of interest to declare.

Funding
This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (No. 2020R1I1A1A01065199 and RS-2023-00245300).

Availability of Data
The datasets are not publicly available but are available from the corresponding author upon reasonable request.

Authors' Contributions
Conceptualization: all authors; Data curation: all authors; Formal analysis: VK; Funding acquisition: KAK, HSS; Investigation: KAK, HSS; Methodology: VK, HSS; Project administration: KAK, HSS; Resources: KAK, HSS; Software: VK; Supervision: KAK, HSS; Validation: KAK, HSS; Visualization: VK, HSS; Writing–original draft: VK, HSS; Writing–review & editing: all authors. All authors read and approved the final manuscript.

References

1. Health Insurance Review and Assessment Service (HIRA) (KR). Current status of heart disease treatment [Internet]. HIRA; 2023 [cited 2025 Mar 19]. Available from: https://www.hira.or.kr/bbsDummy.do?pgmid=HIRA A020041000100&brdScnBltNo=4&brdBltNo=11037. Korean.
2. Gong FF, Vaitenas I, Malaisrie SC, et al. Mechanical complications of acute myocardial infarction: a review. JAMA Cardiol 2021;6:341–9.
3. Salari N, Morddarvanjoghi F, Abdolmaleki A, et al. The global prevalence of myocardial infarction: a systematic review and meta-analysis. BMC Cardiovasc Disord 2023;23:206.
4. Baviera M, Genovese S, Colacioppo P, et al. Diabetes mellitus duration and mortality in patients hospitalized with acute myocardial infarction. Cardiovasc Diabetol 2022;21:223.
5. Son J, Kim J, Kong ST, et al. Leveraging the generalization ability of deep convolutional neural networks for improving classifiers for color fundus photographs. Appl Sci 2021;11:591.
6. Han C, Song Y, Lim HS, et al. Automated detection of acute myocardial infarction using asynchronous electrocardiogram signals: preview of implementing artificial intelligence with multichannel electrocardiographs obtained from smartwatches: retrospective study. J Med Internet Res 2021;23:e31129.
7. Lee SJ, Lee SH, Choi HI, et al. Deep learning improves prediction of cardiovascular disease-related mortality and admission in patients with hypertension: analysis of the Korean National Health Information Database. J Clin Med 2022;11:6677.
8. Rim TH, Lee CJ, Tham YC, et al. Deep-learning-based cardiovascular risk stratification using coronary artery calcium scores predicted from retinal photographs. Lancet Digit Health 2021;3:e306–16.
9. Liu N, Koh ZX, Goh J, et al. Prediction of adverse cardiac events in emergency department patients with chest pain using machine learning for variable selection. BMC Med Inform Decis Mak 2014;14:75.
10. Krittanawong C, Johnson KW, Rosenson RS, et al. Deep learning for cardiovascular medicine: a practical primer. Eur Heart J 2019;40:2058–73.
11. Garcia-Ordas MT, Bayon-Gutierrez M, Benavides C, et al. Heart disease risk prediction using deep learning techniques with feature augmentation. Multimed Tools Appl 2023;82:31759–73.
12. Palanivinayagam A, Damasevicius R. Effective handling of missing values in datasets for classification using machine learning methods. Information 2023;14:92.
13. Stang J, Hartvigsen T, Reitan J. The effect of data quality on data mining: improving prediction accuracy by generic data cleansing. In: Proceedings of the 15th International Conference on Information Quality; 2010 Nov 12–14; Little Rock, AR. ICIQ; 2010.
14. Dhany HW, Sutarman S, Izhari F. Exploratory data analysis (EDA) methods for healthcare classification. J Intell Decis Support Syst 2023;6:209–15.
15. Girden ER. ANOVA: repeated measures. Sage; 1992.
16. Tibshirani R. Regression shrinkage and selection via the lasso. J R Stat Soc Series B Stat Methodol 1996;58:267–88.
17. Guyon I, Weston J, Barnhill S, et al. Gene selection for cancer classification using support vector machines. Mach Learn 2002;46:389–422.
18. Van der Maaten L, Hinton G. Visualizing data using t-SNE. J Mach Learn Res 2008;9:2579–605.
19. Pearson K. LIII. On lines and planes of closest fit to systems of points in space. London Edinburgh Dublin Philos Mag J Sci 1901;2:559–72.
20. Chawla NV, Bowyer KW, Hall LO, et al. SMOTE: synthetic minority over-sampling technique. J Artif Intell Res 202;16:321–57.
21. Blagus R, Lusa L. SMOTE for high-dimensional class-imbalanced data. BMC Bioinformatics 2013;14:106.
22. Ramezankhani A, Pournik O, Shahrabi J, et al. The impact of oversampling with SMOTE on the performance of 3 classifiers in prediction of type 2 diabetes. Med Decis Making 2016;36:137–44.
23. Bohning D. Multinomial logistic regression algorithm. Ann Inst Stat Math 1992;44:197–200.
24. Ho TK. Random decision forests. In: Proceedings of 3rd International Conference on Document Analysis and Recognition; 1995 Aug 14–16; Montreal, CA. IEEE; 1995. p. 278–82.
25. Charbuty B, Abdulazeez A. Classification based on decision tree algorithm for machine learning. J Appl Sci Technol Trends 2021;2:20–8.
26. Cortes C, Vapnik V. Support-vector networks. Mach Learn 1995;20:273–97.
27. Altman NS. An introduction to kernel and nearest-neighbor. Am Stat 1992;46:175–85.
28. Chen T, Guestrin C. Xgboost: a scalable tree boosting system. In: Proceedings of the 22nd ACM SIGKDD International Conference on Knowledge Discovery and Data Mining; 2016 Aug 13–17; New York, NY. ACM; 2016. p. 785–94.

https://doi.org/10.24171/j.phrp.2025.0120

29. Kohavi R. A study of cross-validation and bootstrap for accuracy estimation and model selection. In: Proceedings of the International Joint Conference on Artificial Intelligence; 1995 Aug 20–25; Montreal, CA. IJCAI; 1995. p. 1137–45.
30. Schmidhuber J. Deep learning in neural networks: an overview. Neural Netw 2015;61:85–117.
31. Ioffe S, Szegedy C. Batch normalization: accelerating deep network training by reducing internal covariate shift. In: Proceedings of the International Conference on Machine Learning; 2015 Jul 6–11; Lille, FR, PMLR. 2015. p. 448–56.
32. Agarap AF. Deep learning using rectified linear units (ReLU). arXiv [Preprint] 2018 Mar 22. https://doi.org/10.48550/arXiv.1803.08375.
33. Srivastava N, Hinton G, Krizhevsky A, et al. Dropout: a simple way to prevent neural networks from overfitting. J Mach Learn Res 2014; 15:1929–58.
34. Kingma DP, Ba J. Adam: a method for stochastic optimization. arXiv [Preprint] 2014 Dec 22. https://doi.org/10.48550/arXiv.1412.6980.
35. Han J, Pei J, Tong H. Data mining: concepts and techniques. 3rd ed. Morgan Kaufmann; 2022.
36. Lundberg SM, Nair B, Vavilala MS, et al. Explainable machine-learning predictions for the prevention of hypoxaemia during surgery. Nat Biomed Eng 2018;2:749–60.

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2025.0052
Osong Public Health Res Perspect 2025;16(4):348-356

Original Article

Genetic diversity and spatiotemporal population structure of *Anopheles sinensis* in the Republic of Korea based on the mitochondrial cytochrome c oxidase subunit I (*COI*) marker

Haneul Jung[1], Bo Gyeong Han[1], Hyun-Il Shin[2], Myoung-Ro Lee[1], Jung-Won Ju[1], Hee-Il Lee[1]

[1]Division of Vectors and Parasitic Diseases, Korea Disease Control and Prevention Agency, Cheongju, Republic of Korea
[2]Division of Zoonotic and Vector Borne Disease Control, Korea Disease Control and Prevention Agency, Cheongju, Republic of Korea

ABSTRACT

Objectives: *Anopheles sinensis* is a predominant malaria vector found throughout the Republic of Korea (ROK). Population genetic analysis can provide insights into the origins and migration patterns of malaria vectors by assessing genetic variation and distribution among populations.
Methods: In this study, we examined the population genetic structure of *An. sinensis* in the ROK using mitochondrial cytochrome c oxidase subunit I (*COI*) marker.
Results: A total of 903 specimens of *An. sinensis* were collected from 9 sampling regions, including malaria-endemic and non-endemic areas. All populations displayed characteristics typical of migrant populations, with high haplotype diversity (*Hd*) and low nucleotide diversity (*Pi*). Network analysis identified 124 haplotypes grouped into 2 clusters. Both clusters included haplotypes from malaria-endemic and non-endemic areas. Cluster I shared its most recent common ancestry with Chinese reference sequences, while cluster II had related with Japanese reference sequences. Pairwise genetic distance (F_{ST}) analysis indicated generally low genetic differentiation among populations. Furthermore, F_{ST} values tended to increase proportionally with geographical distance between regions. Analysis of molecular variance confirmed that individual mosquitoes within the population had a large effect on the overall variation. Neutrality tests using 4 methods (Tajima's D, Fu's Fs, Fu and Li's D, and Fu and Li's F) yielded negative values, suggesting that *An. sinensis* populations are expanding in all studied regions.
Conclusion: This study characterizes the genetic attributes of *An. sinensis* in the ROK, providing valuable insights into the biology of this important malaria vector and contributing useful data for malaria control strategies.

Keywords: *Anopheles sinensis*; Malaria; Population genetic structure

Received: February 18, 2025
Revised: April 2, 2025
Accepted: May 6, 2025

Corresponding author:
Hee-Il Lee
Division of Vectors and Parasitic Diseases, Korea Disease Control and Prevention Agency, 187 Osongsaengmyeong 2-ro, Osong-eup, Heungdeok-gu, Cheongju 28159, Republic of Korea
E-mail: isak@korea.kr

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Korea Disease Control and Prevention Agency and Korea National Institute of Health.

© 2025 Korea Disease Control and Prevention Agency.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Malaria causes approximately half a million deaths worldwide each year and contributes significantly to morbidity and mortality in humans [1]. The *Plasmodium* parasite that causes malaria is transmitted by infected female *Anopheles* mosquitoes, and 5 species of malaria parasite can infect humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi* [2]. *P. falciparum* is predominantly found in malaria-endemic countries in Africa, whereas *P. vivax* is found in Southeast Asia. These 2 species occasionally cause co-infections in South America, Southeast Asia, and the Western Pacific regions [3]. *P. malariae* and *P. ovale* are broadly distributed geographically but often remain undiagnosed due to diagnostic difficulties, low parasitemia levels, and relatively mild virulence [4]. *P. knowlesi* occurs infrequently in Malaysia and Indonesia [3]. Notably, only *P. vivax* is prevalent in the Republic of Korea (ROK), with endemic areas primarily situated near the demilitarized zone bordering the Democratic People's Republic of Korea (DPRK) [5].

Anopheline mosquitoes form genetic structures at different spatial scales, particularly in large, homogeneous geographic regions, due to recent expansions or extensive short-range gene flow [6]. Approximately 70 species of anopheline mosquitoes have been reported to have the ability to transmit malaria parasites to humans [7]. Among these, 8 species (*Anopheles belenrae*, *Anopheles kleini*, *Anopheles koreicus*, *Anopheles lesteri*, *Anopheles lindesayi japonicus*, *Anopheles pullus*, *Anopheles sinensis*, and *Anopheles sineroides*) of anopheline mosquitoes inhabit in the ROK [8]. Of these, *An. sinensis* has been confirmed to exhibit a broad geographical distribution and large population sizes [8,9]. *An. sinensis* is considered the primary malaria transmission vector in Asia [7], and its vector competence has been demonstrated through artificial sporozoite infection experiments [10]. Thus, *An. sinensis* is regarded as an important malaria vector in the ROK.

Mitochondrial DNA (mtDNA) serves as a valuable marker for analyzing population genetic structures in various species, including fish, arthropods, and amphibians [11–15]. A Chinese study examining the population genetics of *An. sinensis* with the mtDNA cytochrome c oxidase subunit I (*COI*) marker identified 2 regional clusters [16]. Similarly, in the ROK, genetic structuring of *An. sinensis* was investigated in 2015 using the mtDNA control region, revealing 2 clusters separated by the Sobaek and Taebaek mountain ranges, which acted as a genetic barrier [17,18].

Recent scientific information on the genetic structure of *An. sinensis* in the ROK remains insufficient. Approximately 10 years after the previous study, the present study aimed to confirm the genetic characteristics of *An. sinensis* using the *COI* marker and provide comprehensive molecular and biological data for this mosquito species, recognized as the main vector of malaria in the ROK.

HIGHLIGHTS

- The Republic of Korea is endemic for malaria, with *Anopheles sinensis* as a major vector.
- Genetic analysis of the *Anopheles sinensis* populations collected in this study showed small regional genetic differentiation.
- The cytochrome c oxidase subunit I haplotype network for *An. sinensis* comprised 2 distinct clusters, and certain haplotypes demonstrated homology with reference sequences from China.

Materials and Methods

Mosquito Collection and Identification

Mosquitoes were collected from 7 malaria-endemic and 2 malaria-non-endemic areas from June to October 2022 (Figure 1). In malaria-endemic regions, mosquitoes were collected from outdoor of civilian houses using black light traps (Shin-Young Comm. System), while in non-endemic areas, they were collected in livestock sheds using light-emitting diode traps (Biotrap). Collected mosquitoes were visually identified in the laboratory based on morphological taxonomic characteristics and subsequently confirmed through molecular identification by multiplex polymerase chain reaction (PCR) using primers targeting the internal transcribed spacer 2 region [19,20].

DNA Extraction and *COI* Gene Amplification

DNA was individually extracted from the whole body of mosquitoes using DNAzol (Thermo Fisher Scientific) or the automated nucleic acid extraction system QIAamp 96 DNA QIAcube HT Kit (QIAGEN). The mitochondrial *COI* gene (710 bp) was amplified using AccuPower PCR PreMix (Bioneer) with forward primer LCO1490 (5'-GGTCAACAAAT CATAAAGATATTGG-3') and reverse primer HCO2198 (5'-TA AACTTCAGGGTGACCAAAAAATCA-3') [21]. PCR conditions were as follows: initial denaturation at 95 °C for 5 minutes; followed by 40 cycles consisting of denaturation at 95°C for 30 seconds, annealing at 53 °C for 30 seconds, elongation at 72 °C for 30 seconds; and a final extension at 72 °C for 10 minutes. The sizes of the amplified PCR products were confirmed using the QIAxcel capillary electrophoresis system

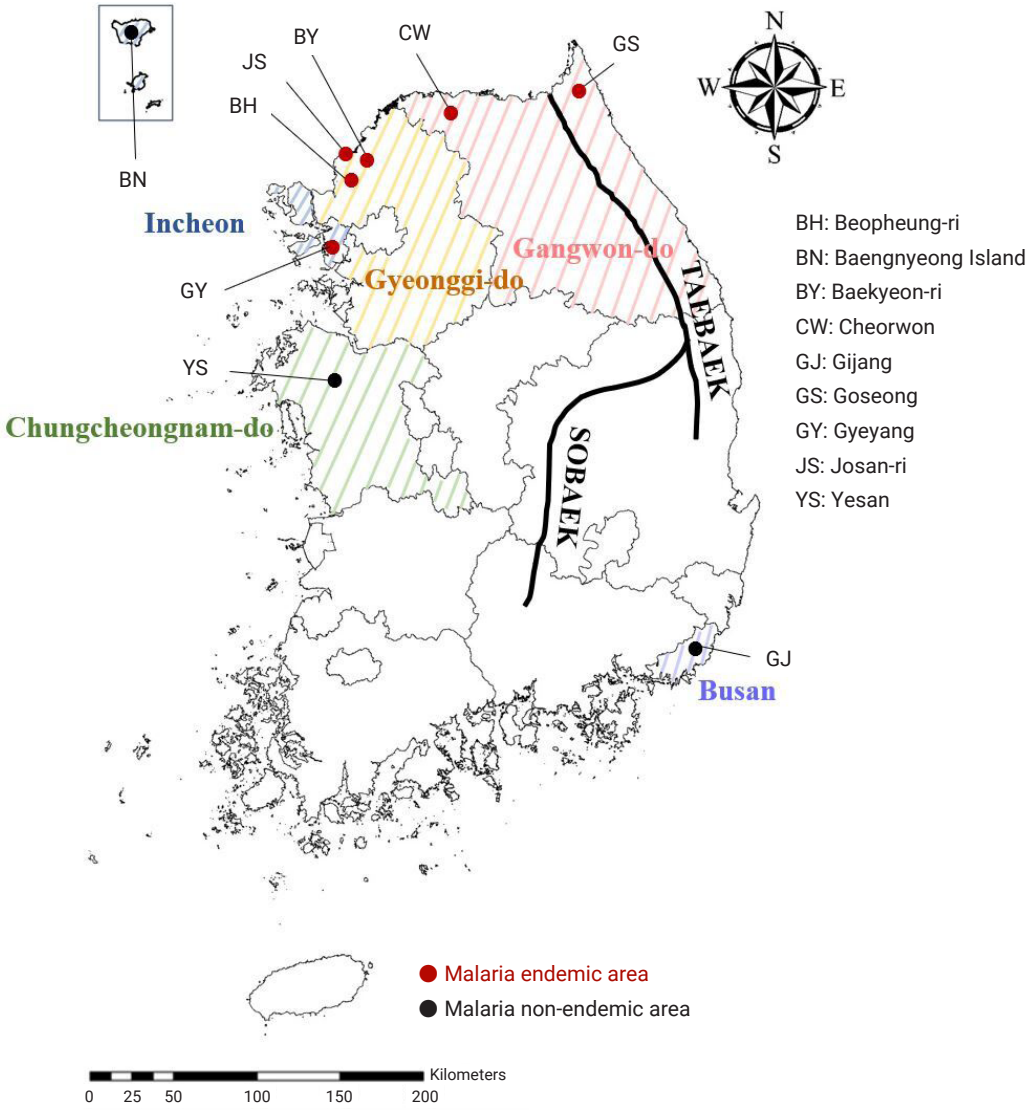


Figure 1. *Anopheles sinensis* sampling sites on the map. The site names are abbreviated.

(Qiagen). PCR amplicons were subjected to bidirectional sequencing using Sanger sequencing technology via a commercial sequencing service (BIOFACT).

Data Analyses

Chromatograms of the amplified *COI* sequences were assembled using DNASTAR Lasergene SeqMan Pro, and consensus sequences were aligned using MEGA 11 (MEGA software). Sequence variation metrics—including the number of haplotypes (H), number of segregating sites (*S*), average number of nucleotide differences (*K*), average number of mutations per sequence (θ), haplotype diversity (*Hd*), and nucleotide diversity (*Pi*)—were calculated using DnaSP (version 5.10.01; Universitat de Barcelona) [22]. The haplotype network of *An. sinensis* was generated using the median-joining method in Network 10.2 software (Fluxus Technology Ltd.) [23]. Pairwise genetic distances (F_{ST}) among regions were calculated using DnaSP to examine genetic differentiation, and gene flow (*N*m) was inferred from the pairwise F_{ST} values using the formula $Nm = (1-F_{ST})/4F_{ST}$ [24]. Analysis of molecular variance (AMOVA) was performed using Arlequin (ver. 3.5.2.2; University of Geneva) [25] to quantify genetic variation within and among populations. Finally, neutrality tests were conducted using 4 different methods—Tajima's D, Fu's Fs, Fu and Li's D, and Fu and Li's F—using DnaSP software [22].

Results

Characteristics of Sequences

All 903 mosquito samples were successfully amplified

https://doi.org/10.24171/j.phrp.2025.0052

and sequenced with assembled chromatograms (Table 1). After trimming the consensus sequences, 625 bp of the initial 710 bp was used for analysis. Sequence alignment identified 79 variable and 546 conserved sites. *S* (number of segregating sites) varied from 18 to 49 sites based on the mosquito collection regions. *K* (average number of nucleotide difference) was 4.35360 ± 0.63288 and *θ* (average number of mutations per sequence) was 7.38100 ± 1.29023. *Hd* (haplotype diversity) was high (0.82159 ± 0.08791), whereas *Pi* (nucleotide diversity) was low (0.00697 ± 0.00101) (Table 2).

COI Haplotype Network

A total of 124 haplotypes were identified from the 903 analyzed *An. sinensis* sequences. The proportion of haplotype confirmed for the population by region was higher in the endemic regions of Goseong (14/22, 63.6%) and Josan-ri (13/21, 61.9%) compared to other regions (Table 2). Across all 9 collection regions, haplotype 10 (38.6%, 349/903) was most frequently observed, followed by haplotype 20 (6.6%, 60/903), being dominant and subdominant haplotypes, respectively. In total, 24 haplotypes were identified across multiple regions, while 75 haplotypes were represented by only a single individual each. The most dominant haplotype (haplotype 10), representing approximately one-third of the total population, was first detected in June in southern and central regions and became increasingly prevalent over time in northern regions. Similarly, haplotype 20 was initially identified in the southern and central regions in June and subsequently found in northern regions. Haplotype patterns found across multiple regions typically emerged first in the southern and central regions before July (summer) and were later confirmed in northern areas from summer onward (Figure 2). Haplotypes identified from single individuals occurred sporadically, predominantly after summer.

The *COI* haplotype network of *An. sinensis* was separated into 2 distinct clusters. Cluster I included mosquitoes collected from all 9 sampling locations and showed relationships to 7 Chinese reference sequences. In particular, haplotypes 6, 20, 34, and 38 shared identical *COI* sequences with mosquitoes from Jiangsu, Anhui, and Chongqing, China. Conversely, cluster II included mosquitoes from 8 locations (excluding Josan-ri) and was closely related to 1 Japanese reference sequence (Figure 3).

Table 1. Information on the collection sites and collected sample number by month and sites

Location ID	Coordinates	No. of collected samples					Total
		June	July	August	September	October	
BH	37°47'01.2"N 126°42'27.3"E	0	11	71	85	2	169
BN	37°57'28.9"N 124°39'52.5"E	0	96	96	0	0	192
BY	37°55'08.0"N 126°44'03.3"E	0	13	53	23	1	90
CW	38°15'49.0"N 127°09'51.7"E	1	8	81	51	0	141
GJ	35°11'55.7"N 129°12'09.1"E	20	21	23	24	0	88
GS	38°32'43.7"N 128°23'59.4"E	0	1	20	1	0	22
GY	37°34'49.0"N 126°44'51.6"E	0	11	17	52	0	80
JS	37°54'37.3"N 126°43'53.0"E	0	3	6	2	10	21
YS	36°40'27.5"N 126°41'39.4"E	44	52	4	0	0	100

BH, Beopheung-ri; BN, Baengnyeong Island; BY, Baekyeon-ri; CW, Cheorwon; GJ, Gijang; GS, Goseong; GY, Gyeyang; JS, Josan-ri; YS, Yesan.

Table 2. Summary data for sequence characteristics of *Anopheles sinensis*

Location ID	*n*	H	*S*	*K*	*θ*	*Hd*	*Pi*
BH	169	39	43	4.18618	8.06430	0.81298	0.00670
BN	192	47	49	4.97044	9.08763	0.86971	0.00795
BY	90	24	34	3.60175	6.70418	0.72659	0.00576
CW	141	30	34	4.32006	6.33780	0.77285	0.00691
GJ	88	38	41	4.83490	8.71484	0.92659	0.00774
GS	22	14	28	5.36364	7.68100	0.90043	0.00858
GY	80	24	37	4.51867	8.07595	0.68006	0.00723
JS	21	13	18	3.49524	5.00314	0.91905	0.00559
YS	100	29	33	3.89152	6.76018	0.78606	0.00623

n, sample size; H, no. of haplotypes; *S*, no. of segregating sites; *K*, average no. of nucleotide difference; *θ*, average no. of mutations per sequence; *Hd*, haplotype diversity; *Pi*, nucleotide diversity; BH, Beopheung-ri; BN, Baengnyeong Island; BY, Baekyeon-ri; CW, Cheorwon; GJ, Gijang; GS, Goseong; GY, Gyeyang; JS, Josan-ri; YS, Yesan.

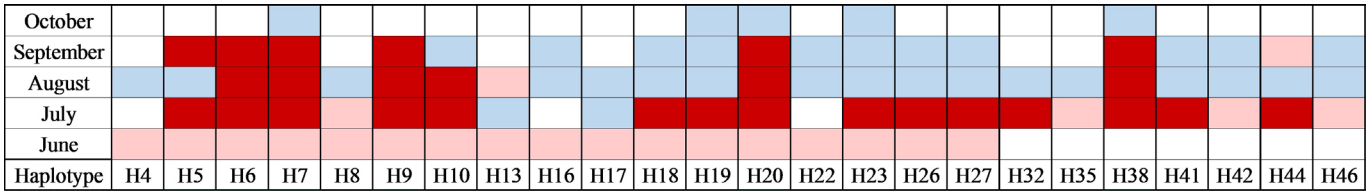


Figure 2. Monthly occurrence of haplotypes simultaneously identified in multiple regions. Pink indicates southern and central regions (Gijang and Yesan), blue indicates northern regions (Beopheung-ri, Baengnyeong Island, Baekyeon-ri, Cheorwon, Goseong, Gyeyang, and Josan-ri), and red indicates both northern, central, and southern regions.

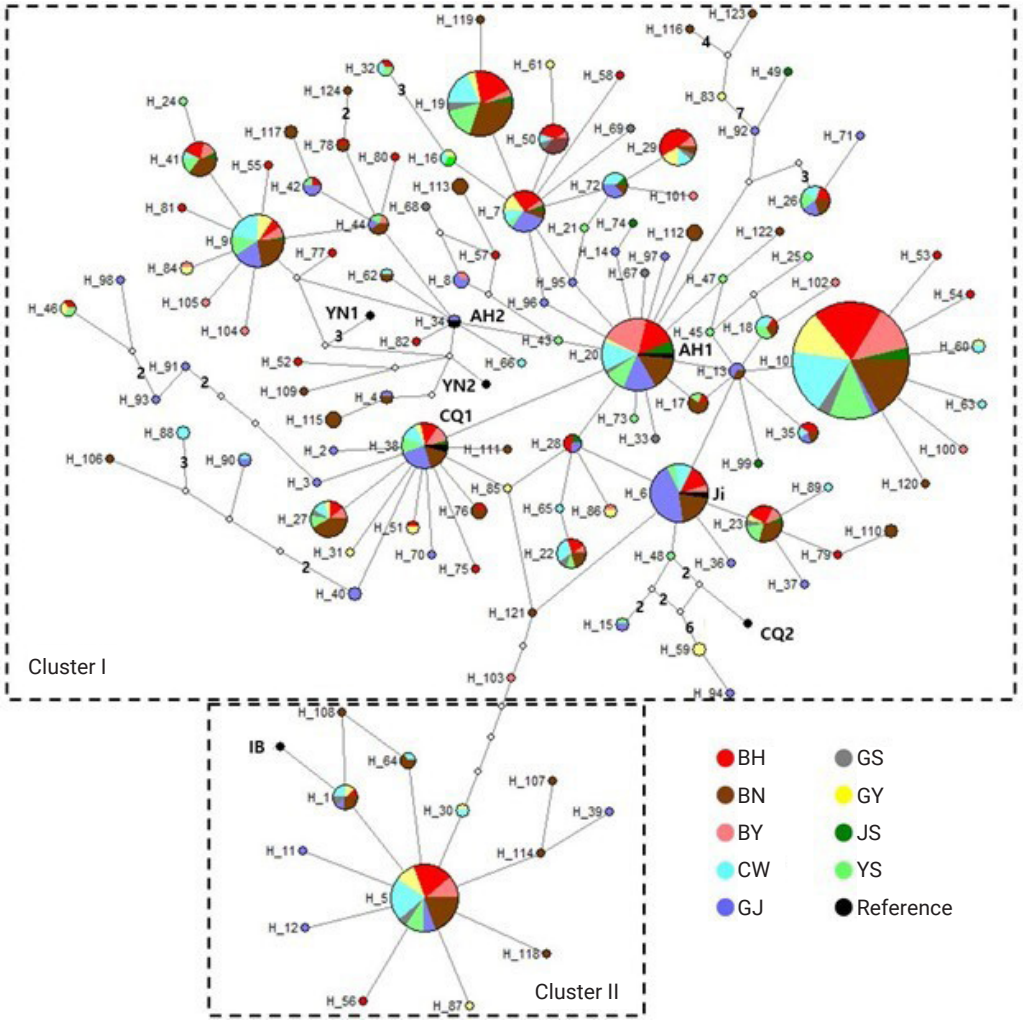


Figure 3. Haplotype network based on the cytochrome c oxidase subunit I (*COI*) gene as calculated by Network 10.2. Each color circle represents individuals from Beopheung-ri (BH), Baengnyeong island (BN), Baekyeon-ri (BY), Cheorwon (CW), Gijang (GJ), Gyeyang (GY), Josan-ri (JS), and Yesan (YS). The black circle symbolizes reference sequence: Anhui (AH1: MG816536, China; AH2: MG816544, China), Chongqing (CQ1: MG816556, China; CQ2: MG816550, China), Ibaraki (IB: LC054430, Japan), Jiangsu (Ji: MF322628, China), and Yunnan (YN1: MG816568, China; YN2: MG816562, China). White dots are a median vector (undetected hypothetical haplotypes). The size of the circle is proportional to the number of individuals.

Population Genetic Structure

Pairwise F_{ST} was used to calculate the genetic distance between populations in the regions where mosquitoes were collected. According to the classification of He et al. [26], F_{ST} values between regions were categorized into small genetic differentiation (0–0.05) and moderate genetic differentiation

https://doi.org/10.24171/j.phrp.2025.0052

(0.05–0.15). Small genetic differentiation predominated, indicating that the genetic distance between the regions was not large. Gene flow was calculated based on F_{ST}; however, it could not be computed when F_{ST} values were negative (Table 3). When comparing F_{ST} values to straight-line geographic distances between regions, a general trend of increasing F_{ST} values with increasing geographic distance was observed, although exceptions occurred (Table 4).

AMOVA was conducted by dividing the populations into 3 groups based on geographic barriers: east (Gijang and Goseong) and west (Beopheung-ri, Baekyeon-ri, Cheorwon, Gyeyang, Josan-ri, and Yesan) range of the mountain, and island (Baengnyeong). The AMOVA results indicated that genetic variation within populations was substantially greater than the variation among groups or among populations within groups (Table 5). This highlights that individual mosquito within populations significantly influenced the total genetic variation, more than among groups and among populations within groups.

Neutrality Test

Four neutrality tests—Tajima's D, Fu's Fs, Fu and Li's D, and Fu and Li's F—were performed using DnaSP, and produced all negative values (Table 6). These results indicate that the *An. sinensis* populations are undergoing population with expansion, characterized by an excess of low-frequency mutations. Notably, the Beopheung-ri region showed strong negative values ($p < 0.05$) for Fu and Li's D and Fu and Li's F tests, suggesting heightened sensitivity to neutrality deviations compared to other regions.

Discussion

Understanding the population genetic structure, migration patterns, and gene flow of vectors provides crucial information for effectively controlling vector-borne diseases [27]. The present study assessed the genetic structure of *An. sinensis* populations in the ROK using the *COI* gene, a representative marker in population genetics studies [28].

An. sinensis is widely distributed across most regions of the ROK [18,29] and recognized as the primary malaria

Table 3. F_{ST} (pairwise genetic distance) and *Nm* (gene flow) values among populations

F_{ST}	Nm								
	BH	BN	BY	CW	GJ	GS	GY	JS	YS
BH		63.5255	117.6745	−5,000.2500	4.0685	−26.1032	43.5328	14.5429	−115.4574
BN	0.0039		26.4309	51.8333	7.2598	−48.5125	16.8968	13.8028	92.0009
BY	0.0021	0.0094		−833.5833	2.7675	23.5369	−111.3611	5.5090	−76.9371
CW	−0.0001	0.0048	−0.0003		3.4039	−52.5513	−80.6359	5.4526	567.9318
GJ	0.0579	0.0333	0.0829	0.0684		10.3793	2.6712	7.3465	3.5581
GS	−0.0097	−0.0052	0.0105	−0.0048	0.0235		44.7950	861.8190	57.3537
GY	0.0057	0.0146	−0.0023	−0.0031	0.0856	0.0056		3.6963	36.8420
JS	0.0169	0.0178	0.0434	0.0438	0.0329	0.0003	0.0634		11.7577
YS	−0.0022	0.0027	−0.0033	0.0004	0.0657	0.0043	0.0067	0.0208	

BH, Beopheung-ri; BN, Baengnyeong Island; BY, Baekyeon-ri; CW, Cheorwon; GJ, Gijang; GS, Goseong; GY, Gyeyang; JS, Josan-ri; YS, Yesan.

Table 4. F_{ST} (pairwise genetic distance) and straight-line distance (km) between collection sites

F_{ST}	Distance								
	BH	BN	BY	CW	GJ	GS	GY	JS	YS
BH		181.33	15.23	66.79	364.04	170.93	23.07	14.29	123.29
BN	0.0039		181.91	221.23	507.23	332.03	187.74	181.71	228.91
BY	0.0021	0.0094		53.71	374.31	161.43	37.63	0.98	138.04
CW	−0.0001	0.0048	−0.0003		386.00	112.01	84.04	54.61	180.96
GJ	0.0579	0.0333	0.0829	0.0684		378.80	344.28	373.03	279.04
GS	−0.0097	−0.0052	0.0105	−0.0048	0.0235		179.95	162.02	257.72
GY	0.0057	0.0146	−0.0023	−0.0031	0.0856	0.0056		36.73	100.80
JS	0.0169	0.0178	0.0434	0.0438	0.0329	0.0003	0.0634		137.32
YS	−0.0022	0.0027	−0.0033	0.0004	0.0657	0.0043	0.0067	0.0208	

BH, Beopheung-ri; BN, Baengnyeong Island; BY, Baekyeon-ri; CW, Cheorwon; GJ, Gijang; GS, Goseong; GY, Gyeyang; JS, Josan-ri; YS, Yesan.

Table 5. Analysis of molecular variance results for *Anopheles sinensis*

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variance (%)	*F*-index	*p*
Among groups	2	27.930	0.04714	2.10	0.02094	0.11241 ± 0.00945
Among populations within groups	6	17.926	0.00926	0.41	0.00420	0.06940 ± 0.00785
Within populations	894	1,961.704	2.19430	97.49	0.02506	0.0000 ± 0.0000
Total	902	2,007.560	2.25070	100		

Table 6. Neutrality test for *Anopheles sinensis*

Location ID	Neutrality tests			
	Tajima's *D*	Fu's *Fs*	Fu and Li's D	Fu and Li's F
BH	−1.44182	−19.13100	−2.46241*	−2.43457*
BN	−1.35769	−24.04200	−1.25227	−1.56128
BY	−1.44273	−8.64200	−1.52977	−1.79123
CW	−0.94790	−9.51100	−0.52387	−0.83794
GJ	−1.42160	−23.13900	−2.17915	−2.25224
GS	−1.15273	−3.90100	−1.43444	−1.57528
GY	−1.42160	−23.13900	−2.17915	−2.25224
JS	−1.12075	−5.40000	−0.81795	−1.05626
YS	−1.31062	−12.68300	−1.82358	−1.94258

BH, Beopheung-ri; BN, Baengnyeong Island; BY, Baekyeon-ri; CW, Cheorwon; GJ, Gijang; GS, Goseong; GY, Gyeyang; JS, Josan-ri; YS, Yesan.
*$p < 0.05$.

vector in the region, as evidenced by its confirmed sporozoite infection capability in artificial infection experiments [10]. In this study, 903 specimens of *An. sinensis* collected from 9 different regions in the ROK were analyzed for population genetic structure using the *COI* marker. Our results indicate that the *An. sinensis* populations exhibit characteristics of migrant populations, characterized by high *Hd* and low *Pi*. In particular, Josan-ri, located near the DPRK border, likely represents a strong migrant population due to its notably high *Hd* and low *Pi* values (Table 2).

Haplotypes were initially identified in southern and central regions and subsequently appeared in northern regions over time (Figure 2). Although the number of collected mosquitoes is different by month, all collected individuals were analyzed without selective bias. *An. sinensis* is known to be cold-intolerant in the ROK [30], and adult mosquitoes in the field start emerging at a minimum temperature of approximately 15.3 °C [31]. In June, collection site temperatures were 18.6 °C in Gijang and 19.86 °C in Yesan, approximately 0.5–3.4 °C warmer than in northern regions. While various factors, including habitat and nutritional availability, influence adult mosquito emergence, temperature likely has a significant impact. Hence, earlier detection of haplotypes in southern regions is presumably due to warmer conditions. Additionally, by tracking the months in which haplotypes first appeared, we observed increases in mosquito numbers and haplotype diversity during the warmer summer months (July and August; Table 1). Given that developmental cycles of *An. sinensis* stimulate with rising temperatures [32], mutations may also occur more frequently during the summer due to accelerated mosquito growth cycles.

Network analysis of *An. sinensis* revealed 2 distinct clusters (Figure 3). Cluster I contained specimens from all 9 sampling regions and showed close relationships with 7 reference sequences from China. Specifically, haplotypes 20, 34, and 38 had identical *COI* sequences to those from Anhui and Chongqing provinces in China. Previous studies have documented insect pests, such as *Nilaparvata lugens*, *Sogatella furcifera*, and *Laodelphax striatellus*, introduced from China to the ROK via jet streams [33]; mosquitoes may have similarly migrated. Cluster II, lacking haplotypes from Josan-ri (the northernmost site), was related to a Japanese reference sequence and comprised diverse haplotypes from Gijang. This strong relationship likely arises from Gijang's geographical proximity to Japan. However, a limitation of this study is the inclusion of only 1 southern sampling site (Gijang); thus, further studies from additional southern locations are necessary to confirm this connection. Both clusters exhibited associations with international sequences, likely reflecting the geographic position of the ROK between China and Japan.

F_{ST} was used to assess regional population differentiation.

https://doi.org/10.24171/j.phrp.2025.0052

According to the classification by He et al. [26] in 2022, F_{ST} values indicate small (0–0.05), moderate (0.05–0.15), large (0.15–0.25), or very large (≥0.25) genetic differentiation. In our analysis, small genetic differentiation was predominant among the *An. sinensis* populations in the ROK (Table 3). Given the broad distribution of *An. sinensis* across the ROK, we analyzed F_{ST} values relative to straight-line geographic distances between sampling regions (Table 4). Among the sampled regions, Gijang exhibited moderate genetic differentiation, likely due to its significant distance from other regions. Interestingly, Josan-ri showed genetic differentiation from all other regions, including Baekyeon-ri, located less than 1 km away. This distinct differentiation suggests unique regional characteristics, identifying Josan-ri mosquitoes as a strong migrant group. Baengnyeong, an island in the northwest ROK, was anticipated to exhibit high genetic differentiation due to its isolation. However, the results differed from expectations, possibly due to inland mosquito movement influenced by prevailing westerly winds

AMOVA results revealed that genetic variation among regions was predominantly driven by individual mosquitoes within populations. Despite group divisions based on geographic barriers, these barriers did not significantly influence genetic differentiation. Neutrality tests (Tajima's D, Fu's Fs, Fu and Li's D, and Fu and Li's F) consistently yielded negative values, indicating that *An. sinensis* populations in the ROK are undergoing population expansion following a recent bottleneck or selection event. Such demographic expansions typically arise through haplotype mutations, but sufficient time may not have elapsed for extensive sequence differences to accumulate [28].

Previous studies examined the population genetic structure of *An. sinensis* in the ROK using the mitochondrial control region marker, reporting southern and northern genetic groups separated by the Sobaek and Taebaek mountain ranges [17,18]. Due to differences in genetic markers, direct comparisons are difficult; however, the *COI* marker employed in our study likely provided greater resolution, successfully identifying genetic differentiation even among regions previously classified into the same northern group. One limitation of our study was the occurrence of negative F_{ST} values. To overcome this, future studies may consider incorporating additional markers, such as alternative mitochondrial regions or microsatellite loci.

Mosquito movement and migration are challenging to predict, as they are influenced by geographical barriers, environmental factors, and human activities [34]. The present study utilized *COI* markers to elucidate the genetic population structure of *An. sinensis* in the ROK, confirming significant regional genetic differences. Such molecular biological analyses provide foundational data on malaria vectors, enhancing our understanding of malaria epidemiology and control strategies.

Notes

Ethics Approval
Not applicable.

Conflicts of Interest
The authors have no conflicts of interest to declare.

Funding
This research was funded by the Korea Disease Control and Prevention Agency (Grant No. 6300-6332-305).

Availability of Data
All data generated or analyzed during this study are included in this published article. For other data, there may be requested through the corresponding author.

Authors' Contributions
Conceptualization: HIS, HIL; Data curation: HJ; Formal analysis: HJ; Investigation: BGH; Methodology: HIS; Project administration: HIS, MRL, JWJ, HIL; Visualization: HJ; Writing–original draft: HJ; Writing–review & editing: all authors. All authors read and approved the final manuscript.

Additional Contributions
We are thankful to the Gangwon State Institute of Health and Environment, Gyeonggi Province Institute of Health and Environment, Incheon Metropolitan City Institute of Health and Environment, Chungcheongnam-do Institute of Health and Environment, and Busan Metropolitan City Institute of Health and Environment for their assistance in collecting mosquito samples.

References

1. Garrido-Cardenas JA, Gonzalez-Ceron L, Manzano-Agugliaro F, et al. Plasmodium genomics: an approach for learning about and ending human malaria. Parasitol Res 2019;118:1–27.
2. Milner DA. Malaria pathogenesis. Cold Spring Harb Perspect Med 2018;8:a025569.
3. World Health Organization (WHO) (CH). World malaria report 2023 [Internet]. WHO; 2023 [cited 2025 Feb 2]. Available from: https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2023.
4. Poespoprodjo JR, Douglas NM, Ansong D, et al. Malaria. Lancet 2023;402:2328–45.
5. Seong H, Suh J, Choi JY, et al. Development of a Plasmodium vivax malaria model for evaluating the effects of control strategies on the malaria burden in democratic people's Republic of Korea. Front Public Health 2024;12:1423004.
6. Besansky NJ, Lehmann T, Fahey GT, et al. Patterns of mitochondrial variation within and between African malaria vectors, Anopheles gambiae and An. arabiensis, suggest extensive gene flow. Genetics 1997;147:1817–28.
7. Sinka ME, Bangs MJ, Manguin S, et al. A global map of dominant

malaria vectors. Parasit Vectors 2012;5:69.
8. Rueda LM, Brown TL, Kim HC, et al. Species composition, larval habitats, seasonal occurrence and distribution of potential malaria vectors and associated species of Anopheles (Diptera: Culicidae) from the Republic of Korea. Malar J 2010;9:55.
9. Foley DH, Klein TA, Kim HC, et al. Geographic distribution and ecology of potential malaria vectors in the Republic of Korea. J Med Entomol 2009;46:680–92.
10. Lee HW, Cho SH, Shin EH, et al. Experimental infection of Anopheles sinensis with Korean isolates of Plasmodium vivax. Korean J Parasitol 2001;39:177–83.
11. Bunmee K, Thaenkham U, Saralamba N, et al. Population genetic structure of the malaria vector Anopheles minimus in Thailand based on mitochondrial DNA markers. Parasit Vectors 2021;14:496.
12. Joshi BD, Johnson JA, Negi T, et al. Understanding genetic diversity and population genetic structure of three Cyprinidae fishes occupying the same habitat from Uttarakhand, India. Mitochondrial DNA B Resour 2019;4:2956–61.
13. King'ori EM, Obanda V, Nyamota R, et al. Population genetic structure of the elephant tick Amblyomma tholloni from different elephant populations in Kenya. Ticks Tick Borne Dis 2022;13:101935.
14. Nakamura Y, Yamagishi J, Hayashida K, et al. Genetic diversity and population structure of Glossina morsitans morsitans in the active foci of human African trypanosomiasis in Zambia and Malawi. PLoS Negl Trop Dis 2019;13:e0007568.
15. Zhang L, Sechi P, Xie J, et al. Genetic diversity and population structure of the endangered salamander hynobius yiwuensis inferred from mitochondrial DNA Sequences. Life (Basel) 2024;14:769.
16. Feng X, Huang L, Lin L, et al. Genetic diversity and population structure of the primary malaria vector Anopheles sinensis (Diptera: Culicidae) in China inferred by cox1 gene. Parasit Vectors 2017;10:75.
17. Jung J, Jung Y, Min GS, et al. Analysis of the population genetic structure of the malaria vector Anopheles sinensis in South Korea based on mitochondrial sequences. Am J Trop Med Hyg 2007;77:310–5.
18. Kang S, Jung J, Kim W. Population genetic structure of the malaria vector anopheles sinensis (Diptera: Culicidae) sensu stricto and evidence for possible introgression in the Republic of Korea. J Med Entomol 2015;52:1270–81.
19. Multiplex PCR assay for the identification of eight Anopheles species belonging to the Hyrcanus, Barbirostris and Lindesayi groups. Malar J 2021;20:287.
20. Joshi D, Park MH, Saeung A, et al. Multiplex assay to identify Korean vectors of malaria. Mol Ecol Resour 2010;10:748–50.
21. Folmer O, Black M, Hoeh W, et al. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 1994;3:294–9.
22. Librado P, Rozas J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 2009;25:1451–2.
23. Bandelt HJ, Forster P, Rohl A. Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 1999;16:37–48.
24. Slatkin M. Gene flow and the geographic structure of natural populations. Science 1987;236:787–92.
25. Excoffier L, Lischer HE. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 2010;10:564–7.
26. He ZQ, Zhang QQ, Wang D, et al. Genetic polymorphism of circumsporozoite protein of Plasmodium falciparum among Chinese migrant workers returning from Africa to Henan Province. Malar J 2022;21:248.
27. Zhang HD, Gao J, Li CX, et al. Genetic diversity and population genetic structure of Aedes albopictus in the Yangtze River Basin, China. Genes (Basel) 2022;13:1950.
28. Chaiphongpachara T, Laojun S, Changbunjong T, et al. Genetic diversity, haplotype relationships, and kdr mutation of Malaria Anopheles Vectors in the most plasmodium knowlesi-Endemic Area of Thailand. Trop Med Infect Dis 2022;7:412.
29. Lee JH, Kim HW, Mustafa B, et al. The relationships between microbiome diversity and epidemiology in domestic species of malaria-mediated mosquitoes of Korea. Sci Rep 2023;13:9081.
30. Lee J, Lee IY, Yong TS, et al. Preliminary observation on the gradient distribution of hibernating females of Anopheles sinensis and Anopheles pullus in the Republic of Korea. J Am Mosq Control Assoc 2008;24:121–2.
31. Mogi M, Okazawa T. Development of Anopheles sinensis immatures (Diptera: Culicidae) in the field: effects of temperature and nutrition. Med Entomol Zool 1996;47:355–62.
32. Feng X, Zhang S, Huang F, et al. Biology, bionomics and molecular biology of Anopheles sinensis Wiedemann 1828 (Diptera: Culicidae), main Malaria vector in China. Front Microbiol 2017;8:1473.
33. Park T, Choe H, Jeong H, et al. Spatial pattern analysis for distribution of migratory insect pests at paddy field in Jeolla-province. Korean J Appl Entomol 2018;57:361–72. Korean.
34. Md Naim D, Kamal NZ, Mahboob S. Population structure and genetic diversity of Aedes aegypti and Aedes albopictus in Penang as revealed by mitochondrial DNA cytochrome oxidase I. Saudi J Biol Sci 2020;27:953–67.

https://doi.org/10.24171/j.phrp.2025.0052

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2025.0113
Osong Public Health Res Perspect 2025;16(4):357-372

Original Article

Assessment of healthcare workers' knowledge and attitudes toward mpox and acceptance of its vaccine: a health belief model-based analysis in the Gulf region

Mona Gamal Mohamed[1,2], Eman Abdelaziz Ahmed Dabou[3,4], Fatma M. Ibrahim[5,6], Shaimaa Abdelsamad[7], Jibin Kunjavara[8], Kamaruddeen Mannethodi[8], Moayad Ahmad Wishah[9], Abdullah Y. Shihab[10], Khaled Mohammed Al-Sayaghi[11], Hammad Ali Fadlalmola[12], Mohammed Musaed Al-Jabri[13]

[1]Adult Health Nursing, RAK College of Nursing, RAK Medical and Health Sciences University, Ras Al Khaimah, United Arab Emirates
[2]Medical Surgical Nursing Department, Faculty of Nursing Sohag University, Sohag, Egypt
[3]Adult Health Nursing, RAK College of Nursing, RAK Medical and Health Sciences University, Ras Al Khaimah, United Arab Emirates
[4]Medical Surgical Nursing Department, Faculty of Nursing Alexandria University, Alexandria, Egypt
[5]RAK College of Nursing, RAK Medical and Health Sciences University, Ras Al Khaimah, United Arab Emirates
[6]Faculty of Nursing, Mansoura University, Mansoura, Egypt
[7]RAK College of Nursing, RAK Medical and Health Sciences University, Ras Al Khaimah, United Arab Emirates
[8]Nursing and Midwifery Research Department, Hamad Medical Corporation, Doha, Qatar
[9]Clinical Resources, Emirates Health Services, Ras Al Khaimah, United Arab Emirates
[10]Department of Community Health Nursing, College of Nursing, University of Baghdad, Baghdad, Iraq
[11]Department of Medical-Surgical Nursing, College of Nursing, Taibah University, Al-Madinah Al-Munawwarah, Saudi Arabia
[12]Department of Community Health Nursing, College of Nursing, Taibah University, Al-Madinah Al-Munawwarah, Saudi Arabia
[13]Nursing Department, College of Applied Medical Sciences, Prince Sattam Bin Abdulaziz University, Wadi Addawasir, Saudi Arabia

Received: April 13, 2025
Revised: April 22, 2025
Accepted: May 19, 2025

Corresponding author:
Mona Gamal Mohamed
Adult Health Nursing, RAK College of Nursing, RAK Medical and Health Sciences University, Ras Al Khaimah 11172, United Arab Emirates
E-mail: mona.mohamed2100@yahoo.com

ABSTRACT

Objectives: This study aimed to (1) assess healthcare workers' (HCWs) knowledge and attitudes toward mpox across 3 Gulf countries, and (2) examine factors influencing their acceptance of the mpox vaccine using the health belief model (HBM). This model evaluated perceptions related to susceptibility, severity, benefits, barriers, cues to action, and self-efficacy. Additionally, the study explored the impact of prior coronavirus disease 2019 (COVID-19) vaccination history on mpox vaccine acceptance.

Methods: A cross-sectional study was conducted among 764 HCWs from the United Arab Emirates (UAE), Saudi Arabia, and Qatar. Data were collected through a structured online questionnaire based on HBM constructs. Logistic regression and structural equation modeling were employed to analyze predictors of vaccine acceptance.

Results: Knowledge levels varied across the countries, with the highest proportion of good knowledge reported in the UAE (59%). UAE HCWs also demonstrated higher perceived

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Korea Disease Control and Prevention Agency and Korea National Institute of Health.

© 2025 Korea Disease Control and Prevention Agency.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

susceptibility, perceived benefits, and intention to vaccinate compared to those in Saudi Arabia and Qatar ($p < 0.001$). Receiving 4 doses of the COVID-19 vaccine was associated with greater acceptance of the mpox vaccine. Among the HBM constructs, perceived benefits and self-efficacy were identified as the strongest predictors of vaccine acceptance. Perceived barriers, such as fear of side effects and concerns about vaccine safety, were more prominent among HCWs in Saudi Arabia and Qatar.

Conclusion: The HBM effectively explains the factors influencing mpox vaccine acceptance among Gulf HCWs. Targeted interventions that improve perceived benefits, reduce barriers, and improve self-efficacy may facilitate greater vaccine uptake and preparedness for emerging infectious diseases.

Keywords: Attitude; Gulf region; Health belief model; Healthcare workers; Knowledge; Monkeypox vaccine; Vaccine acceptance

Introduction

Healthcare workers (HCWs) play a crucial role in combating infectious disease outbreaks [1], serving as frontline defenders, educators, and influencers of public health behavior [2,3]. Their acceptance of vaccines directly influences workplace safety, patient outcomes, and community trust [4]. During the coronavirus disease 2019 (COVID-19) pandemic, HCWs' vaccine hesitancy—driven by misinformation, safety concerns, and distrust—highlighted the necessity of addressing behavioral determinants of vaccination [5]. With the global mpox outbreak in 2022, understanding HCWs' vaccination intentions has become urgent once again [6], particularly in high-risk regions such as the Gulf Cooperation Council (GCC), where HCWs frequently interact with transient populations and international travelers [7].

A viral zoonosis endemic to Central and West Africa, mpox re-emerged in 2022 as a multi-country outbreak, with over 87,000 cases reported globally [8]. Unlike previous outbreaks, this epidemic was characterized by sustained human-to-human transmission through close contact, sexual networks, and fomites [9]. Symptoms, including painful rashes and systemic complications [10], alongside stigma—especially among men who have sex with men (MSM)—have complicated containment efforts [11]. Although smallpox vaccines (e.g., JYNNEOS) provide protection, global disparities in vaccine access and hesitancy have impeded uptake [12]. In the Gulf, where mass religious gatherings (e.g., Hajj) and tourism hubs elevate outbreak risks, HCWs' preparedness to manage mpox is critical [13–15].

The socio-demographic landscape of the GCC region, characterized by dense expatriate labor populations, hyper-urbanization, and global connectivity, heightens susceptibility to imported and locally transmitted pathogens [16]. For instance, Saudi Arabia hosts 2 to 3 million pilgrims annually during Hajj [17], while multicultural cities in the United Arab Emirates (UAE) attract millions of tourists [18]. These dynamics, combined with limited public awareness of zoonotic diseases and cultural sensitivities [19] regarding stigmatized transmission routes (e.g., sexual contact), create unique challenges for HCWs [20,21]. Misconceptions about mpox transmission and prevention could increase occupational exposure risks and delay outbreak responses [22,23].

Vaccine hesitancy in the Gulf region is influenced by cultural, religious, and systemic factors [24]. During the COVID-19 pandemic, concerns regarding mRNA technology's compatibility with Islamic principles, distrust of Western pharmaceuticals, and fears related to vaccine side effects reduced vaccine uptake among HCWs [25]. For mpox, hesitancy might be further compounded by stigma toward

HIGHLIGHTS

- This study is the first to apply the health belief model to assess mpox vaccine acceptance among healthcare workers in the Gulf region.
- The findings emphasize the critical roles of perceived susceptibility, benefits, and self-efficacy in predicting vaccine acceptance.
- The study provides evidence-based strategies for policymakers to design culturally tailored educational interventions to reduce vaccine hesitancy and enhance public health responses to emerging infectious diseases.
- Addressing perceived barriers, including fear of side effects and social stigma, is essential for increasing vaccine uptake.

https://doi.org/10.24171/j.phrp.2025.0113

MSM, a key affected population in conservative Gulf societies, where open discussions about sexuality are limited [26]. Additionally, hierarchical workplace cultures may discourage HCWs from voicing concerns or seeking clarification regarding vaccination policies [27].

HCWs' ability to advocate for vaccination is dependent on their knowledge of disease transmission, prevention, and treatment. Studies have identified gaps in HCWs' understanding of mpox, including confusion with other poxviruses and underestimation of non-sexual transmission routes [28,29]. In the Gulf region, linguistic diversity and reliance on expatriate HCWs from varied educational backgrounds could further fragment knowledge dissemination [30]. Misinformation propagated via social media, such as myths about vaccine-induced infertility, might deepen skepticism, underscoring the need for targeted training [31].

The health belief model (HBM), a psychological theory developed by Rosenstock [32], posits that health behaviors are influenced by 6 constructs: perceived susceptibility, severity, benefits, barriers, cues to action, and self-efficacy [33]. It has been widely applied to predict vaccine uptake, including influenza and human papilloma virus vaccinations [34]. For instance, during the COVID-19 pandemic, HCWs' perceived susceptibility to infection and trust in institutional guidance were critical predictors of vaccine acceptance [3]. However, the model's utility in explaining mpox vaccination behavior, particularly in contexts where cultural norms influence risk perception, remains untested.

Objectives

This study aimed to achieve 2 main objectives. First, it assessed HCWs knowledge and attitudes toward mpox across 3 Gulf countries. Second, it examined factors influencing mpox vaccine acceptance using the HBM. Specifically, the study evaluated HCWs' perceptions of susceptibility, severity, benefits, barriers, cues to action, and self-efficacy, and how these constructs predicted vaccine acceptance. It also explored the relationship between prior COVID-19 vaccination history and attitudes toward mpox vaccination.

Materials and Methods

Study Design and Setting

This was a multicenter, quantitative, cross-sectional study designed to assess HCWs knowledge, attitudes, and perceptions regarding mpox, as well as their intention to receive the mpox vaccine. The study was conducted across public and private hospitals in 3 Gulf countries: the UAE, the Kingdom of Saudi Arabia, and the State of Qatar. These countries were selected due to their leading roles in regional healthcare infrastructure, active public health surveillance systems, and diverse, multinational healthcare workforces, making them ideal for examining cross-country variations in vaccine perceptions and health beliefs. Data collection occurred from April 1 to October 30, 2024, through an online survey platform (Figure 1).

Participants and Sample Size

Eligible participants included HCWs (physicians, nurses, and allied health professionals) with at least 1 year of clinical experience at healthcare facilities in the Gulf region. HCWs who were in the process of resigning or unwilling to participate were excluded. A non-probability convenience sampling method was used due to logistical feasibility and the multicenter nature of the study.

The required sample size was calculated using the formula for proportions, assuming a 95% confidence level (Z = 1.96), a margin of error of 5% (e = 0.05), and a conservative prevalence estimate of 50% ($p = 0.5$) for adequate mpox knowledge, due to the absence of prior regional data. This calculation yielded a minimum sample size of 384. To improve statistical power and account for incomplete responses, the target was increased. Ultimately, 764 HCWs participated, including 256 from the UAE, 187 from Saudi Arabia, and 321 from Qatar.

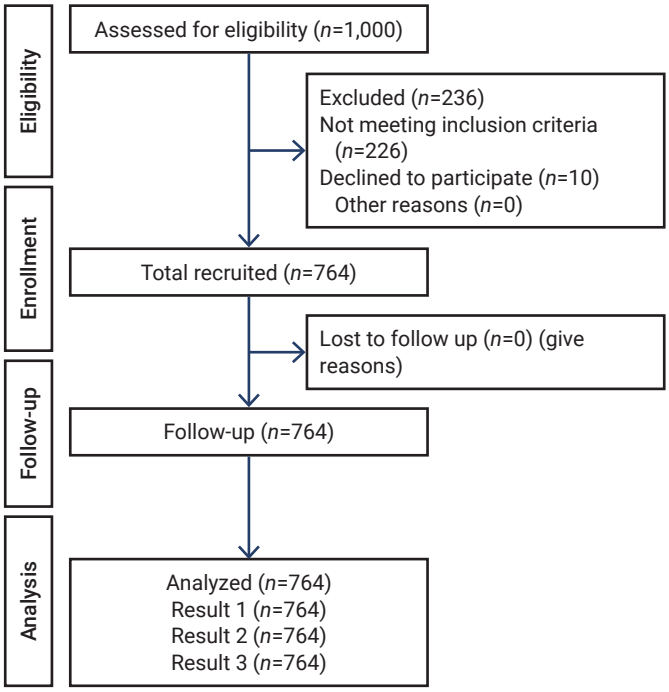


Figure 1. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) flowchart.

Participants and Sample Size

Eligible participants included HCWs (physicians, nurses, and allied health professionals) with at least 1 year of clinical experience at healthcare facilities in the Gulf region. HCWs who were in the process of resigning or unwilling to participate were excluded. A non-probability convenience sampling method was used due to logistical feasibility and the multicenter nature of the study.

The sample size was calculated using the standard formula for estimating proportions in cross-sectional studies:

$$n = \frac{Z^2 \cdot p \cdot (1-p)}{e^2}.$$

Assuming a 95% confidence level (Z=1.96), a margin of error of 5% (e=0.05), and a conservative prevalence estimate of 50% (p=0.5), the minimum required sample size was calculated to be 384. To compensate for an estimated non-response rate of 40% and a dropout rate of 10%, the final target sample size was increased to approximately 770 participants. This allowed for better representation and completeness of the data. Ultimately, 764 HCWs participated in the study: 256 from the UAE, 187 from Saudi Arabia, and 321 from Qatar.

Data Collection and Measures

Data were collected via a structured, self-administered online questionnaire, disseminated through institutional emails and internal communication systems. Department heads facilitated the survey's distribution. Participation was voluntary, and informed consent was implied by completing the survey.

The questionnaire was divided into 3 sections.

Socio-demographic characteristics

This section collected data on participants' age, sex, marital status, education level, country of residence, specialty, type of workplace, years of medical practice, primary sources of mpox information, and COVID-19 vaccination status (number of doses received).

HCWs' knowledge and attitudes about mpox infection

The knowledge assessment included 16 items measuring participants' understanding of mpox transmission, symptoms, and preventive measures. These items were adapted from previously validated tools used in similar studies among HCWs in Iraq, Saudi Arabia, Rwanda, and other international contexts [3,35,36]. Responses were rated on a 3-point Likert scale ("I know," "Uncertain," and "I do not know"), with a Cronbach's alpha value of 0.76, indicating good internal consistency. Participants who correctly answered at least 70% of the knowledge items (i.e., 12 out of 16 questions) were classified as having "good knowledge," while those scoring below this threshold were considered to have "poor knowledge." This criterion defined satisfactory knowledge (Table S1).

The attitude assessment consisted of 5 items rated on a 5-point Likert scale (ranging from "strongly agree" to "strongly disagree"), evaluating participants' perspectives on the seriousness and impact of mpox. These attitude items were adapted from validated instruments and systematic reviews assessing HCWs' attitudes toward mpox [37,38]. Each item was scored from 1 (strongly disagree) to 5 (strongly agree), with higher scores indicating more positive attitudes. The total possible score ranged from 5 to 25. Participants scoring 70% or more of the total score (≥18 out of 25) were classified as having a positive attitude, while those below this threshold were considered to have a negative attitude. This section aimed to assess the depth of knowledge and attitudes among HCWs using standardized, evidence-based constructs (Tables S2, S3).

Mpox vaccine acceptance and intention based on the HBM

This section explored HCWs' willingness to receive the mpox vaccine and recommend it to others, along with concerns about side effects and payment preferences. It incorporated 24 items structured according to the HBM, encompassing 6 dimensions: perceived susceptibility, perceived severity, perceived benefits, perceived barriers, cues to action, and perceived intention to vaccinate. The items were adapted from validated instruments employed in previous HBM-based studies on vaccine hesitancy, particularly related to COVID-19 and mpox [3,39,40]. These references provided a robust theoretical and empirical foundation for measuring behavioral predictors of vaccine acceptance among HCWs.

Bias Mitigation

Efforts to minimize bias included collecting responses anonymously to reduce social desirability bias and employing a standardized online data collection method to ensure consistency across countries. Reminder emails were sent to enhance response rates and minimize non-response bias.

The content validity of the draft self-administered questionnaire was evaluated by an expert committee composed of specialists in public health, infectious diseases, and health psychology. The committee provided feedback and suggested modifications, resulting in the final version of the questionnaire. Construct validity was assessed through confirmatory factor analysis, which indicated a good model fit (Tucker-Lewis index [TLI], 0.904; root mean square error of approximation [RMSEA], 0.046; 95% confidence interval [CI], 0.040–0.051).

Following approval from respective facility authorities,

https://doi.org/10.24171/j.phrp.2025.0113

invitations containing the survey link were disseminated to department heads across participating hospital facilities. The invitations included an information sheet describing the study's objectives. Data collection took place via an online survey platform (Google Forms) between April 1 and October 30, 2024. To maximize participation, 2 follow-up reminder emails were sent. The survey employed implied consent, meaning participation was voluntary, and survey completion indicated participant consent.

Handling of Quantitative Variables

Quantitative variables such as age and years of professional experience were analyzed as continuous variables. When appropriate, categorical groupings (e.g., COVID-19 vaccination doses categorized as 0–1, 2, 3, and 4 doses) were used for subgroup analyses.

Statistical analysis

All statistical analyses were conducted using IBM SPSS ver. 26.0 (IBM Corp.) and jamovi, an R-based open software. The following statistical methods were employed.

Descriptive statistics

Categorical variables (e.g., sex, marital status, education level) were presented using frequencies (*n*) and percentages (%). Ordinal and continuous variables, including attitude, perception, and knowledge scores, were summarized using means and standard deviations.

Normality testing

The Shapiro-Wilk test was used to assess the normality of the distribution of continuous and ordinal variables. A significance level of $p \leq 0.05$ indicated significant deviation from a normal distribution.

Inferential statistics

The chi-square test and Fisher exact test were used to compare categorical variables across groups (e.g., HCWs from different countries). Analysis of variance was conducted to compare means of normally distributed continuous variables among multiple groups. Non-parametric tests, including the Kruskal-Wallis (H) and Mann-Whitney (U) tests, were applied to compare ordinal and continuous variables between groups when data did not meet normality assumptions.

Univariate analysis

Univariate binary logistic regression was performed to identify relationships between socio-demographic characteristics and outcome variables (knowledge, attitudes, and willingness to vaccinate). Variables with $p \leq 0.05$ in univariate analyses were considered for inclusion in subsequent multivariate models. Odds ratios (ORs) with 95% CIs were calculated for each predictor of vaccine acceptance.

Health belief model analysis

Structural equation modeling (SEM) was employed to evaluate the fit of the HBM, incorporating key constructs—perceived susceptibility, severity, benefits, barriers, self-efficacy, and cues to action—hypothesized to influence HCWs' vaccine acceptance. Model fit was assessed using standard indices, including the comparative fit index (CFI), RMSEA, and TLI.

Subgroup and interaction analysis

Subgroup analyses were conducted based on country of residence, years of professional experience, and COVID-19 vaccination status to identify differences in knowledge, attitudes, and vaccine acceptance across groups. Interaction terms were tested to evaluate whether relationships between predictors and outcomes differed among subgroups.

Handling of missing data

Missing data were addressed using multiple imputation techniques (if applicable) to preserve sample size and minimize biases associated with incomplete data. Participants with significant missing data (i.e., more than 20% missing responses within any questionnaire section) were excluded from analyses. Sensitivity analyses were conducted to evaluate the impact of data imputation on study findings.

Sensitivity analyses

Sensitivity analyses assessed the robustness of findings by testing different assumptions related to handling missing data (e.g., excluding cases with missing data versus using imputation techniques) and examining alternative analytical models (e.g., varying cutoff points for socio-demographic variables).

Statistical significance

A 2-tailed significance threshold of $p \leq 0.05$ was employed for all statistical tests. Results with *p*-values below this threshold were considered statistically significant.

Ethics statement

This study received ethical approval from the RAK Medical and Health Sciences University Research Committee (approval number: RAKMHSU-HEC-43-2023/24-N-F) and the Ministry of Health and Prevention (MOHAP) Research Ethics Committee (approval number: MOHAP/REC/2024/71-2024 F-N). All participants provided informed consent prior to data collection. The study was conducted in accordance with

ethical guidelines outlined in the Declaration of Helsinki.

Results

The study included a diverse group of HCWs, predominantly aged between 30 to 39 years (46.5%), followed by those aged ≥40 years (27.7%) and those aged 20 to 29 years (25.8%). Women comprised 75.5% of participants, and men 24.5%. Most participants were married (72.6%), followed by single individuals (25.7%), while a small percentage were separated, divorced, or widowed (1.7%). Participants primarily held bachelor's degrees (73.6%), with smaller proportions holding diplomas (10.3%), master's degrees (13.5%), and PhD/fellowships (2.6%). Most respondents were from Qatar (42.0%), followed by the UAE (33.5%) and Saudi Arabia (24.5%). The majority worked in general hospitals (83.9%), with fewer working in community healthcare (13.4%) and private hospitals (2.7%). Nurses represented the largest specialty group (93.5%), followed by physicians (3.1%), pharmacists (1.8%), and other healthcare roles (1.6%). Most participants reported 5 to 10 years of medical practice experience (54.6%). The primary sources of mpox information included HCWs (43.5%), social media (33.2%), and medical books or studies (23.3%). Most participants were vaccinated against COVID-19 (94.4%), with the largest group having received 3 doses (59.4%) (Table 1).

The data revealed significant associations between overall knowledge and several socio-demographic characteristics. Participants aged 30 to 39 had the highest proportion of good knowledge (39.5%), whereas those aged ≥40 had the lowest proportion (29.7%). Women exhibited a higher proportion of good knowledge (72.8%) compared to men (27.2%). Additionally, marital status, educational level, country of residence, type of workplace, specialty, medical practice experience, and primary source of mpox information were significantly associated with overall knowledge. For instance, participants from the UAE demonstrated the highest proportion of good knowledge (59.0%), and those holding bachelor's degrees represented 80.5% of participants with good knowledge (Table 2).

The analysis identified significant associations between overall attitudes and several socio-demographic characteristics. Participants aged 30 to 39 had the highest proportion of positive attitudes (46.3%), while participants aged 20 to 29 had the lowest proportion of negative attitudes (22.0%). Women reported more negative attitudes (83.1%) than men (16.9%). Marital status, country of residence, and primary source of mpox information were significantly associated with overall attitudes. For example, participants from Qatar had the highest proportion of negative attitudes (50.4%)

Table 1. Socio-demographic characteristics among Gulf healthcare workers (*n* = 764)

Characteristic	Value
Age (y)	
20–29	197 (25.8)
30–39	355 (46.5)
≥40	212 (27.7)
Sex	
Female	577 (75.5)
Male	187 (24.5)
Marital status	
Single	196 (25.7)
Married	555 (72.6)
Separated/divorced/widowed	13 (1.7)
Educational level	
Diploma	79 (10.3)
Bachelor's degree	562 (73.6)
Postgraduate degree	123 (16.1)
Country of residence	
Saudi Arabia	187 (24.5)
United Arab Emirates	256 (33.5)
Qatar	321 (42.0)
Type of workplace	
General hospital	641 (83.9)
Community health care	102 (13.4)
Private hospital	21 (2.7)
Specialty	
Nurse	714 (93.5)
Pharmacist	14 (1.8)
Physician	24 (3.1)
Others (allied health, therapist, etc.)	12 (1.6)
Medical practice experience (y)	
≤5	608 (79.6)
6–10	76 (9.9)
11–15	53 (6.9)
>15	79 (10.3)
Source of information about mpox	
Healthcare workers (peers, workshop, CDC)	332 (43.5)
Medical books or during my studies	178 (23.3)
Social media	254 (33.2)
COVID-19 vaccination	
Vaccinated	721 (94.4)
Not-vaccinated	43 (5.6)
If vaccinated, number of COVID-19 vaccine doses (*n* = 721)	
1	1 (0.1)
2	159 (22.1)
3	428 (59.4)
4	133 (18.4)

Data are presented as *n* (%).
CDC, Centers for Disease Control and Prevention; COVID-19, coronavirus disease 2019.

https://doi.org/10.24171/j.phrp.2025.0113

Table 2. Associations between overall knowledge and socio-demographic characteristics among participants

Variable	Good (*n* = 195)	Poor (*n* = 569)	*p*
Age (y)			0.060
20–29	60 (30.8)	137 (24.1)	
30–39	77 (39.5)	278 (48.9)	
≥ 40	58 (29.7)	154 (27.1)	
Sex			0.335
Female	142 (72.8)	435 (76.4)	
Male	53 (27.2)	134 (23.6)	
Marital status			0.002
Single	68 (34.9)	128 (22.5)	
Married	122 (62.6)	433 (76.1)	
Separated/divorced/widowed	5 (2.6)	8 (1.4)	
Educational level			0.017
Diploma	19 (9.7)	60 (10.5)	
Bachelor's degree	157 (80.5)	405 (71.2)	
Postgraduate degree	19 (9.7)	104 (18.3)	
Country of residence			< 0.001
Saudi Arabia	25 (12.8)	162 (28.5)	
United Arab Emirates	115 (59.0)	141 (24.8)	
Qatar	55 (28.2)	266 (46.7)	
Specialty			0.003
Nurse	179 (91.8)	535 (94.0)	
Pharmacist	0 (0)	14 (2.5)	
Physician	12 (6.2)	12 (2.1)	
Others (allied health, therapist, etc.)	4 (2.1)	8 (1.4)	
Medical practice experience (y)			< 0.001
≤ 5	60 (30.8)	133 (23.4)	
6–10	80 (41.0)	336 (59.1)	
11–15	29 (14.9)	47 (8.3)	
> 15	26 (13.3)	53 (9.3)	
Source of information about mpox			< 0.001
Healthcare workers (peers, workshop, CDC)	88 (45.1)	244 (42.9)	
Medical books or during my studies	77 (39.5)	101 (17.8)	
Social media	30 (15.4)	224 (39.4)	
COVID-19 vaccination			0.305
Vaccinated	186 (95.4)	535 (94.0)	
Not-vaccinated	9 (4.6)	34 (6.0)	
If vaccinated, no. of COVID-19 vaccine doses (*n* = 721)			< 0.001
1	0 (0)	1 (0.2)	
2	41 (22)	118 (22.1)	
3	90 (48.4)	338 (63.1)	
4	55 (29.6)	78 (14.6)	

Data are presented as *n* (%).
CDC, Centers for Disease Control and Prevention; COVID-19, coronavirus disease 2019.

(Table 3).

The analysis also indicated significant associations between vaccine acceptance and willingness to receive the mpox vaccine with several socio-demographic characteristics. Participants aged 30 to 39 demonstrated the highest willingness to vaccinate (44.6%), whereas participants aged ≥ 40 showed the lowest willingness (21.7%). Women reported a higher proportion of unwillingness (80.0%) compared to men (20.0%). Marital status, country of residence, and source of mpox information were significantly associated with vaccine willingness. Specifically, participants from the UAE reported the highest proportion of willingness (52.1%),

Table 3. Associations between overall attitude and socio-demographic characteristics among participants

Variable	Negative (*n* = 427)	Positive (*n* = 337)	*p*
Age (y)			0.007
20–29	94 (22.0)	103 (30.6)	
30–39	199 (46.6)	156 (46.3)	
≥ 40	134 (31.4)	78 (23.1)	
Sex			< 0.001
Female	355 (83.1)	222 (65.9)	
Male	72 (16.9)	115 (34.1)	
Marital status			0.001
Single	88 (20.6)	108 (32.0)	
Married	329 (77.0)	226 (67.1)	
Separated/divorced/widowed	10 (2.3)	3 (0.9)	
Country of residence			< 0.001
Saudi Arabia	96 (22.5)	91 (27.0)	
United Arab Emirates	116 (27.2)	140 (41.5)	
Qatar	215 (50.4)	106 (31.5)	
Specialty			0.295
Nurse	402 (94.1)	312 (92.5)	
Pharmacist	9 (2.1)	5 (1.5)	
Physician	9 (2.1)	15 (4.5)	
Others (allied health, therapist, etc.)	7 (1.6)	5 (1.5)	
Medical practice experience (y)			< 0.001
≤ 5	82 (19.2)	111 (32.9)	
6–10	265 (62.1)	151 (44.8)	
11–15	40 (9.4)	36 (10.7)	
> 15	40 (9.3)	39 (11.6)	
Source of information about mpox			< 0.001
Healthcare workers (peers, workshop, CDC)	209 (48.9)	123 (36.5)	
Medical books or during my studies	65 (15.2)	113 (33.5)	
Social media	153 (35.8)	101 (30.0)	
COVID-19 vaccination			< 0.001
Vaccinated	403 (94.4)	318 (94.4)	
Not-vaccinated	24 (5.6)	19 (5.6)	
If vaccinated, no. of COVID-19 vaccine doses (*n* = 721)			0.003
1	0 (0)	1 (0.3)	
2	81 (20.1)	78 (24.5)	
3	261 (64.8)	167 (52.6)	
4	61 (15.1)	72 (22.6)	

Data are presented as *n* (%).
CDC, Centers for Disease Control and Prevention; COVID-19, coronavirus disease 2019.

and those who received information from HCWs showed the highest proportion of willingness (39.2%) (Table 4).

The analysis further identified significant predictors of mpox vaccine perceptions among HCWs. Younger participants (aged 20–29) reported higher perceived susceptibility and greater intention to vaccinate compared to older age groups, with significant *p*-values of 0.041 and < 0.001, respectively. Men had higher perceived susceptibility and vaccination intention than women, with significant *p*-values of < 0.001 and 0.030, respectively. Single HCWs reported higher perceived susceptibility and vaccination intention compared to married or separated/divorced/widowed participants, with significant *p*-values of 0.009 and < 0.001, respectively. Participants holding bachelor's degrees reported higher perceived susceptibility and vaccination intention compared to other educational levels, with significant *p*-values of 0.010 and 0.003, respectively. Additionally, HCWs from the UAE showed higher perceived susceptibility, benefits, and vaccination intention compared to those from Saudi Arabia and Qatar, with significant *p*-values of < 0.001, < 0.001, and

https://doi.org/10.24171/j.phrp.2025.0113

Table 4. Associations between overall acceptance and willingness to receive the mpox vaccine and socio-demographic characteristics

Variable	No (*n* = 524)	Yes (*n* = 240)	*p*
Age (y)			0.001
20–29	116 (22.1)	81 (33.8)	
30–39	248 (47.4)	107 (44.6)	
≥ 40	160 (30.5)	52 (21.7)	
Sex			< 0.001
Female	419 (80.0)	158 (65.8)	
Male	105 (20.0)	82 (34.2)	
Marital status			0.001
Single	114 (21.8)	82 (34.2)	
Married	399 (76.1)	56 (23.3)	
Separated/divorced/widowed	11 (2.1)	2 (0.8)	
Country of residence			< 0.001
Saudi Arabia	151 (28.8)	36 (15.0)	
United Arab Emirates	131 (25.0)	125 (52.1)	
Qatar	242 (46.2)	79 (32.9)	
Specialty			0.457
Nurse	492 (93.9)	222 (92.5)	
Pharmacist	7 (1.3)	7 (2.9)	
Physician	16 (3.1)	8 (3.3)	
Others (allied health, therapist, etc.)	9 (1.7)	3 (1.3)	
Medical practice experience (y)			0.007
≤ 5	115 (21.9)	78 (32.5)	
6–10	304 (58.0)	112 (46.7)	
11–15	49 (9.4)	27 (11.3)	
> 15	56 (10.7)	23 (9.6)	
Source of information about mpox			< 0.001
Healthcare workers (peers, workshop, CDC)	238 (45.4)	94 (39.2)	
Medical books or during my studies	90 (17.2)	88 (36.7)	
Social media	196 (37.4)	58 (24.2)	
COVID-19 vaccination			0.028
Vaccinated	488 (93.1)	233 (97.1)	
Not-vaccinated	36 (6.9)	7 (2.9)	
If vaccinated, no. of COVID-19 vaccine doses (*n* = 721)			< 0.001
1	0 (0)	1 (0.4)	
2	101 (20.7)	58 (24.9)	
3	318 (65.2)	110 (47.2)	
4	69 (14.1)	64 (27.5)	

Data are presented as *n* (%).
CDC, Centers for Disease Control and Prevention; COVID-19, coronavirus disease 2019.

0.001, respectively. Participants who received 4 COVID-19 vaccine doses reported higher perceived susceptibility, benefits, and vaccination intention compared to those with fewer doses, with significant *p*-values of < 0.001, < 0.001, and 0.001, respectively. These findings highlight the importance of socio-demographic-demographic factors in shaping vaccine perceptions and acceptance strategies (Table 5, Tables S4, S5).

Univariate binary logistic regression analysis identified several predictors associated with high knowledge, positive attitudes, and willingness to vaccinate against mpox. Significant predictors included age, sex, marital status, educational level, country of residence, specialty, medical practice experience, primary information source, and COVID-19 vaccination status. For example, participants aged 20 to 29 demonstrated higher odds of exhibiting positive attitudes (OR, 1.882) and willingness to vaccinate (OR, 2.149) compared to those aged ≥ 40. Male participants had higher odds of positive attitudes (OR, 2.554) and willingness to

Table 5. Predictors of mpox vaccine perceptions according to the health belief model among healthcare workers

Variable	Perceived susceptibility	*p*	Perceived severity	*p*	Perceived benefits	*p*	Perceived barriers	*p*	Cues to action	*p*	Perceived intention	*p*
Age (y)		0.041		0.075		0.543		0.002		0.459		<0.001
20–29	14.1±3.3		13.3±3.5		14.6±3.1		10.3±3.2		18±4.1		13.9±3	
30–39	13.7±3.1		13.9±3.4		14.9±3.1		11.1±2.7		17.8±3.7		13.1±3.6	
≥40	13.3±3.3		14±3.5		14.7±3		11.2±2.8		17.5±3.5		12.3±3.9	
Sex		<0.001		0.057		0.402		<0.001		0.038		0.030
Female	13.5±3.2		13.7±3.5		14.7±3.1		11.2±2.9		17.6±3.8		12.9±3.6	
Male	14.5±3.1		14.2±3.2		14.9±2.9		10.2±2.8		18.2±3.5		13.6±3.7	
Marital status		0.009		0.232		0.372		0.001		0.004		<0.001
Single	14.3±3.5		13.7±3.7		15±3.3		10.3±3.2		18.5±4.3		14±3.4	
Married	13.5±3.1		13.8±3.4		14.6±3		11.2±2.7		17.5±3.5		12.8±3.6	
Separated/ divorced/ widowed	13±3		12.2±3.5		15±2.1		11.1±2.6		16.3±4.3		12±4.8	
Country of residence		<0.001		<0.001		<0.001		<0.001		<0.001		0.001
Saudi Arabia	13.2±3		12.8±3		13.1±2.6		10.8±2.6		16.5±3.6		13.1±2.5	
United Arab Emirates	14.5±3.2		14.4±3.5		15.8±2.8		10.3±2.9		18.9±3.5		13.7±3.6	
Qatar	13.4±3.2		13.9±3.6		14.8±3.2		11.5±2.9		17.6±3.7		12.6±4.1	
Type of workplace		0.100		0.688		0.115		0.244		0.064		0.001
General hospital	14.1±3.2		13.8±3.4		14.6±3.1		10.5±2.9		18±3.8		13.7±3.2	
Community health care	13.5±2.8		13.6±3.2		15.4±2.6		10.9±2.6		16.8±3.3		12±3.3	
Private hospital	12.5±2.1		13±0.4		14.1±1.9		9.5±1		18.3±1.8		13.2±2.1	
Specialty		0.737		0.157		0.061		0.670		0.530		0.505
Nurse	13.7±3.2		13.8±3.5		14.8±3.1		10.9±2.9		17.8±3.8		13.1±3.6	
Pharmacist	12.8±3.3		12.1±1.5		12.9±1.4		10.4±1.2		16.8±1.5		11.9±3.9	
Physician	13.6±2.7		14±2.4		14.4±2.4		11.2±2.4		18±2.4		12.5±4.4	
Others (allied health, therapist, etc.)	13.8±3.4		15.2±3		13.5±4.4		11.7±2.7		16.6±4.9		12.9±4.5	
Medical practice experience (y)		0.011		0.436		0.078		0.008		0.459		0.002
≤5	14.3±3.1		13.4±3.2		14.2±3.1		10.4±3.1		17.7±3.9		13.9±2.9	
6–10	13.4±3.3		13.8±3.6		14.9±3.2		11.3±2.9		17.7±3.9		12.7±3.9	
11–15	13.4±3.1		14.1±3.1		14.9±3		10.7±2.5		18.1±3.3		13.1±3.8	
>15	14.5±3.2		14.2±3.9		15±2.7		10.4±2.7		18.1±3.3		13.4±3	
Source of information about mpox		0.475		0.568		0.015		0.064		0.177		0.013
Healthcare workers (peers, workshop, CDC)	13.7±3.2		13.9±3.6		15±3.1		11.1±2.7		17.8±3.8		12.8±3.9	
Medical books or during my studies	13.9±2.9		13.8±3.2		14.9±2.9		10.5±3.1		18.1±3.9		13.7±3.2	
Social media	13.6±3.3		13.6±3.4		14.3±3.2		11±3		17.5±3.6		13±3.4	

(Continued on the next page)

https://doi.org/10.24171/j.phrp.2025.0113

Table 5. Continued

Variable	Perceived susceptibility	*p*	Perceived severity	*p*	Perceived benefits	*p*	Perceived barriers	*p*	Cues to action	*p*	Perceived intention	*p*
COVID-19 vaccination		0.439		0.743		< 0.001		0.034		0.092		0.422
Vaccinated	13.7 ± 3.2		13.8 ± 3.5		14.9 ± 3.1		10.9 ± 3		17.8 ± 3.8		13.1 ± 3.6	
Not-vaccinated	14.1 ± 2.6		13.6 ± 2.1		12.6 ± 1.9		11.8 ± 1.2		16.8 ± 3.2		12.6 ± 3.5	
If vaccinated, number of COVID-19 vaccine doses (*n* = 721)		< 0.001		< 0.001		< 0.001		0.001		< 0.001		0.001
2	13.5 ± 3.3		13.5 ± 3.7		14.9 ± 3.5		11 ± 3		18.5 ± 3.7		13.3 ± 4.1	
3	13.3 ± 3		13.4 ± 3.4		14.4 ± 3		11.1 ± 2.9		17.2 ± 3.7		12.7 ± 3.4	
4	15.3 ± 3.3		15.3 ± 3.3		16.2 ± 2.5		10.1 ± 3.1		19 ± 3.5		14.1 ± 3.5	

Data are presented as mean ± standard deviation.
CDC, Centers for Disease Control and Prevention; COVID-19, coronavirus disease 2019.

vaccinate (OR, 2.071) compared to female participants (Table 6).

SEM analysis indicated a good model fit, evidenced by an RMSEA of 0.111 (95% CI, 0.108–0.115), TLI of 0.986, and CFI of 0.988 (Figure 2).

Discussion

The findings of this study provide valuable insights into factors influencing mpox vaccine acceptance among HCWs in the Gulf region. Utilizing the HBM, the study identified important predictors of vaccine acceptance related to knowledge, perceived risk, and attitudes toward vaccination.

The analysis revealed that overall knowledge significantly correlated with several socio-demographic-demographic factors, including marital status, educational level, country of residence, specialty, medical experience, and sources of information. Notably, HCWs from the UAE demonstrated the highest levels of good knowledge, and those holding a bachelor's degree were more likely to exhibit good mpox knowledge.

These results align closely with a study conducted in Lebanon [41], which reported significantly better mpox knowledge and attitudes associated with postgraduate education and older age, supporting our finding that higher educational attainment is associated with greater knowledge. Similarly, another Lebanese study showed that physicians had significantly higher mpox knowledge compared to other healthcare occupations [42], supporting the broader trend observed in our research where physicians exhibited better knowledge.

Regarding mpox knowledge, recent studies have reported insufficient knowledge among Saudi Arabian HCWs [43]. Our findings similarly showed that only 30.8% of younger HCWs (aged 20–29 years) demonstrated good knowledge, compared to 39.5% among those aged 30 to 39 years. This highlights variability in knowledge across age groups, likely due to differences in professional experience.

Moreover, prior studies have demonstrated that previous vaccination behavior significantly influences vaccine uptake [44,45]. Moreover, studies have demonstrated that prior vaccination behavior is a key factor in determining vaccine uptake.

Our study's finding that only 30.8% of younger HCWs had good mpox knowledge, compared to 39.5% among those aged 30 to 39 years, underscores a critical knowledge gap between age groups, emphasizing the need for targeted educational interventions. These findings are consistent with previous research indicating that 60.5% of Nigerian HCWs exhibited adequate knowledge [46], which is lower than the 70% rate of good knowledge reported among Jordanian HCWs [47], but higher than the 27% observed among Italian medical professionals [48]. Additionally, our study identified nurses as having lower knowledge scores than other healthcare professionals, paralleling disparities noted in global contexts.

Social media emerged as a prevalent source of health information (51.1%) among HCWs, followed by official local statements (57.6%) and international health authorities such as World Health Organization and Centers for Disease Control and Prevention (59.8%) [49]. Our study found the highest proportion of negative attitudes (35.9%) among HCWs relying on social media for information, confirming that misinformation negatively impacts vaccine perceptions.

A systematic review and meta-analysis assessing global

Table 6. Univariate binary logistic regression analysis of predictive factors associated with high knowledge, positive attitudes, and willingness scores regarding mpox

Variable	Knowledge	*p*	Attitudes	*p*	Willingness	*p*
Age (y)						
20–29	1.163 (0.758–1.784)	0.490	1.882 (1.268–2.794)	0.002	2.149 (1.408–3.278)	<0.001
30–39	0.735 (0.496–1.090)	0.126	1.347 (0.950–1.909)	0.095	1.328 (0.902–1.954)	0.151
≥40	Reference					
Sex						
Female	Reference					
Male	1.212 (0.837–1.754)	0.309	2.554 (1.820–3.585)	<0.001	2.071 (1.471–2.915)	<0.001
Marital status						
Single	0.850 (0.268–2.699)	0.783	4.091 (1.092–15.322)	0.037	3.956 (0.854–18.327)	0.079
Married	0.451 (0.145–1.403)	0.169	2.290 (0.623–8.413)	0.212	2.150 (0.471–9.812)	0.323
Separated/divorced/widowed	Reference					
Country of residence						
Saudi Arabia	0.746 (0.447–1.245)	0.262	1.923 (1.329–2.781)	0.001	0.730 (0.469–1.138)	0.165
United Arab Emirates	3.945 (2.696–5.772)	<0.001	2.448 (1.745–3.434)	<0.001	2.923 (2.054–4.160)	<0.001
Qatar	Reference					
Specialty						
Nurse	Reference					
Physician	2.989 (1.319–6.772)	0.009	2.147 (0.928–4.972)	0.074	1.108 (0.467–2.627)	0.816
Others (allied health, therapist, etc.)	1.494 (0.445–5.022)	0.516	0.920 (0.289–2.927)	0.888	0.739 (0.198–2.755)	0.652
Medical practice experience (y)						
≤5	5.323 (1.218–23.262)	0.026	3.641 (1.462–9.069)	0.006	2.812 (1.017–7.776)	0.046
6–10	2.857 (0.662–12.339)	0.160	1.547 (0.636–3.764)	0.337	1.547 (0.570–4.202)	0.392
11–15	7.404 (1.628–33.682)	0.010	2.443 (0.920–6.487)	0.073	2.314 (0.784–6.832)	0.129
>15	Reference					
Source of information about mpox						
Healthcare workers (peers, workshop, CDC)	Reference					
Medical books or during my studies	2.114 (1.440–3.103)	<0.001	2.954 (2.025–4.310)	<0.001	2.476 (1.695–3.615)	<0.001
Social media	0.371 (0.236–0.584)	<0.001	1.122 (0.802–1.569)	0.503	0.749 (0.513–1.093)	0.134
COVID-19 vaccination						
Yes	Reference					
No	0.761 (0.358–1.617)	0.478	1.003 (0.540–1.864)	0.992	0.407 (0.179–0.929)	0.033
No. of COVID-19 vaccine doses						
2	0.493 (0.300–0.809)	0.005	0.816 (0.514–1.294)	0.387	0.619 (0.387–0.990)	0.045
3	0.378 (0.249–0.572)	<0.001	0.542 (0.366–0.803)	0.002	0.373 (0.249–0.558)	<0.001
4	Reference					

Data are presented as odds ratio (95% confidence interval).
CDC, Centers for Disease Control and Prevention; COVID-19, coronavirus disease 2019.

knowledge and attitudes towards mpox among HCWs revealed that only 26.0% had good knowledge, and 34.6% had positive attitudes [37]. Our study found comparatively higher knowledge levels among Gulf HCWs, suggesting greater awareness within our study population relative to the global average. Moreover, higher knowledge among older professionals supports the notion that experience contributes significantly to better awareness.

The results indicated significant associations between overall attitudes and several socio-demographic-demographic characteristics. A recent study in Saudi Arabia reported that 74.7% of respondents were motivated by the need to protect their health, family, and friends [50]. Our study similarly found that positive attitudes were more common among HCWs with greater professional experience, reinforcing the importance of personal and professional responsibility in shaping vaccine-related attitudes. Key reasons for vaccine hesitancy included insufficient information (56.1% strongly agreed), fear of unknown side effects (45.6% strongly agreed), and doubts regarding vaccine effectiveness (46.4% strongly agreed and 26.8% agreed) [51]. In alignment with these findings, negative attitudes in our study were most

https://doi.org/10.24171/j.phrp.2025.0113

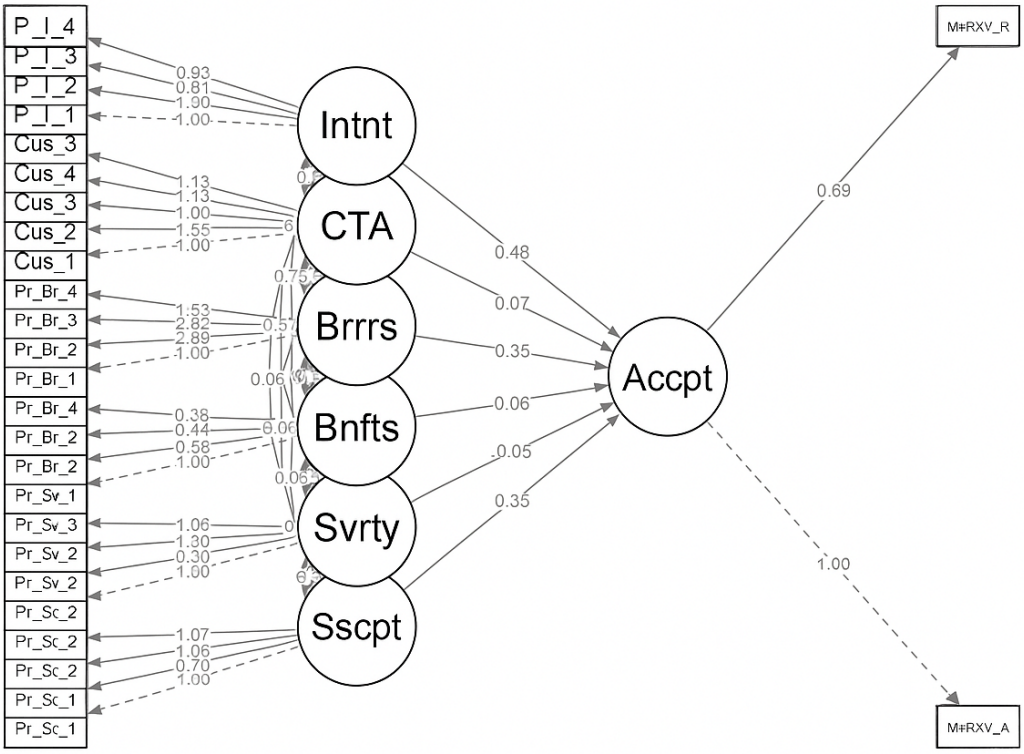


Figure 2. Structural equation model of Gulf healthcare workers' perceptions and acceptance of HMPXV vaccines ($n = 764$). Structural equation modeling depicting the relationships among perceived susceptibility (Sscpt), perceived severity (Svrty), perceived benefits (Bnfts), perceived barriers (Brrrs), cues to action (CTA), perceived intention (Intnt), acceptance (Accpt), vaccine recommendation (HMPXV vaccine_R), and vaccine acceptance (HMPXV vaccine_A). The model demonstrated a good fit, with root mean square error of approximation = 0.111 (95% confidence interval, 0.108–0.115), Tucker-Lewis index = 0.986, and comparative fit index = 0.988.

frequent among those relying on social media for information (35.8%), compared to those using healthcare professionals as sources (48.9%), confirming the detrimental impact of misinformation. Furthermore, cues to action (e.g., governmental recommendations) significantly predicted vaccine acceptance, highlighting the critical role of institutional trust in shaping vaccine attitudes.

Our findings indicate that sex plays a role in shaping attitudes toward mpox vaccination. Additionally, adequate knowledge and a positive vaccine attitude significantly influenced HCWs' willingness to recommend the vaccine to high-risk groups. This relationship between knowledge and attitude helps explain why attitudes might be more negative in contexts of lower vaccine awareness [52].

Interestingly, our study found that female HCWs had higher willingness to receive the mpox vaccine, consistent with a study from Pakistan showing higher vaccine acceptance rates among women, contrary to common global trends in vaccine hesitancy [49]. Additionally, the prevalence of chronic conditions among men (34.9%) was slightly higher than among women (31.9%), and prior research shows that individuals with chronic diseases tend to have higher vaccine hesitancy [53,54]. Our study demonstrated that perceived barriers, such as concerns about side effects, were significantly associated with chronic disease status, reinforcing the role health conditions play in vaccine perception.

Our results indicate higher vaccine willingness among Gulf HCWs, suggesting they perceive mpox as a significant health threat. In contrast, only 50% of HCWs from Kurdistan had positive attitudes, with 82.4% unwilling to vaccinate [3].

The analysis of HBM components indicated that perceived barriers, including fear of side effects and the social stigma associated with mpox, significantly deterred vaccine acceptance. These findings align with research from Egypt and Jordan, where social stigma and misinformation were major factors influencing vaccine hesitancy [28,55]. Additionally, younger HCWs and those with prior exposure to COVID-19 vaccination campaigns exhibited lower vaccine hesitancy, highlighting the positive impact of prior pandemic experiences on current vaccination behavior.

Finally, HCWs from the UAE demonstrated significantly higher perceived susceptibility, perceived benefits, and vaccination intention compared to those from Saudi Arabia and Qatar, as indicated by highly significant *p*-values (< 0.001, < 0.001, and 0.001, respectively). These differences underscore the effectiveness of the UAE's healthcare infrastructure and public health communication strategies. Additionally, HCWs who received 4 COVID-19 vaccine doses had higher perceived susceptibility, perceived benefits, and vaccination intention compared to those with fewer doses, reflecting the influence of prior vaccination experiences in shaping positive attitudes toward future vaccines.

Study Limitations, Interpretation, and Generalizability

This study has several limitations. The cross-sectional design restricts the ability to infer causal relationships, and reliance on self-reported data may introduce recall and social desirability biases. Additionally, conducting the survey online potentially excluded HCWs with limited internet access or insufficient digital literacy.

Despite these limitations, the findings indicate moderate to high levels of mpox knowledge among HCWs in the Gulf region. Vaccine acceptance was influenced by various socio-demographic and psychological factors. Specifically, the HBM effectively identified perceived severity, perceived benefits, and cues to action as significant predictors of vaccine willingness.

While the inclusion of participants from the UAE, Saudi Arabia, and Qatar enhances the regional relevance of these findings within the Gulf, employing convenience sampling may have reduced the sample's representativeness, particularly for rural or offline populations. Therefore, caution should be exercised when generalizing these results to other healthcare contexts or geographic regions.

Conclusion

This study applied the HBM to elucidate factors influencing mpox vaccine acceptance among HCWs in the Gulf region. Findings revealed that perceived susceptibility, perceived benefits, and self-efficacy were the most influential predictors of vaccine acceptance. Conversely, perceived barriers, such as fears of side effects and misinformation, impeded vaccine uptake. Notably, HCWs from the UAE demonstrated higher vaccine acceptance compared to those from Saudi Arabia and Qatar, reflecting the positive influence of prior COVID-19 vaccination experiences and the effectiveness of robust healthcare infrastructure. These results emphasize the importance of implementing targeted educational programs aimed at enhancing perceived benefits, addressing vaccine-related concerns, and strengthening self-efficacy to improve vaccine acceptance and preparedness for future infectious disease outbreaks.

Supplementary Material

Table S1. Healthcare workers' knowledge about mpox infection (3-point Likert scale) (*n* = 764); **Table S2.** Healthcare workers' Attitude about mpox infection (5-point Likert scale) (*n* = 764); **Table S3.** The willingness of healthcare workers to be vaccinated against mpox (5-point Likert scale) (*n* = 764); **Table S4.** Mpox vaccine perceptions according to the health belief model (*n* = 764); **Table S5.** Mpox vaccine perceptions according to the health belief model. Supplementary data are available at https://doi.org/10.24171/j.phrp.2025.0113.

Notes

Ethics Approval

This study was approved by the RAK Medical and Health Sciences University Research Committee (RAKMHSU-HEC-43-2023/24-N-F) and the MOHAP Research Ethics Committee (MOHAP/REC/2024/71-2024 F-N). All participants provided informed consent prior to data collection, and the study was conducted in accordance with the guidelines set forth in the Declaration of Helsinki.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

None.

Availability of Data

The datasets are not publicly available but are available from the corresponding author upon reasonable request.

Authors' Contributions

Conceptualization: MGM, MMAJ, MAW; Data curation: MGM, FMI, KM, SA; Formal analysis: MGM, JK, HAF; Funding acquisition: MMAJ; Investigation: MGM, EAAD, FMI, KM, SA; Methodology: MGM, EAAD, SA; Project administration: MGM; Resources: MGM; Software: MAW; Supervision: MAW, KMAS, FMI, MMAJ; Validation: MGM, KMAS; Visualization: MGM, SA; Writing–original draft: MGM, SA; Writing–review & editing: all authors. All authors read and approved the final manuscript.

References

1. Ravi RK, Rasheed HM, Gamal M. Acceptance of and concerns about Covid-19 vaccination among parents: a cross-sectional survey in Ras Al-Khaimah, United Arab Emirates. Afr J Nurs Midwifery 2022;24:9.
2. Biswas N, Mustapha T, Khubchandani J, et al. The nature and extent of COVID-19 vaccination hesitancy in healthcare workers. J Community Health 2021;46:1244–51.
3. Ahmed SK, Abdulqadir SO, Omar RM, et al. Knowledge, attitudes, and willingness of healthcare workers in Iraq's Kurdistan Region to vaccinate against human monkeypox: a nationwide cross-sectional

study. Vaccines (Basel) 2023;11:1734.
4. Makwana MN, Shekhda HJ, Rupani MP. Acceptance and expectations of healthcare workers and community during the COVID-19 vaccine rollout in Bhavnagar city, western India: a qualitative exploration. BMC Health Serv Res 2024;24:386.
5. Limbu YB, Gautam RK, Pham L. The health belief model applied to COVID-19 vaccine hesitancy: a systematic review. Vaccines (Basel) 2022;10:973.
6. Ahmed SK, Dabou EA, Ibrahim FM, et al. Challenges and counteracting strategies including optimum health service practices for frontline nurses during the mpox outbreak and futuristic vision. SAGE Open Nurs 2024;10:23779608241256209.
7. Zeidan RK, Shukla A, Hussein A, et al. Assessment of knowledge, perceptions, and attitudes during the global mpox outbreak in June 2022: a cross-sectional study from the United Arab Emirates. Int J Public Health 2023;68:1606080.
8. World Health Organization (WHO) (CH). Background and challenges. WHO; 2023.
9. Mohamed MG, Islam MR, Ahmed SK, et al. Assessment of knowledge, attitude, anxiety level and perceived mental healthcare needs toward mpox infection among nursing students: a multi-center cross-sectional study. Glob Transit 2024;6:203–11.
10. Ahmed SK, Dabou EA, Abdelsamad S, et al. Monkeypox virus infection and myocarditis: a review of current evidence and possible pathogenesis. Narra J 2023;3:e104.
11. Ahmed SK, Saied AA, Ravi RK, et al. The 2022 monkeypox outbreak and associated psychiatric morbidities. Int J Surg 2022;106:106913.
12. Ahmed SK, El-Kader RG, Lorenzo JM, et al. Hospital-based salient prevention and control measures to counteract the 2022 monkeypox outbreak. Health Sci Rep 2023;6:e1057.
13. Shoaib HM. Monkeypox as a potential emerging pandemic: a discussion on future risks and preparedness in Saudi Arabia. Front Public Health 2023;11:1254545.
14. Ahmed SK, Omar RM, Hussein SH, et al. Middle East countries preparedness for monkeypox outbreak: a call to action. Int J Surg 2022;106:106948.
15. Mohamed MG, Al-Yafeai TM, Adam S, et al. The significance of emotional intelligence in academic stress, resilience, and safe transition from high school to university: an SEM analysis among Northern Emirati university students. Glob Transit 2025;7:109–17.
16. Ali A, Cochrane L. Residency and citizenship in the Gulf: recent policy changes and future implications for the region. Comp Migr Stud 2024; 12:16.
17. Parker S, Steffen R, Rashid H, et al. Sacred journeys and pilgrimages: health risks associated with travels for religious purposes. J Travel Med 2024;31:taae122.
18. Shakeela A, Jose S. Cross-cultural perceptions and tourist satisfaction in the Middle Eastern context: a case study of Abu Dhabi's destination image. Consum Behav Tour Hosp 2025;20:75–87.
19. Elbus LM, Shaheen RA, Khalaf SA, et al. Work addiction among critical care nurses: exploring its impact on creativity and professional quality of life. Worldviews Evid Based Nurs 2025;22:e70032.
20. Acharya A, Kumar N, Singh K, et al. "Mpox in MSM: tackling stigma, minimizing risk factors, exploring pathogenesis, and treatment approaches". Biomed J 2025;48:100746.
21. Ahmed SK, Ahmed Rashad EA, Mohamed MG, et al. The global human monkeypox outbreak in 2022: an overview. Int J Surg 2022;104:106794.
22. Ahmed SK, Mohamed MG, Essa RA, et al. Global reports of myocarditis following COVID-19 vaccination: a systematic review and meta-analysis. Diabetes Metab Syndr 2022;16:102513.
23. Lawrence A. Assessing vaccine intentions, knowledge, self-efficacy, and trust: a cross-sectional study on perceptions of monkeypox vaccination and public health risk awareness in Makurdi, Benue State, Nigeria. Cureus 2024;16:e72131.
24. Khalid Ahmed S, Gamal Mohamed M, Abdulrahman Essa R, et al. Global reports of takotsubo (stress) cardiomyopathy following COVID-19 vaccination: a systematic review and meta-analysis. Int J Cardiol Heart Vasc 2022;43:101108.
25. Alsuwaidi AR, Hammad HA, Elbarazi I, et al. Vaccine hesitancy within the Muslim community: Islamic faith and public health perspectives. Hum Vaccin Immunother 2023;19:2190716.
26. Bergman A, McGee K, Farley J, et al. Combating stigma in the era of monkeypox: is history repeating itself? J Assoc Nurses AIDS Care 2022;33:668–75.
27. Mektebi A, Elsaid M, Yadav T, et al. Mpox vaccine acceptance among healthcare workers: a systematic review and meta-analysis. BMC Public Health 2024;24:4.
28. Ghazy RM, Hussein M, Abdu SM, et al. The intention of Egyptian healthcare workers to take the monkeypox vaccine: is urgent action required? BMC Health Serv Res 2024;24:1204.
29. Miraglia Del Giudice G, Della Polla G, Folcarelli L, et al. Knowledge and attitudes of health care workers about monkeypox virus infection in Southern Italy. Front Public Health 2023;11:1091267.
30. Boyle R. Language contact in the United Arab Emirates. World Englishes 2012;31:312–30.
31. Smith TC, Gorski DH. Infertility: a common target of antivaccine misinformation campaigns. Vaccine 2024;42:924–9.
32. Rosenstock IM. The health belief model and preventive health behavior. Health Educ Monogr 1974;2:354–86.
33. Jones CL, Jensen JD, Scherr CL, et al. The health belief model as an explanatory framework in communication research: exploring parallel, serial, and moderated mediation. Health Commun 2015;30:566–76.
34. Zampetakis LA, Melas C. The health belief model predicts vaccination intentions against COVID-19: a survey experiment approach. Appl Psychol Health Well Being 2021;13:469–84.
35. Ntawuyamara E, Ingabire T, Yandemye I, et al. Assessing healthcare workers' knowledge and confidence in the diagnosis, management and prevention of monkeypox. World J Clin Cases 2025;13:99884.
36. Sobaikhi NH, Alshahrani NZ, Hazazi RS, et al. Health workers' knowledge and attitude towards monkeypox in Southwestern Saudi

Arabia: a cross-sectional study. Diseases 2023;11:81.
37. Jahromi AS, Jokar M, Sharifi N, et al. Global knowledge and attitudes towards mpox (monkeypox) among healthcare workers: a systematic review and meta-analysis. Int Health 2024;16:487–98.
38. Amer FA, Nofal HA, Gebriel MG, et al. Grasping knowledge, attitude, and perception towards monkeypox among healthcare workers and medical students: an Egyptian cross-sectional study. Front Cell Infect Microbiol 2024;14:1339352.
39. Riad A, Drobov A, Rozmarinova J, et al. Monkeypox knowledge and vaccine hesitancy of czech healthcare workers: a Health Belief Model (HBM)-based study. Vaccines (Basel) 2022;10:2022.
40. Han Y, Wang X, Li X, et al. The willingness of healthcare workers to be vaccinated against monkeypox and their knowledge about monkeypox: a systematic review and meta-analysis. Heliyon 2024;10:e35196.
41. Swed S, Bohsas H, Patwary MM, et al. Knowledge of mpox and its determinants among the healthcare personnel in Arabic regions: a multi-country cross-sectional study. New Microbes New Infect 2023; 54:101146.
42. Malaeb D, Sallam M, Salim NA, et al. Knowledge, attitude and conspiracy beliefs of healthcare workers in Lebanon towards monkeypox. Trop Med Infect Dis 2023;8:81.
43. Alarifi AM, Alshahrani NZ, Sah R. Are Saudi healthcare workers willing to receive the monkeypox virus vaccine?: evidence from a descriptive-baseline survey. Trop Med Infect Dis 2023;8:396.
44. Du M, Deng J, Yan W, et al. Mpox vaccination hesitancy, previous immunisation coverage, and vaccination readiness in the African region: a multinational survey. EClinicalMedicine 2024;80:103047.
45. Jongen VW, Groot Bruinderink ML, Boyd A, et al. What determines mpox vaccination uptake?: assessing the effect of intent-to-vaccinate versus other determinants among men who have sex with men. Vaccine 2024;42:186–93.
46. Kiros T, Erkihun M, Wondmagegn M, et al. Assessment of knowledge, attitude, and associated factors of mpox among healthcare professionals at Debre Tabor Comprehensive Specialized Hospital, Northwest Ethiopia, 2024: a cross-sectional study. Health Sci Rep 2025;8:e70371.
47. AlBalas S, AlMaqableh HO, Al Smadi AN, et al. Assessing knowledge, attitude, and practice (KAP) towards monkeypox among healthcare workers in Jordan: a cross-sectional survey. Jordan J Pharm Sci 2024; 17:254–66.
48. Raccagni AR, Gianotti N, Moro M, et al. Mpox virus: control of in-hospital occupational transmission experience from a tertiary level hospital in Milan, Italy. Life (Basel) 2023;13:1705.
49. Ali Z, Malik A, Malik J, et al. Monkeypox vaccine hesitancy among healthcare workers in Pakistan. J Community Hosp Intern Med Perspect 2024;14:50–7.
50. Naik S, Vellappally S, Al Kheraif AA, et al. Monkeypox virus outbreak 2022: assessment of knowledge among healthcare workers in two countries—India and Saudi Arabia. Int J Clin Pract 2025;2025:1–7.
51. Yang Y, Zhang W, Han B, et al. Mpox knowledge and vaccination hesitancy among healthcare workers in Beijing, China: a cross-sectional survey. Vaccine X 2024;16:100434.
52. Lawrence A. Socio-demographic factors influencing monkeypox vaccination intentions among healthcare workers and the general population of Makurdi, Nigeria: a cross-sectional study. Cureus 2024; 16:e71828.
53. Yappalparvi A, Khatib MN, Balaraman AK, et al. Prevalence of mpox vaccine acceptance and hesitancy among people living with HIV: a comprehensive systematic review and meta-analysis. AIDS Res Ther 2025;22:31.
54. Ahmed SK, Mohamed MG, Dabou EA, et al. Monkeypox (mpox) in immunosuppressed patients. F1000Res 2023;12:127.
55. Sah R, Mohanty A, Reda A, et al. Stigma during monkeypox outbreak. Front Public Health 2022;10:1023519.

https://doi.org/10.24171/j.phrp.2025.0113

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2025.0103
Osong Public Health Res Perspect 2025;16(4):373-380

Original Article

Development and evaluation of the Korea Disease Control and Prevention Agency's scenario-based training program for effective response to emerging infectious disease outbreaks

Mi Yu[1], Eun Kyoung Kim[1], Kyungnam Kim[1], Jin Lee[1], Jin Hwan Jeon[1], Soyeon Kim[1], Sangwon Lee[2], Yunhyung Kwon[1]

[1]Division of Disease Control Capacity Building, Korea Disease Control and Prevention Agency, Cheongju, Republic of Korea
[2]Department of Disease Data Science, Korea Disease Control and Prevention Agency, Cheongju, Republic of Korea

ABSTRACT

Objectives: This study aimed to strengthen the core capabilities of epidemiological investigation and response teams (ERTs) at the Korea Disease Control and Prevention Agency, drawing on lessons learned from the Middle East respiratory syndrome and coronavirus disease 2019 pandemics. The primary objectives were to facilitate rapid and accurate data collection and analysis, improve communication skills, and simulate real-world on-site responses.
Methods: The training program was developed using a scenario involving a novel infectious disease from the World Health Organization priority list entering a community. ERTs collected and analyzed epidemiological data and implemented control measures as they addressed the missions of the scenario. Improvement in competencies was assessed through self-evaluation surveys completed before and after training.
Results: A total of 49 participants, including disease control officers, epidemiological investigation officers, researchers, and administrative officials, attended the training. Participant satisfaction with the training was high, scoring 6.4 out of 7, and the improvement in competency was statistically significant (pre-training score, 5.2; post-training score, 6.2; $p < 0.001$).
Conclusion: Scenario-based training can effectively enhance the capabilities of ERTs. Such training is essential, as emerging and re-emerging infectious diseases can exhibit unpredictable spread and potentially severe impacts. Therefore, developing diverse scenarios and conducting regular training sessions are necessary to improve the capacities of ERTs.

Keywords: Disease; Epidemiology; Investigation; Outbreak response; Training; Unknown

Received: March 26, 2025
Revised: May 7, 2025
Accepted: June 1, 2025

Corresponding author:
Yunhyung Kwon
Division of Disease Control Capacity Building, Korea Disease Control and Prevention Agency, 187 Osongsaengmyeong 2-ro, Osong-eup, Heungdeok-gu, Cheongju 28159, Korea
E-mail: yhhodori@korea.kr

Introduction

The expansion of cross-border trade and increased international travel have facilitated

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Korea Disease Control and Prevention Agency and Korea National Institute of Health.

© 2025 Korea Disease Control and Prevention Agency.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

the spread of infectious diseases across national borders, increasing the risk of a global pandemic. Infectious disease pandemics impose heavy burdens on healthcare systems and have substantial social and economic impacts. In particular, the coronavirus disease 2019 (COVID-19) pandemic has caused millions of deaths globally and overwhelmed healthcare systems, underscoring the urgent need to strengthen the capacities of health authorities and healthcare organizations to effectively combat infectious diseases [1,2].

Significant past pandemics and outbreaks, including severe acute respiratory syndrome (2003), H1N1 influenza (2009), Zika virus infection (2014), Middle East respiratory syndrome (2015), COVID-19 (2020), and Mpox (2022, previously known as monkeypox), have exerted substantial socioeconomic effects. The frequency and scale of such epidemics have increased, and infectious disease specialists warn of a high likelihood of another global pandemic in the near future [3–5].

The increasingly frequent occurrence and significant impacts of infectious disease outbreaks have highlighted the necessity of on-site response training for emerging, re-emerging, and unidentified diseases. Unidentified infectious diseases pose challenges due to initial diagnostic difficulties and uncertainties regarding transmission and infection mechanisms, complicating prompt responses by health authorities [6,7]. In Korea, trainings and drills are regularly held for public officials to respond to emerging and re-emerging infectious diseases. However, the field capabilities of personnel directly involved in epidemiological investigations require improvement [8]. Epidemiological investigation and response teams (ERTs), critical to managing disease crises, must swiftly reach outbreak sites and implement effective control measures. However, due to limited practical experience, such as in patient interviews and associated epidemiological data collection, rigorous scenario-based training is essential to hone their response skills. Therefore, this study aimed to develop a training scenario for responding to an unknown infectious disease outbreak and to evaluate its effectiveness in improving the practical skills of response personnel. Recognizing the critical role of ERTs in mitigating harm and controlling outbreaks, especially of emerging diseases, the Korea Disease Control and Prevention Agency (KDCA) has analyzed various domestic and international situations. Accordingly, the agency has developed scenarios to practice implementing preventive measures and integrated these into its training program [9]. These scenarios are designed to simulate real-world situations, requiring trainees to navigate a wide range of variables and uncertainties. In the present study, after the training was administered, a self-evaluation was used to assess participants' changes in awareness and response capabilities. Based on the results, this study aimed to identify areas for future improvement of outbreak response training.

HIGHLIGHTS

- This study aimed to improve the competencies of epidemiological investigation and response teams through scenario-based training, enabling rapid and accurate data collection and analysis.
- The scenario-based training program produced significant improvements in participant competencies, with self-evaluation scores increasing from 5.2 to 6.2 (p<0.001).
- Regular scenario-based training is crucial to effectively prepare for unpredictable infectious disease outbreaks.

Materials and Methods

Exercise Framework

The purpose of this exercise was to enhance the capacity of ERTs to effectively respond to infectious disease outbreaks in the field. Specifically, the training was designed to familiarize each ERT member with their roles and responsibilities when deployed for field epidemiological investigations and responses, develop their skills regarding information gathering and data collection (e.g., interviewing), familiarize them with response procedures, train them in implementing evidence-based control measures to curb the spread of infection, and strengthen their communication and teamwork skills.

The training lasted 7 hours and was conducted in a hands-on format, utilizing hypothetical scenarios that closely mirrored real-world field situations. It covered the entire process, from pre-deployment to the implementation of disease control measures. Each participant was assigned a specific role and actively engaged in resolving scenario-based missions. After the training, outcomes from each team were presented and evaluated by external experts. At the end of the session, a self-evaluation survey was administered (Figure 1).

Participants

The ERTs consisted of 145 public officials, including epidemiological investigation officers (EIOs) and administrative officials (as of July 24, 2024). A total of 49 participants (33.8% of the ERT personnel) attended this training. The trainees were divided into 6 teams, each with 7 to 8 members.

https://doi.org/10.24171/j.phrp.2025.0103

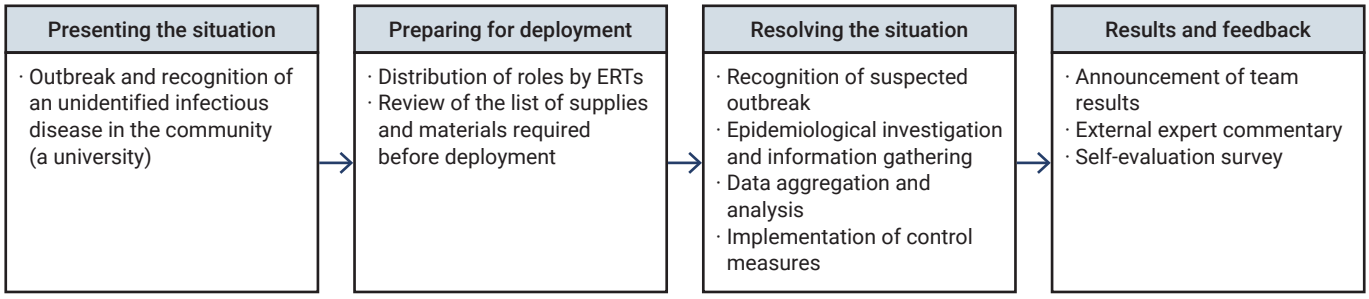


Figure 1. Flowchart of the training process. ERT, epidemiological investigation and response team.

Materials

Exercise scenario

The scenarios in the exercise were developed internally to simulate a localized outbreak of an unidentified infectious disease. These scenarios covered the entire field-based information collection process, including data compilation and analysis, as well as the implementation of on-site control measures. The exercise was divided into a pre-deployment and a post-deployment mission, with 3 contingencies assigned during the second mission. The scenario involved an outbreak of an unidentified infectious disease at a university. Initially, a student developed symptoms including tingling in the hands and a cough, which were not immediately recognized as signs of an infectious disease. However, after a university festival, many more students at the university and its affiliated high school rapidly developed similar symptoms. In response, the KDCA mobilized an ERT to the site. The scenario included 3 contingencies: first, a mutated case of the Hendra virus was detected abroad, prompting the World Health Organization to declare it a serious public health threat; second, a suspected Hendra virus case was reported in Korea and information was leaked from laboratory testing conducted by public health authorities, resulting in unexpected media coverage; and third, the university expressed concerns that the outbreak might disrupt students' upcoming final exams, both at the university and nationally. Further details of the scenario are provided in Supplementary Material 1.

Self-evaluation survey for training

An evaluation survey was developed to assess the effectiveness of the training. The survey included 25 items, scored on a 7-point Likert scale (1=strongly disagree, 7=strongly agree), covering training satisfaction, pre-training understanding, and on-site response competencies. The survey tool was developed through internal discussions among the authors, ensuring that the items effectively reflected the extent to which training objectives were achieved. The tool was developed with reference to previous studies, which suggest that using a 7-point Likert scale, rather than a 5-point scale, tends to yield more accurate responses due to reduced central tendency bias. Additionally, the 7-point scale is associated with fewer careless or overly consistent responses, thus increasing the reliability of the assessment.

The items assessing pre-training understanding were intended to evaluate participants' perceptions of the on-site response team's role and their competencies in infectious disease response before training. Items related to data collection and assessment measured whether the exercise improved participants' skills in information gathering and data assessment. Items on field investigation competencies evaluated participants' understanding of defining investigation cases and conducting contact tracing during a suspected outbreak. Items assessing situation analysis and problem-solving skills measured participants' abilities to apply evidence-based recommendations for control measures and to identify additional suspected cases. Lastly, items on communication and collaboration evaluated participants' perceptions of the importance of communicating findings and their experiences collaborating with teammates.

The full list of survey items is provided in Supplementary Material 2.

Statistical analysis

Descriptive statistics were calculated for continuous variables, including mean, standard deviation, minimum, and maximum values. Frequencies and percentages were calculated for nominal variables. The difference between pre- and post-test competencies was analyzed using a paired samples t-test. Statistical analysis was performed using jamovi ver. 2.6.24 for Windows (https://www.jamovi.org/).

Ethics Approval

This study qualifies for exemption from institutional review by the KDCA Institutional Review Board (IRB), in accordance

with the agency's IRB guidelines. The study used publicly available information and did not collect identifiable personal data from participants. It involved no direct manipulation of human participants, nor did it include research on individuals in vulnerable settings.

Results

General Characteristics of Exercise Participants

The mean age of the participants was 41.4 years, with a mean work experience of 4 years and 5 months. Of the participants, 18 (36.7%) were male and 31 (63.3%) were female. Seven were disease control officers (14.3%), 31 were EIOs (63.3%), 8 were public health researchers (16.3%), and 3 were administrative officials (6.1%). Regarding training, 32 participants (66.7%) had completed the Korea Field Epidemiology Training Program, 3 participants (6.3%) were currently enrolled, and for 13 participants (27.1%), the program was either not started or not applicable. For affiliations, 2 participants (4.1%) were from a quarantine station, 15 (30.6%) were from regional disease control centers, and 32 (65.3%) were from KDCA headquarters.

Regarding on-site response experience since January 2023, 23 participants (46.9%) had participated in 1 to 5 field responses, while 12 participants (24.5%) had no field response experience. Concerning prior central ERT training, first-time attendees were the largest group, totaling 20 participants (40.8%), followed by 14 participants (28.6%) with 2 prior training experiences and 9 participants (18.4%) who had attended once previously (Table 1).

Exercise Task Performance Results

Mission 1: pre-deployment tasks

The first task was to define the scope of the initial field response debriefing. All teams emphasized the importance

Table 1. General characteristics of training participants ($n = 49$)

Characteristic	Value
Sex	
Male	18 (36.7)
Female	31 (63.3)
Age (y)	41.4±7.3
Experience in infection disease control and response (mo)	53.2±31.9
Position	
Disease control officers	7 (14.3)
EIOs	31 (63.3)
Public health researchers (senior researchers, laboratory scientists, etc.)	8 (16.3)
Administrative and public health officials	3 (6.1)
KFETP course	
Completed	33 (67.3)
In progress	3 (6.1)
Not started or not applicable	13 (26.5)
Affiliation	
KDCA headquarters	32 (65.3)
Regional disease control center	15 (30.6)
Quarantine station	2 (4.1)
No. of on-site response experiences (since January 2023)	
1–5	23 (46.9)
6–10	5 (10.2)
≥11	9 (18.4)
None	12 (24.5)
Participation in previous central ERT training (since 2023)	
None	20 (40.8)
1	9 (18.4)
2	14 (28.6)
3	6 (12.2)

Data are presented as *n* (%) or mean±standard deviation.
EIO, epidemiological investigation officer; KFETP, Korea Field Epidemiological Training Program; KDCA, Korea Disease Control and Prevention Agency; ERT, epidemiological investigation and response team.

https://doi.org/10.24171/j.phrp.2025.0103

of collaboration among health officials, schools, and healthcare providers during an outbreak. Additionally, the Health and Environmental Research Institute, a diagnostic testing organization, was involved from the start of the project, highlighting the necessity of active diagnosis.

The second task was to request and collect necessary information prior to the meeting. Each member submitted requests to the healthcare center, university, and healthcare organizations regarding the first suspected cases. This included student enrollment lists for relevant courses, dormitory occupancy data, drug utilization review information, and patient movement data gathered via the KDCA Epidemiological Investigation Support System.

The third task was to organize the roles of the field response teams. Within each team, members were designated to conduct in-person interviews with the index case, healthcare workers, and a school health officer. Interviews with the index case were designed to determine patient movement routes, symptom onset dates, and post-interview control plans. Interviews with healthcare workers were used to verify patient medical records, including symptoms, test results, and diagnoses. They also assessed specimen availability, contacts within the healthcare organization, and protective equipment use among healthcare providers. Interviews with school health staff aimed to identify potential areas of exposure within the school, including lists of students who had contact with the patient, students who shared a dormitory with the patient, visitors to the health room, and shuttle bus users. The team leader coordinated task allocation, liaised with headquarters and relevant agencies, and thoroughly reviewed the investigation findings.

During the field epidemiological interviews, certain participants were tasked with inspecting their preparation lists for field investigation. Many teams listed personal protective equipment and laptops, while others emphasized accurate information gathering by including items such as recorders. Certain teams focused on specimen collection and analysis, incorporating environmental culture containers into their methodologies. Additionally, a few teams considered equipment such as ventilation measurement devices, while others prioritized simpler information-gathering tools.

Mission 2: outbreak control tasks

The first task involved discussing the criteria for identifying suspected cases to investigate, as well as the methodology for reporting any suspected cases or symptoms observed. All teams agreed that cases with observed symptoms and an epidemiological link should meet the selection criteria. Some teams proposed a broader approach, allowing symptomatic individuals and their family members to report directly.

The second task was establishing a framework to prioritize individuals for testing. Typically, individuals exhibiting symptoms and those who had close contact with confirmed cases were prioritized. Some teams prioritized healthcare workers who treated patients or individuals in communal settings such as dormitories, whereas others emphasized symptomatic individuals within schools or the broader community. Several sample collection options were proposed, including the use of negative-pressure rooms in healthcare facilities and the establishment of temporary testing stations at schools and public health centers.

The third task involved discussing control measures related to schools. While most teams concluded that school closures were unnecessary, some recommended closures due to potential community impact. Those favoring school closures advised a closure period of 16 days, considering the incubation period, or up to 18 days post-exposure to prevent additional cases. Additionally, given the widespread fear surrounding the unidentified infectious disease and its potential disruption of academic events like final and national exams, each team considered various alternative strategies. These included separating infected from non-infected students during exams and implementing non–face-to-face educational methods.

The fourth task was focused on identifying key criteria for selecting individuals for isolation or determining whether to implement active or passive surveillance. Given the disease's high fatality rate and potential for person-to-person transmission, all teams stressed the importance of preventing further spread and reducing the severity of illness. Teams recommended adaptable surveillance intensity, particularly when no clear epidemiological link was evident. Isolation methods for quarantined individuals were chosen based on symptom severity, including inpatient isolation at a healthcare facility or home isolation.

The final task was centered on developing a tracking method to identify additional suspected cases. Teams proposed various surveillance and tracking strategies, such as expanding community surveillance networks, encouraging early reporting via short message service and press releases, strengthening border quarantine measures, and improving risk communication management. Additionally, some teams evaluated the epidemiological correlation between the index case's overseas travel history and symptom onset, using this information to refine infection prevention strategies.

Self-Evaluation Results for Training

Each item in the self-evaluation survey was rated on a scale from 1 to 7. The mean score for pre-training understanding

was 5.2, while the score for pre-training capacity was 4.9. Post-training scores, used to assess improvement in competencies, were as follows: 6.1 for field information gathering and management, 6.1 for field investigation, 6.2 for situation analysis and problem-solving skills, and 6.3 (the highest score) for communication and cooperation. The mean score for training satisfaction was 6.4 (Table 2).

A comparison between pre-training understanding and post-training competency scores revealed a significant improvement from 5.2 ± 1.4 to 6.2 ± 0.8 ($p < 0.001$) (Table 3).

Discussion

Unlike previous ERT exercises, this training closely simulated real field scenarios. Information collected through on-site interviews with the index case, healthcare workers, and school health staff was compiled and analyzed to inform the control measures implemented. Contingencies were developed to provide a framework for anticipating and managing potential challenges in the field. Notably, each interview site was designed to mimic a real-world situation, enabling the team to practice handling difficulties in gathering and addressing field information. Scenario-based training programs like this have become increasingly popular in healthcare education, promoting effectiveness in crisis response preparedness through repeated exposure to realistic scenarios [10].

This training program was particularly meaningful because it involved not only EIOs but also disease control officers, scientific researchers, laboratory specialists, and administrative officials, thereby strengthening the communication capabilities of the entire organization during a crisis response. As response training is designed to improve preparedness by integrating various organizational components [11,12], this program aligns well with the goals of other major training initiatives.

Furthermore, as demonstrated by the ongoing contributions of Field Epidemiology Training Programs to COVID-19 responses and broader global infectious disease control efforts, structured and practice-oriented programs such as scenario-based exercises play a pivotal role in building

Table 3. Comparison of competencies between pre- and post-training (paired samples t-test)

	Pre-training	Post-training	*p*
Self-evaluation of competencies	5.2 ± 1.4	6.2 ± 0.8	< 0.001

Data are presented as mean ± standard deviation.

Table 2. Results of self-evaluation (7-point scale) ($n = 49$)

	Mean ± SD	Min–max
Pre-training understanding	5.2 ± 1.4	1.5–7
Understanding of the role and mission of field response	5.5 ± 1.4	1–7
Self-capacity of infectious disease field response	4.9 ± 1.6	1–7
Post-training self-evaluation of competencies (1–4)	6.2 ± 0.8	4–7
1. On-site information gathering and management	6.1 ± 0.9	4–7
Collecting necessary information more effectively than before	6.1 ± 1.0	4–7
Managing collected data more systematically than before	6.1 ± 0.9	4–7
2. On-site investigation competencies	6.1 ± 0.9	4–7
Understanding the process of case definition and case selection	6.1 ± 1.1	3–7
Understanding and conducting contact investigation and tracing	6.1 ± 0.9	4–7
3. Situational analysis and problem solving	6.2 ± 1.0	4–7
Evidence-based control measure suggestions	6.2 ± 0.9	4–7
Understanding various methods to identify additional suspected cases	6.1 ± 1.1	3–7
4. Communication and collaboration competencies	6.3 ± 0.9	4–7
Effectively communicating the results of investigations	6.3 ± 1.0	4–7
Understanding the importance of collaborating effectively with colleagues	6.4 ± 0.9	4–7
Satisfaction with the training	6.4 ± 0.8	4–7
Usefulness of the training to infectious disease outbreak response	6.2 ± 1.3	1–7
Attainment of the training goals	6.5 ± 0.8	4–7
Adequate understanding of the training content	6.4 ± 0.8	4–7
Usefulness of educational materials and method	6.4 ± 1.0	3–7
Seamless provision of guidance and support for the training	6.5 ± 0.7	4–7

SD, standard deviation.

https://doi.org/10.24171/j.phrp.2025.0103

frontline response capabilities [13].

Importantly, we confirmed the effectiveness of the training through a comparison of pre-training understanding and post-training competency self-evaluation scores. One limitation of our analysis is that the pre- and post-training evaluations did not consist of identical questions, potentially hindering the direct comparability of the results. However, since both evaluations assessed overall competencies, we believe the comparison remains meaningful.

External experts who observed the entire training program noted that it appeared to closely replicate real-world outbreak responses, incorporating elements such as uncertainty and time constraints in the context of a mutant virus capable of person-to-person transmission. Additionally, they emphasized that responding to an unidentified infectious disease requires careful, rapid, and consistent decision-making, especially when scientific evidence about the emerging disease is limited. The group presentations also provided diverse learning opportunities by allowing teams to share results and insights.

ERTs consist of various professionals, including disease control officers; however, only 31% of disease control officers attended this training. Future training efforts should encourage and increase attendance to ensure a more integrated response across the agency.

Since an efficient response to a disease pandemic crisis requires a well-coordinated system involving multiple ministries [14], future training programs should also expand to integrate ERTs with other relevant agencies.

The developed scenario has been officially registered as intellectual property with the Korea Copyright Commission (January 14, 2025). Moving forward, efforts will focus on developing diverse training topics and methods, incorporating these into training programs, and integrating them into an ERT scenario bank. The competencies of local governmental ERTs will be enhanced through standardized training based on these developed scenarios.

The KDCA will continue to strengthen response capabilities for infectious disease outbreaks to safeguard public health against future outbreaks caused by unidentified infectious agents. Strengthening preparedness and response mechanisms is essential for effectively managing future public health emergencies.

Conclusion

This training program was found to be highly effective, as it simulated a realistic response environment, enhanced participant competencies, and fostered greater communication and collaboration among disease control officers, EIOs, laboratory scientists, scientific researchers, and public officials. The notable improvement in participants' self-assessed competency scores from pre- to post-training underscores the program's success in preparing responders for crisis situations. Future training initiatives should expand to include other relevant ministries and refine scenarios to address emerging threats. Continued development of diverse training topics and scenarios will further strengthen the capabilities of both central and local ERTs, ensuring more effective responses to infectious disease emergencies.

Supplementary Material

Supplementary Material 1. Development scenario. **Supplementary Material 2.** Self-evaluation survey for the training. Supplementary data are available at https://doi.org/10.24171/j.phrp.2025.0103.

Notes

Ethics Approval
Not applicable.

Conflicts of Interest
The authors have no conflicts of interest to declare.

Funding
None.

Availability of Data
All study-related data are included in the publication or provided as supplementary information.

Authors' Contributions
Conceptualization: MY, EKK, YK, KK; Formal analysis: MY; Software: JL, JHJ, SK; Supervision: EKK, YK; Validation: YK, SL, MY, EKK; Methodology: MY, YK, EKK, KK, JL, JHJ; Writing–original draft: MY, EKK, YK; Writing–review & editing: all authors. All authors read and approved the final manuscript.

References

1. Chung H, Kim S, Choi H, et al. Trends and changes in social risks following the COVID-19. Korea Institute for Health and Social Affairs; 2023. Korean.
2. Gi YH, Ko M. A study on analyzing the results of non-pharmaceutical intervention of COVID-19 and suggesting policy countermeasures applicable to future, infectious disease epidemics. National Evidence-Based Healthcare Collaborating Agency; 2024. Korean.
3. Coulson M. Defining disease X [Internet]. Johns Hopkins Bloomberg School of Public Health; 2024 [cited 2024 Feb 15]. Available from: https://publichealth.jhu.edu/2024/what-is-disease-x.
4. Alfani G, Murphy TE. Plague and lethal epidemics in the pre-industrial world. J Econ Hist 2017;77:314–43.
5. Centers for Disease Control and Prevention (CDC) (US). Outbreaks [Internet]. CDC; 2024 [cited 2024 Feb 15]. Available from: https://

www.cdc.gov/outbreaks/index.html.
6. Iserson KV. The next pandemic: prepare for “Disease X”. West J Emerg Med 2020;21:756–8.
7. World Health Organization (WHO) (CH). Research response to pathogen X during a pandemic [Internet]. WHO; 2024 [cited 2024 Feb 15]. Available from: https://www.who.int/news-room/events/detail/2024/01/19/default-calendar/Research-response-to-pathogen-X-during-a-pandemic.
8. Korea Disease Control and Prevention Agency (KDCA) (KR). Internal report, improvement plan for the operation of central and local epidemiological investigation teams. KDCA; 2024.
9. World Health Organization (WHO) (CH). WHO R&D roadmaps [Internet]. WHO; 2024 [cited 2024 Feb 15]. Available from: https://www.who.int/observatories/global-observatory-on-health-research-and-development/analyses-and-syntheses/who-r-d-blueprint/who-r-d-roadmaps.
10. Baek HJ, Kim YM, Jung SK, et al. Development and pilot applications of simulation-based emergency department crisis management team training program. J Korean Soc Emerg Med 2008;19:603–10.
11. Kim IH, Jang JH, Jo SK, et al. 2019 Tabletop exercise for laboratory diagnosis and analyses of unknown disease outbreaks by the Korea Centers for Disease Control and Prevention. Osong Public Health Res Perspect 2020;11:280–5.
12. Kim YS, Kim HM, So BH, et al. Experience of the simulation based hospital disaster preparation training program by the polls of before and after training program. J Korean Soc Emerg Med 2016;27:618–32.
13. Bell E, Mittendorf C, Meyer E, et al. Continuing contributions of field epidemiology training programs to global COVID-19 response. Emerg Infect Dis 2022;28:S129–37.
14. Choa M, Kang H. Education and training in disaster medicine. Hanyang Med Rev 2015;35:174–9.

https://doi.org/10.24171/j.phrp.2025.0103

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2025.0102
Osong Public Health Res Perspect 2025;16(4):381-391

Original Article

Impact of direct prehospital transport on mortality in patients with severe trauma based on the injury severity score: a nationwide observational study in the Republic of Korea

Sun Ju Kim[1], KeunKyun Kim[2], Oh Hyun Kim[1], Chan Yong Park[3]

[1]Department of Emergency Medicine, Yonsei University Wonju College of Medicine, Wonju, Republic of Korea
[2]Cancer Data Center, National Cancer Control Institute, National Cancer Center, Seoul, Republic of Korea
[3]Department of Surgery, Seoul National University College of Medicine, Seoul, Republic of Korea

ABSTRACT

Objectives: Severe trauma remains a leading cause of death in the Republic of Korea. In response, regional trauma centers were established in 2012. This study evaluated the impact of direct prehospital transport to trauma centers on in-hospital mortality among patients with severe trauma using the national trauma registry.

Methods: This nationwide observational study utilized data from the Korean community-based trauma registry between 2016 and 2020. Patients with an injury severity score >15 who were directly transported from the prehospital setting were included. Variables analyzed encompassed demographics, injury mechanism, transport time, hospital level, and outcomes. The primary outcome was in-hospital mortality. Both logistic regression and decision tree models were employed.

Results: A total of 24,567 patients were included. Overall mortality decreased by 5.2% during the study period. Patients transported to level 1 or 2 trauma centers had a lower mortality rate (23.6%) compared to those transported to level 3 or 4 hospitals (28.0%). The proportion of direct transports to high-level centers increased from 46.7% to 64.1% between 2016 and 2020. Despite longer transport times, patients transported to high-level institutions exhibited improved survival, particularly when transport exceeded 30 minutes, suggesting that hospital selection may be more critical than minimizing transport time.

Conclusion: Direct transport to high-level trauma centers improves survival among patients with severe trauma, even when transport times are prolonged. These findings support the importance of a well-organized trauma system that emphasizes hospital capability in prehospital triage decisions.

Keywords: Emergency medical services; Injury severity score; Korea; Mortality; Trauma centers

Received: April 27, 2025
Revised: June 29, 2025
Accepted: June 30, 2025

Corresponding author:
Chan Yong Park
Department of Surgery, Seoul National University College of Medicine, 103 Daehak-ro, Jongno-gu, Seoul 03080, Republic of Korea
E-mail: trauma-park@naver.com

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Korea Disease Control and Prevention Agency and Korea National Institute of Health.

© 2025 Korea Disease Control and Prevention Agency.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Severe trauma is a leading cause of death worldwide [1]. It requires a national, systematic approach due to its significant social and economic impact, particularly among younger patients, resulting in substantial national losses and social disruption [2]. In the Republic of Korea, severe trauma is among the 3 major causes of death, along with cancer and cardiovascular disease. As of 2010, the preventable trauma death rate was approximately 30%, notably higher than in high-income countries, which led to the creation of a national trauma treatment system [3–5].

In response, the Republic of Korea launched the nationwide regional trauma center (RTC) initiative in 2012 to improve the identification and prioritization of patients with severe trauma. RTCs have been operational since 2014. According to Kwon et al. [6], the Republic of Korea's preventable trauma death rate declined to 15.7% by 2019, with RTCs playing a key role in improving survival rates. However, most recent studies on severe trauma include patients who were transferred between facilities. While these studies highlight overall survival improvements, they offer limited insight into outcomes for patients assessed and transported directly from the prehospital setting.

Given its impact on patient prognosis, the American College of Surgeons recommends that patients with severe trauma be classified and triaged at the prehospital stage, with transport to an appropriate facility, such as a level 1 trauma center [7]. Hospital location, regional characteristics, trauma center classification, and injury mechanisms all play important roles in trauma mortality research. The concordance rate between prehospital and hospital classifications for severe trauma is typically defined using an injury severity score (ISS) threshold of 15, though international differences complicate direct comparisons [8]. Furthermore, few studies have examined outcomes for patients with severe trauma who are directly transported from the prehospital stage in the Republic of Korea.

Therefore, this study analyzed the impact of direct prehospital transport on outcomes and mortality in patients with ISS >15 using national data.

HIGHLIGHTS

- This nationwide study analyzed mortality outcomes among patients with severe trauma (injury severity score >15) who were directly transported from the prehospital stage, utilizing national trauma registry data from 2016 to 2020.
- Patients transported to high-level institutions (level 1 or 2) experienced significantly lower mortality compared to those transported to lower-level hospitals, even when transport times exceeded 30 minutes.
- The findings underscore the importance of a well-organized trauma system and suggest that selecting an appropriate hospital is more crucial than minimizing transport time in trauma care.

Materials and Methods

Study Design and Setting

This community-based observational study utilized data from a nationwide trauma registry in the Republic of Korea. The country has a population of 51.7 million and covers an area of 100,210 km^2, comprising 17 administrative provinces. The emergency medical service (EMS), operated by the Korea National Fire Agency, provides care throughout all regions, managing a range of conditions from minor injuries to severe trauma and cardiac arrest.

In the Republic of Korea, EMS personnel are authorized to perform advanced airway management and fluid resuscitation under physician supervision. However, all patients must be transported to a hospital, as EMS providers do not have the authority to terminate resuscitation efforts. EMS personnel complete ambulance run sheets for every transport. The Ministry of Health and Welfare has developed a network of RTCs, establishing 17 centers, with 9 officially operational by 2019. According to EMS transport protocols, patients identified as having severe trauma are recommended for transfer to the nearest regional trauma or emergency medical center [9].

Data Source

Community-based Severe Trauma Surveillance was first implemented in 2 regions in 2013 and expanded nationwide in 2016 by the Korea Disease Control and Prevention Agency. This study analyzed data from surveys conducted between 2016 and 2020. The surveillance included all cases classified as severe trauma by the national EMS, focusing on hospital transfers and treatment outcomes. A total of 700 medical institutions participated, and patient severity was objectively classified using the ISS system, with national expert investigators overseeing the classification process.

Patients with Severe Trauma in the Prehospital Stage Based on the Registry

In the Republic of Korea, since 2013, detailed records of EMS treatment for patients with severe trauma have

https://doi.org/10.24171/j.phrp.2025.0102

been maintained to improve the quality of EMS activities. Patients included in the "severe trauma detailed treatment" registry are those classified as having severe trauma. This study analyzed all patients recorded in this registry. EMS categorizes severe trauma according to 4 criteria: (1) physiological findings—systolic blood pressure <90 mmHg, respiratory rate <10 or >29, or altered mental status based on verbal response; (2) physical examination findings—penetrating or stab wounds, chest wall deformity, suspected long bone fractures (>2), crush injuries, pulseless wounds, amputations, suspected pelvic fractures, open skull fractures, traumatic paralysis, and similar conditions; (3) mechanism of injury—including falls, high-energy vector traffic accidents, passenger vehicle accidents, motorcycle accidents, bicycle accidents, and others; and (4) additional EMS-based considerations.

Exclusion Criteria

Patients were excluded if there was a collection error in time data (such as invalid timestamps like "99:99" or a 14-month date), errors in emergency room arrival time (for example, if the emergency department [ED] arrival time was recorded as earlier than the EMS dispatch request time), cases without traumatic mechanisms or those exposed to non-trauma-related events (including thermal injuries, respiratory threats, exposure to chemicals or other substances, physical overexertion, or unspecified categories), ISS score <16, or unknown ISS. Patients with prehospital cardiac arrest due to severe trauma were also excluded, as these cases are associated with a high mortality rate and different mortality patterns [10].

Data Collection and Variables

Collected data included general characteristics (age, sex, region, and hospital transfer level), accident details (time of accident, rescue request, EMS arrival, and ED arrival), intentionality, toxic exposure, and trauma mechanisms (traffic accidents, falls/slips, violence, blunt trauma, penetrating trauma, mechanical injury, thermal injury, and chemical injury). Trauma locations were categorized as home, medical institutions, schools, pathways, industrial sites, farms, public spaces, commercial facilities, and outdoor areas. ISS scores and patient disposition outcomes (mortality) were also recorded. Time-related variables included EMS activation time, EMS scene arrival time, and ED arrival time. "Time to ED arrival" was defined as the interval from EMS activation to ED arrival. To accurately assess prehospital transport time, patients transferred from other healthcare facilities were excluded during initial data curation. The study period was divided into 2016–2017 and 2018–2020 to reflect major policy changes implemented in 2018, including revisions to the EMSs Act and the Ministry of Health and Welfare's initiative to strengthen severe trauma care.

Institution types were categorized according to the National Medical Center Statistical Yearbook: (1) level 1, RTCs; level 2, regional emergency centers; level 3, local emergency centers; and level 4, local emergency facilities.

"High-level medical institutions" were defined as level 1 and level 2 facilities, while "lower-level medical institutions" referred to level 3 and level 4 facilities. High-level institutions serve as definitive treatment centers within a regional system, equipped with specialized personnel, facilities, and resources required for advanced care in severe emergency cases. By contrast, lower-level institutions function as frontline or intermediary centers within local communities, primarily managing mild to moderate emergency cases and ensuring the timely transfer of critically ill patients to higher-level centers.

Regional categorization included metropolitan areas such as Seoul, Gyeonggi Province, Incheon, and other regions [11].

Outcomes

The primary outcome was to assess changes in mortality among patients with severe trauma (ISS >15) who were directly transported from the prehospital stage in the Republic of Korea. The secondary outcome was to analyze factors influencing mortality. Mortality was determined at hospital discharge through review of medical records.

Statistical Analysis

To examine the association between patient characteristics and mortality, a cross-analysis was performed. Additionally, binary logistic regression analysis was conducted to assess the effect of specific variables on mortality and determine the relative importance of each factor. A stepwise selection method was used to identify the key variables in the model. Decision tree analysis was used to visualize decision-making rules based on the independent variables most strongly associated with mortality in patients with severe trauma. This technique, widely employed in data mining, enables the integration of expert knowledge into domain-specific analyses [12]. It was applied for group classification and outcome prediction.

All analyses were conducted using SAS ver. 9.4 (SAS Institute Inc.). for data cleaning and cross-analysis, and SAS Enterprise Miner 9.4 for binary logistic regression and interactive decision tree analysis.

Ethics Statement

This study protocol was reviewed and approved by National Approval Statistics (approval number: 117105), and the requirement for informed consent was waived.

Results

Patient Flow

Figure 1 illustrates the patient selection process from the community-based severe trauma surveillance dataset in the Republic of Korea between 2016 and 2020. Initially, 237,616 patients were identified. After excluding 2,321 patients due to collection errors or missing time data—including 2,311 cases with implausible time entries (e.g., "99 hours 99 minutes" or "14th month") and 10 cases where emergency room arrival times were recorded as earlier than EMS dispatch requests—an additional 71,490 patients without traumatic mechanisms or those exposed to non-trauma-related events (such as chemical exposure, physical exertion, or unspecified causes) were excluded. This resulted in 163,805 patients with suspected severe trauma at the prehospital stage. Subsequently, 139,238 patients were excluded based on the following criteria: 2,902 patients with unknown ISS, 118,739 patients with ISS less than 16, and 17,597 patients who had out-of-hospital cardiac arrest or prehospital death. Ultimately, 24,567 patients met the inclusion criteria. The annual distribution was as follows: 4,454 patients in 2016, 4,558 in 2017, 5,097 in 2018, 5,493 in 2019, and 4,965 in 2020.

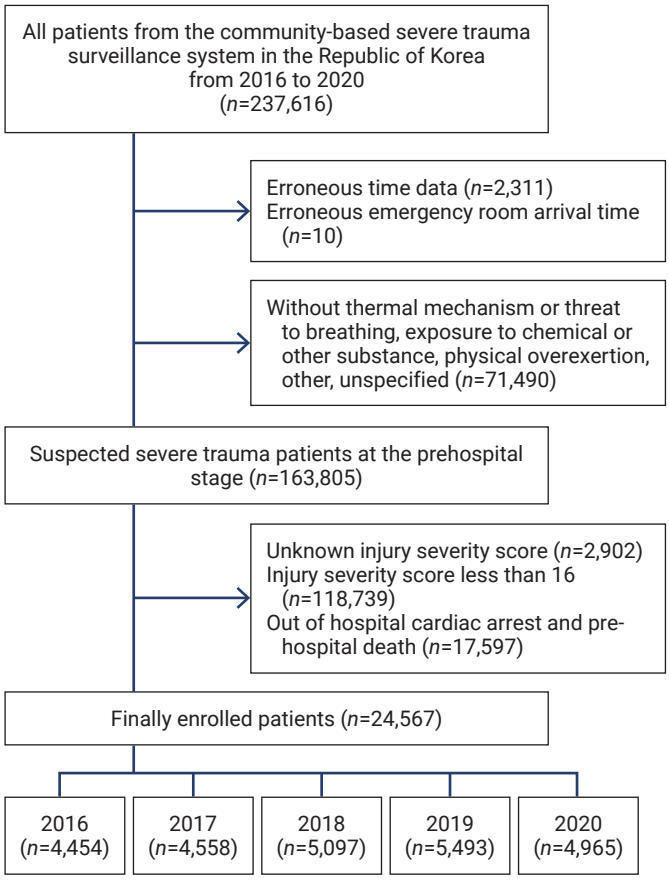


Figure 1. Flow diagram of patient selection for this study.

General Characteristics

Baseline characteristics for the survival and mortality groups are shown in Table 1. Among female patients, 4,449 (71.7%) survived, while 4,509 male patients (24.6%) died. Among patients aged ≥55 years, 4,285 (31.6%) died, and in higher-level medical institutions (levels 1 and 2), 3,352 patients (23.6%) died. During 2018–2020, 11,884 patients (76.4%) survived, and among those with an ISS of 25–75, 3,768 (39.5%) died (Table 1, Table S1).

Comparison of Survival-Mortality by ISS Score Group, Hospital Type, and Year

Table 2 presents survival and mortality rates among patients with severe trauma, categorized by hospital type and ISS, across 2 time periods: 2016–2017 and 2018–2020. In level 1 and 2 hospitals, survival rates were 73.7% (3,108 patients) in 2016–2017 and 77.5% (7,727 patients) in 2018–2020. In level 3 and 4 hospitals, survival rates were 69.0% (3,313 patients) in 2016–2017 and 74.5% (4,157 patients) in 2018–2020. During 2016–2017, 2,107 (46.7%) patients visited level 1 and 2 hospitals annually, while 2,399 (53.3%) visited level 3 and 4 hospitals. In 2018–2020, 3,324 (64.1%) patients visited level 1 and 2 hospitals annually, compared to 1,860 (35.9%) who visited level 3 and 4 hospitals.

By ISS grouping, among patients with an ISS of 25–75, the mortality rate was 43.5% (1,550 patients) in 2016–2017 and 37.1% (2,218 patients) in 2018–2020 (Table 2, Table S2).

Factors Associated with Mortality in Patients with Severe Trauma

Compared to female patients (reference group), male patients had a slightly lower risk of mortality (Exp(B) = 0.921; 95% confidence interval [CI], 0.857–0.989; $p = 0.02$). Age was a strong predictor of survival: patients aged 15–54 years (Exp(B) = 0.440; 95% CI, 0.411–0.471; $p < 0.001$) and those aged <15 years (Exp(B) = 0.497; 95% CI, 0.385–0.642; $p < 0.001$) had significantly lower mortality risk compared to those aged ≥55 years. Patients residing in metropolitan areas (Seoul, Gyeonggi Province, Incheon) had a reduced risk of mortality compared to those in other regions (Exp(B) = 0.920; 95% CI, 0.862–0.982; $p = 0.01$). Treatment at higher-level institutions (level 1 or 2) was associated with a significantly lower risk of death than treatment at lower-level facilities (level 3 or

https://doi.org/10.24171/j.phrp.2025.0102

Table 1. General characteristics of analysis subjects

Characteristic	Category	Survival	Death
Sex	Male	13,856 (75.4)	4,509 (24.6)
	Female	4,449 (71.7)	1,753 (28.3)
Age (y)	<15	346 (78.5)	95 (21.5)
	15–54	8,688 (82.2)	1,882 (17.8)
	≥55	9,271 (68.4)	4,285 (31.6)
Region[a)]	Metropolitan area	6,936 (75.3)	2,276 (24.7)
	Urban area	11,369 (74.0)	3,986 (26.0)
Hospital type[b)]	Level 1, 2	10,835 (76.4)	3,352 (23.6)
	Level 3, 4	7,470 (72.0)	2,910 (28.0)
Year	2016, 2017	6,421 (71.2)	2,591 (28.8)
	2018–2020	11,884 (76.4)	3,671 (23.6)
ISS	16–24	12,534 (83.4)	2,494 (16.6)
	25–75	5,771 (60.5)	3,768 (39.5)
Injury mechanism	Transport accident	10,838 (74.5)	3,708 (25.5)
	Fall/slip down	6,283 (73.4)	2,280 (26.6)
	Other blunt injury	645 (80.2)	159 (19.8)
	Penetrating injury	282 (78.8)	76 (21.2)
	Contact with machinery	257 (86.8)	39 (13.2)
Intent	Accidental	16,934 (74.8)	5,700 (25.2)
	Intentional	680 (76.7)	207 (23.3)
	Violence/assault	283 (79.5)	73 (20.5)
Time to arrival at ED	<30	8,764 (70.5)	3,661 (29.5)
	≥30	9,541 (78.6)	2,601 (21.4)
	Total	18,305 (74.5)	6,262 (25.5)

Data are presented as *n* (%).
ISS, injury severity score; ED, emergency department.
[a)]Metropolitan area: Seoul, Gyeonggi Province, Incheon. [b)]Level 1, regional trauma center; level 2, regional emergency center; level 3, local emergency center; level 4, local emergency facility.

4) (Exp(B) = 0.810; 95% CI, 0.760–0.864; $p < 0.001$). Patients treated during 2016–2017 had a higher risk of mortality than those treated during 2018–2020 (reference group) (Exp(B) = 1.249; 95% CI, 1.171–1.333; $p < 0.001$), indicating improved outcomes in the later period. ISS scores were strongly associated with mortality. Patients with an ISS of 16–24 had a substantially lower risk of death compared to those with scores of 25–75 (Exp(B) = 0.295; 95% CI, 0.277–0.314; $p < 0.001$).

Regarding injury mechanism, falls/slips (Exp(B) = 0.906; 95% CI, 0.845–0.971; $p = 0.005$), other blunt injuries (Exp(B) = 0.774; 95% CI, 0.636–0.943; $p = 0.01$), and contact with machinery (Exp(B) = 0.662; 95% CI, 0.466–0.941; $p = 0.02$) were associated with lower mortality compared to transport-related accidents (reference group). Penetrating injuries did not show a significant difference ($p = 0.27$). Patients with intentional injuries had a significantly higher risk of mortality compared to those with accidental injuries (Exp(B) = 1.380; 95% CI, 1.150–1.657; $p < 0.001$), while violent/assault-related injuries did not show a significant association ($p = 0.36$). Finally, patients who arrived at the ED ≥30 minutes after EMS activation had a significantly lower risk of mortality compared to those who arrived within 30 minutes (Exp(B) = 0.671; 95% CI, 0.630–0.716; $p < 0.001$) (Table 3, Table S3).

Survival Outcomes in Patients with Severe Trauma Based on Time to Arrival at ED, ISS Score, and Region

Among patients who arrived at the ED in less than 30 minutes ($n = 12,425$), the survival rate was 70.5%, while the mortality rate was 29.5%. In this group, patients with an ISS of 16–24 had a survival rate of 79.9% and a mortality rate of 20.0%. For those in metropolitan areas, the survival rate was 81.0%, compared to 79.1% in urban areas. Among patients with an ISS of 25–75, the survival rate was 56.4% and the mortality rate was 43.6%. In metropolitan areas, the survival and mortality rates were 58.7% and 41.3%, respectively, compared to 54.7% and 45.3% in urban areas.

Patients with arrival times of ≥30 minutes ($n = 12,142$) had a survival rate of 78.6% and a mortality rate of 21.4%. For

Table 2. Comparison of survival and mortality rates by institution type and ISS in severe trauma patients

Characteristic	Year	
	2016, 2017	2018–2020
Institution type[a]		
Level 1, 2		
Visits per year	2,107 (46.7)[b]	3,324 (64.1)[c]
Survival	3,108 (73.7)	7,727 (77.5)
Death	1,106 (26.3)	2,246 (22.5)
Level 3, 4		
Visits per year	2,399 (53.3)[b]	1,860(35.9)[c]
Survival	3,313 (69.0)	4,157 (74.5)
Death	1,485 (31.0)	1,425 (25.5)
ISS		
16–24		
Survival	4,405 (80.9)	8,129 (84.8)
Death	1,041 (19.1)	1,453 (15.2)
25–75		
Survival	2,016 (56.5)	3,755 (62.9)
Death	1,550 (43.5)	2,218 (37.1)

Data are presented as *n* (%).
ISS, injury severity score.
[a]Level 1, regional trauma center; level 2, regional emergency center; level 3, local emergency center; level 4, local emergency facility. [b]The ratio of visits per year for levels 1, 2 and levels 3, 4 in 2016 and 2017. [c]The ratio of visits per year for levels 1, 2 and levels 3, 4 in 2018 and 2020.

patients with an ISS of 16–24, the survival rate was 86.9% and the mortality rate was 13.2%. Patients with an ISS of 25–75 had a survival rate of 69.9% and a mortality rate of 35.1%. Among patients arriving at ≥30 minutes with an ISS of 16–24, survival rates were 86.9% in metropolitan areas and 86.8% in urban areas. For patients with an ISS of 25–75, survival was higher in metropolitan areas (67.8%) than in urban areas (63.6%) (Table 4; Figure 2).

Survival Outcomes in Patients with Severe Trauma Based on Time to Arrival at ED, Year, and Institution Level

Patients who arrived at the ED within 30 minutes had a lower survival rate (70.5%) compared to those with arrival times of ≥30 minutes (78.6%). High-level institutions showed higher survival rates (70.1% in 2016–2017 and 72.8% in 2018–2020) compared to lower-level institutions (66.2% and 72.0%, respectively). In contrast, patients arriving at the ED after ≥30 minutes demonstrated consistently higher survival rates, with improvements from 75.8% in 2016–2017 to 79.9% in 2018–2020. Within this group, high-level institutions had superior survival outcomes (77.3% in 2016–2017 and 80.7% in 2018–2020) compared to lower-

Table 3. Factors associated with mortality in severe trauma patients: multivariate logistic regression analysis

Characteristic	Category	Exp(B)	95% CI	*p*
Sex	Male	0.921	0.857–0.989	0.03
	Female	1.000		
Age (y)	<15	0.497	0.385–0.642	<0.001
	15–54	0.440	0.411–0.471	<0.001
	≥55	1.000		
Region[a]	Metropolitan area	0.920	0.862–0.982	0.01
	Urban area	1.000		
Hospital type[b]	Level 1, 2	0.810	0.760–0.864	<0.001
	Level 3, 4	1.000		
Year	2016, 2017	1.249	1.171–1.333	<0.001
	2018–2020	1.000		
ISS	16–24	0.295	0.277–0.314	<0.001
	25–75	1.000		
Injury mechanism	Transport accident	1.000		
	Fall/slip down	0.906	0.845–0.971	0.005
	Other blunt injury	0.774	0.636–0.943	0.01
	Penetrating injury	0.821	0.576–1.169	0.27
	Contact with machinery	0.662	0.466–0.941	0.02
Intent	Accidental	1.000		
	Intentional	1.380	1.150–1.657	0.001
	Violence/assault	1.167	0.838–1.624	0.36
Time to arrival at ED	<30	1.000		
	≥30	0.671	0.630–0.716	<0.001

CI, confidence interval; ED, emergency department.
[a]Metropolitan area: Seoul, Gyeonggi Province, Incheon. [b]Level 1, regional trauma center; level 2, regional emergency center; level 3, local emergency center; level 4, local emergency facility.

https://doi.org/10.24171/j.phrp.2025.0102

Table 4. Numeric summary of mortality from interactive decision tree analysis by time to ED arrival, ISS, and region (based on Figure 2)

Node	Node description	Death (*n*, %)
1	Time to arrival at ED < 30 min & ISS score (16–24) & region (metropolitan area[a)])	3,209 (19.0)
2	Time to arrival at ED < 30 min & ISS score (16–24) & region (urban area)	4,261 (20.9)
3	Time to arrival at ED < 30 min & ISS score (25–75) & region (metropolitan area[a)])	2,124 (41.3)
4	Time to arrival at ED < 30 min & ISS score (25–75) & region (urban area)	2,831 (45.3)
5	Time to arrival at ED ≥ 30 min & ISS score (16–24) & region (metropolitan area[a)])	2,413 (13.1)
6	Time to arrival at ED ≥ 30 min & ISS score (16–24) & region (urban area)	5,145 (13.2)
7	Time to arrival at ED ≥ 30 min & ISS score (25–75) & region (metropolitan area[a)])	1,466 (32.2)
8	Time to arrival at ED ≥ 30 min & ISS score (25–75) & region (urban area)	3,118 (36.4)

ED, emergency department; ISS, injury severity score.
[a)]Metropolitan areas: Seoul, Gyeonggi Province, Incheon.

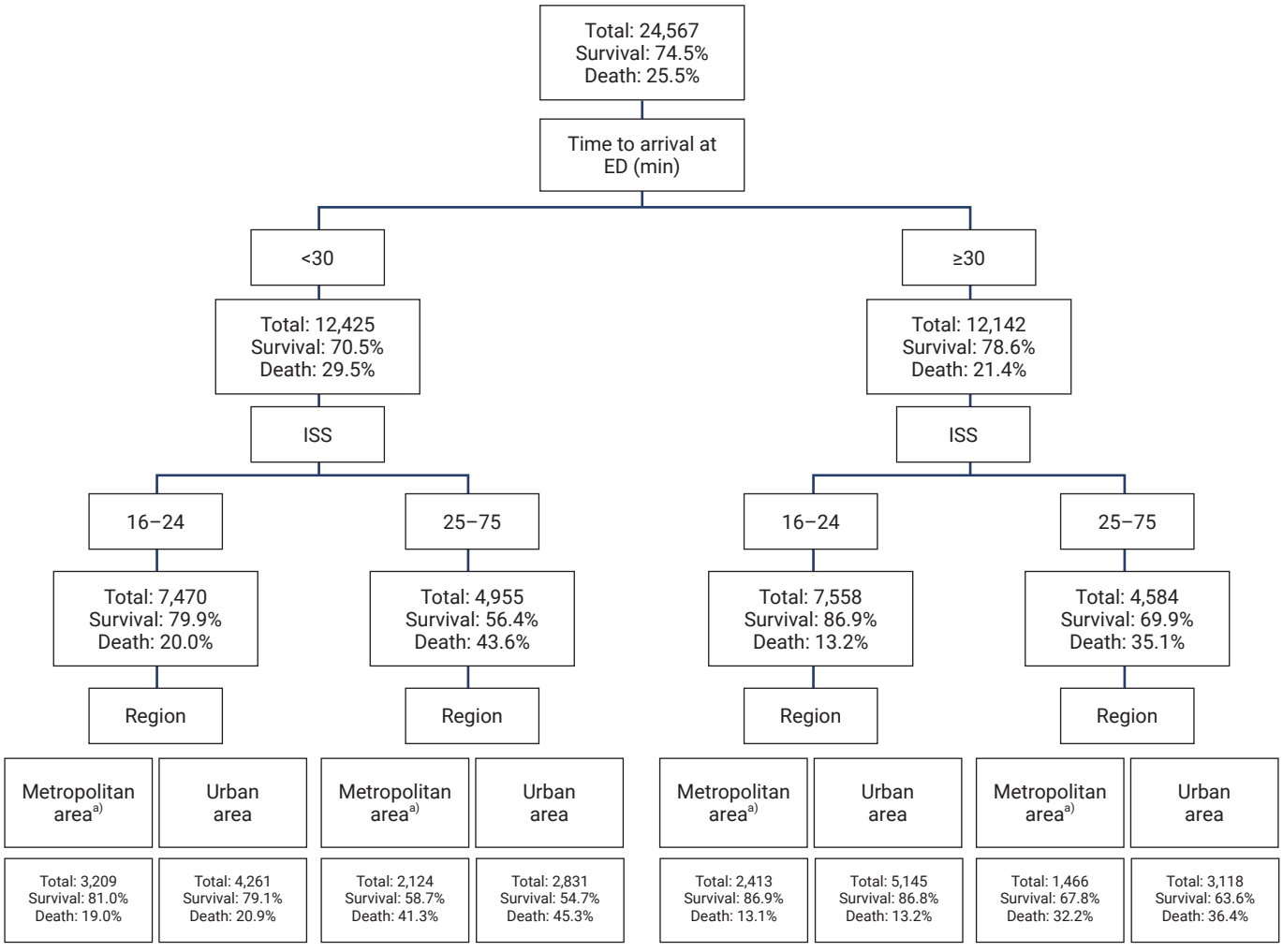


Figure 2. Decision tree analysis of survival outcomes in severe trauma patients based on time to emergency department (ED) arrival, injury severity score (ISS), and region. [a)]Metropolitan areas: Seoul, Gyeonggi Province, Incheon.

level institutions (74.0% and 77.8%, respectively) (Table 5; Figure 3).

Discussion

Our study observed improvements in the Republic of Korea's

Table 5. Numeric summary of mortality from interactive decision tree analysis by time to ED arrival, year, and institution level (based on Figure 3)

Node	Node description	Death (*n*, %)
1	Time to arrival at ED <30 min & year (2016, 2017) & institution (high level[a])	2,100 (29.9)
2	Time to arrival at ED <30 min & year (2016, 2017) & institution (lower level[b])	3,032 (33.9)
3	Time to arrival at ED <30 min & year (2018–2020) & institution (high level[a])	4,074 (27.2)
4	Time to arrival at ED <30 min & year (2018–2020) & institution (lower level[b])	3,219 (28.0)
5	Time to arrival at ED ≥30 min & year (2016, 2017) & institution (high level[a])	2,114 (22.7)
6	Time to arrival at ED ≥30 min & year (2016, 2017) & institution (lower level[b])	1,766 (26.0)
7	Time to arrival at ED ≥30 min & year (2018–2020) & institution (high level[a])	5,899 (19.3)
8	Time to arrival at ED ≥30 min & year (2018–2020) & institution (lower level[b])	2,363 (22.2)

ED, emergency department.
[a]High level: high-level medical institution, corresponding to level 1 (regional trauma center) and level 2 (regional emergency center). [b]Lower level: lower-level medical institution, corresponding to level 3 (local emergency center) and level 4 (local emergency facility).

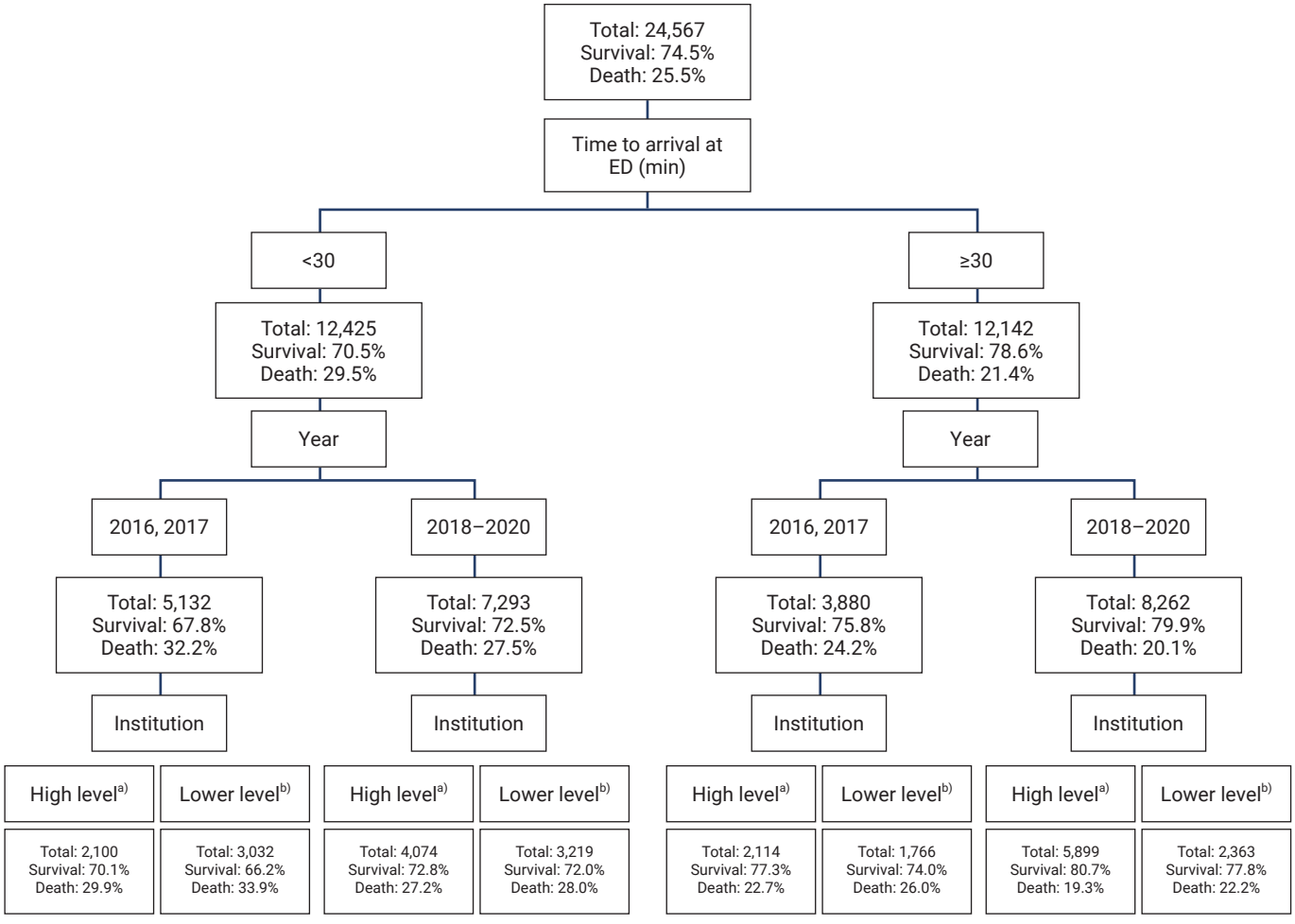


Figure 3. Decision tree analysis of survival outcomes in severe trauma patients based on time to emergency department (ED) arrival, year, and institution level. [a]High level: high-level medical institution, corresponding to level 1 (regional trauma center) and level 2 (regional emergency center). [b]Lower level: lower-level medical institution, corresponding to level 3 (local emergency center) and level 4 (local emergency facility).

https://doi.org/10.24171/j.phrp.2025.0102

trauma system, as indicated by a decreased mortality rate among patients with severe trauma (ISS >15). This reduction included cases involving direct prehospital transportation, which were analyzed by institution, year, and ISS score. Although longer transport times may sometimes be required, selecting an appropriate hospital and transporting patients directly to high-level institutions can enhance survival rates.

This nationwide study in the Republic of Korea analyzed patients with severe trauma (ISS >15) who were directly transported following EMS assessment. Between 2016–2017 and 2018–2020, the overall in-hospital mortality rate decreased by 5.2%, with consistent improvements observed across hospital levels and ISS subgroups (Table 2). Mortality in urban areas was 1.1% lower than in metropolitan areas. However, transport times exceeding 30 minutes were more frequently observed in urban areas (68%), likely due to the relative distance to high-level trauma centers. Subgroup analysis revealed that among patients with higher ISS scores (25–75), survival was better in metropolitan areas, despite longer transport times being less frequent. This suggests that factors related to hospital level—such as available resources and trauma care capacity—may have a greater influence on outcomes than transport time alone. These findings highlight the potential importance of destination hospital capability in prehospital triage decisions and underscore the need for further investigation into regional differences in trauma care accessibility.

Consistent with previous studies [13,14], higher ISS scores were associated with increased mortality, while high-level institutions had an 11.6% lower mortality rate than lower-level institutions (Exp(B) = 0.884, $p < 0.0001$). Between 2016–2017 and 2018–2020, survival rates among severe trauma patients improved significantly, coinciding with a substantial increase in annual visits to high-level institutions—from 2,107 cases (46.7%) to 3,324 cases (64.1%). Additionally, a higher proportion of patients transported to high-level institutions had transport times exceeding 30 minutes (8,003 cases, 65.9%) compared to those transported in under 30 minutes (6,174 cases, 49.7%). Consequently, among directly transported patients, those with transport times longer than 30 minutes exhibited higher survival rates. These results suggest that, for patients with severe trauma, direct transport to high-level institutions—despite longer transport times—may be more beneficial than rapid transport to nearby hospitals.

Kim et al. [15], in a single-center study in the Republic of Korea, demonstrated that direct transport to a high-level trauma center was associated with improved survival rates, even when transport time exceeded 60 minutes. Another study reported that transport times of up to 90 minutes did not significantly impact mortality [16]. Similarly, a pediatric trauma study in Sweden found no significant association between prolonged transport time and 30-day mortality or functional outcomes [17]. However, other studies have shown that each 10-minute delay in total transport time increases the risk of poor functional outcomes by 6%, that transport times exceeding 50 minutes are predictive of poor functional outcomes in trauma patients [18], and that transport times longer than 30 minutes are associated with worse outcomes in patients with penetrating injuries [19]. While findings are mixed, this ongoing debate is influenced by differences in regional and national healthcare systems, inclusion of transferred patients, and interfacility transfer times. Well-designed studies are needed to establish an optimal transport time cutoff in the Republic of Korea, taking into account the healthcare system and transport modalities, including air transport.

Limitations

This study had several limitations. First, final diagnoses and detailed disease categories were unavailable, restricting subgroup analyses and detailed statistical classifications. However, as these data have not been disclosed, our study provides an overall assessment of patients with severe trauma and ISS scores who were directly transported from prehospital settings. Second, we included only EMS-assessed patients with trauma. Patients with severe trauma who were not transported by EMS may have influenced the distribution of ED visits. In the Republic of Korea, approximately 15% to 20% of patients with severe trauma visit emergency institutions directly without EMS involvement [6,20]. Although EMS transport remains predominant, direct-visit patients require different considerations. In another National Emergency Department Information System-based study that analyzed severe trauma using international classification of disease-based injury severity score rather than ISS, 83,646 patients (22.8%) visited ambulatory hospitals, a smaller number than the 217,482 patients (59.2%) transported by public EMS. However, this difference should be considered when interpreting the results, given the differences in underlying data and the definitions of severe trauma patients [21]. Future studies should include these patients for a more comprehensive analysis.

Conclusion

Among patients with severe trauma directly transported from prehospital settings, mortality rates in the Republic of Korea have decreased. Direct transport to appropriate high-level

institutions is associated with reduced mortality, emphasizing the need for a well-structured trauma system and a consensus on optimal transport strategies.

Supplementary Material

Table S1. General characteristics of analysis subjects (institution type: level 4); **Table S2.** Comparison of survival and mortality rates by institution type and ISS in severe trauma patients (institution type: level 4); **Table S3.** Factors associated with mortality in severe trauma patients: multivariate logistic regression analysis (institution type: level 4). Supplementary data are available at Supplementary data are available at https://doi.org/10.24171/j.phrp.2025.0102.

Notes

Ethics Approval

The dataset used in this study was approved by the National Approval Statistics (approval number: 117105), The requirements for informed consent were waived.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

This research was based on Community-based Severe Trauma Surveillance. The Database is administered by the Korea Disease Control and Prevention Agency. There was no financial support for our research.

Availability of Data

The datasets used in this study are publicly available from the Korea Disease Control and Prevention Agent.

Authors' Contributions

Conceptualization: CYP; Data curation: KKK; Formal analysis: KKK; Methodology: SJK, OHK; Project administration: CYP; Resources: OHK; Software: KKK; Supervision: CYP; Validation: SJK; Visualization: KKK; Writing–original draft: SJK; Writing–review & editing: KKK, OHK, CYP. All authors read and approved the final manuscript.

Additional Contributions

The authors thank the Korea Disease Control and Prevention Agency for data provision and support.

References

1. Prin M, Li G. Complications and in-hospital mortality in trauma patients treated in intensive care units in the United States, 2013. Inj Epidemiol 2016;3:18.
2. Herrera-Escobar JP, Rivero R, Apoj M, et al. Long-term social dysfunction after trauma: what is the prevalence, risk factors, and associated outcomes? Surgery 2019;166:392–7.
3. Jung KY, Kim JS, Kim Y. Problems in trauma care and preventable deaths. J Korean Soc Emerg Med 2001;12:45–56.
4. Kim Y, Jung KY, Cho KH, et al. Preventable trauma deaths rates and management errors in emergency medical system in Korea. J Korean Soc Emerg Med 2006;17:385–94.
5. Korean Statistical Information Service (KR). Deaths and death rates by sex, age group [Internet]. Korean Statistical Information Service; 2023 [cited 2025 Mar 13]. Available from: https://kosis.kr/statHtml/statHtml.do?orgId=101&tblId=DT_1B80A13&conn_path=I2&language=en.
6. Kwon J, Lee M, Kim Y, et al. Trauma system establishment and outcome improvement: a retrospective national cohort study in South Korea. Int J Surg 2023;109:2293–302.
7. Sasser SM, Hunt RC, Faul M, et al. Guidelines for field triage of injured patients: recommendations of the national expert panel on field triage, 2011. MMWR Recomm Rep 2012;61(RR-1):1–20.
8. van Rein EA, Houwert RM, Gunning AC, et al. Accuracy of prehospital triage protocols in selecting severely injured patients: a systematic review. J Trauma Acute Care Surg 2017;83:328–39.
9. National Emergency Medical Center (KR). Emergency Medical Statistics annual report 2019. Ministry of Health and Welfare of Korea; 2020.
10. Vianen NJ, Van Lieshout EM, Maissan IM, et al. Prehospital traumatic cardiac arrest: a systematic review and meta-analysis. Eur J Trauma Emerg Surg 2022;48:3357–72.
11. Ministry of Health and Welfare of Korea (KR). Designation of 47 advanced general hospitals for the 5th term ('24-'26) [Internet]. Ministry of Health and Welfare of Korea; 2023 [cited 2025 Mar 12]. Available from: https://www.mohw.go.kr/board.es?act=view&bid=0027&list_no=1479568&mid=a10503000000. Korean.
12. Stravinskiene A, Gudas S, Dabrilaite A. Decision tree algorithms: integration of domain knowledge for data mining. In: Abramowicz W, Domingue J, Wecel K, editors. Business Information Systems Workshops: BIS 2012 International Workshops and Future Internet Symposium; 2012 May 21-23; Vilnius, Lithuania, Springer Berlin Heidelberg. 2012. p. 13–24.
13. Hosseinpour R, Barghi A, Mehrabi S, et al. Prognosis of the trauma patients according to the trauma and injury severity score (TRISS); a diagnostic accuracy study. Bull Emerg Trauma 2020;8:148–55.
14. Kuo SC, Kuo PJ, Chen YC, et al. Comparison of the new exponential injury severity score with the injury severity score and the new injury severity score in trauma patients: a cross-sectional study. PLoS One 2017;12:e0187871.
15. Kim J, Heo Y, Lee JC, et al. Effective transport for trauma patients under current circumstances in Korea: a single institution analysis of treatment outcomes for trauma patients transported via the domestic 119 service. J Korean Med Sci 2015;30:336–42.
16. Petri RW, Dyer A, Lumpkin J. The effect of prehospital transport time on the mortality from traumatic injury. Prehosp Disaster Med 1995;10:24–9.
17. Traff H, Hagander L, Salo M. Association of transport time with adverse outcome in paediatric trauma. BJS Open 2021;5:zrab036.
18. Chen CH, Shin SD, Sun JT, et al. Association between prehospital time and outcome of trauma patients in 4 Asian countries: a cross-

national, multicenter cohort study. PLoS Med 2020;17:e1003360.
19. Tansley G, Schuurman N, Bowes M, et al. Effect of predicted travel time to trauma care on mortality in major trauma patients in Nova Scotia. Can J Surg 2019;62:123–30.
20. Lim SY, Jo YH, Kim S, et al. Emergency department utilization in elderly patients: a report from the National Emergency Department Information System (NEDIS) of Korea, 2018-2022. Clin Exp Emerg Med 2023;10:S26–35.
21. Kim JY, Yoon YH, Park SJ, et al. Mortality and incidence rate of acute severe trauma patients in the emergency department: a report from the National Emergency Department Information System (NEDIS) of Korea, 2018-2022. Clin Exp Emerg Med 2023;10:S55–62.

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2025.0014
Osong Public Health Res Perspect 2025;16(4):392-405

Original Article

Prevalence and determinants of medication adherence in Moroccan patients with type 2 diabetes: a multicenter cross-sectional study

Maryem Arraji, Nadia Al Wachami, Younes Iderdar, Fatima Zahra Bouchachi, Karima Boumendil, Mohamed Chahboune

Laboratory of Health Sciences and Technologies, Higher Institute of Health Sciences, Hassan First University of Settat, Settat, Morocco

ABSTRACT

Objectives: This study was performed to evaluate the prevalence of medication adherence and its determinants among Moroccan patients with type 2 diabetes mellitus.

Methods: A multicenter cross-sectional survey was conducted from February to June 2024, involving 584 patients from the Casablanca-Settat and Rabat-Sale-Kenitra regions of Morocco. Medication adherence was assessed using the general medication adherence scale. Associations between independent variables and adherence were analyzed using chi-square tests and multivariate logistic regression.

Results: Medication adherence was observed in 96.2% of participants. Multivariate analysis revealed significantly lower medication adherence among patients using 2 or more oral antidiabetic drugs (adjusted odds ratio [aOR], 0.026; 95% confidence interval [CI], 0.001–0.642; p=0.026) and those with a diabetes duration of 11 to 15 years (aOR, 0.037; 95% CI, 0.001–0.956; p=0.047).

Conclusion: Despite a high overall adherence rate, patients on dual or polytherapy and those with longer disease duration exhibited lower adherence in multivariate analysis. Targeted interventions are needed to improve adherence in these high-risk groups.

Keywords: Determinants; General medication adherence scale; Medication adherence; Morocco; Type 2 diabetes mellitus

Received: 20 January 2025
Revised: 18 April 2025
Accepted: 11 May 2025

Corresponding author:
Maryem Arraji
Laboratory of Sciences and Health Technologies, Higher Institute of Health Sciences, Hassan First University of Settat, Settat 26000, Morocco
E-mail: m.arraji@uhp.ac.ma

Introduction

Despite substantial efforts to prevent and control diabetes mellitus (DM), this non-communicable disease remains a global crisis, with increasing prevalence in all regions worldwide [1,2]. In 2021, approximately 537 million adults aged 20 to 75 years lived with DM, representing 10.5% of all adults in this age group, according to the International Diabetes

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Korea Disease Control and Prevention Agency and Korea National Institute of Health.

© 2025 Korea Disease Control and Prevention Agency.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Federation [3]. This number is projected to reach around 783 million by 2045, with a global prevalence of 12.2% [3]. Of this increase, 94% is expected to occur in low- to middle-income countries [3]. Additionally, approximately 6.7 million deaths globally were attributed to DM in 2021 [3].

Type 2 diabetes mellitus (T2DM) is the most common form of DM, accounting for over 90% of global cases [3]. The rise in T2DM prevalence and incidence has been driven by rapid economic and social developments that have promoted dietary changes and reduced physical activity [1,2]. Effective T2DM management is complex, extending beyond pharmacological treatments, and includes self-care practices such as healthy eating, regular physical activity, weight control, and smoking cessation [3].

In Morocco, approximately 12.4% of adults had T2DM in 2023, according to World Health Organization data [2]. T2DM is responsible for over 12,000 deaths annually, with an additional 32,000 deaths attributed to complications of high blood glucose levels [4]. This disease imposes a significant socioeconomic burden on Morocco, increasing medical expenses primarily due to chronic complications such as neuropathy, retinopathy, kidney failure, heart attacks, and strokes [5]. These complications require costly treatments and often impair patients' ability to work and perform daily activities, additionally affecting their families and society [6].

In light of this considerable socioeconomic impact, medication adherence for T2DM management is essential. Poor adherence can worsen health outcomes, increase the risk of therapeutic failure and hospitalizations, and impose financial burdens on patients and healthcare systems [7]. Numerous studies have explored medication adherence in patients with T2DM, identifying various socioeconomic, clinical, and behavioral determinants [8–11].

Medication adherence is typically relatively low in developing countries due to resource constraints [2]. Non-adherence rates to antidiabetic treatments vary widely, ranging from 36% to 93% [12,13], and can adversely impact blood glucose control, contributing to complications and reduced quality of life [14]. Additionally, more than half of individuals with T2DM experience poor glycemic control [15], which worsens health outcomes; this issue is particularly common in East African countries such as Ethiopia, Tanzania, Uganda, and Kenya [14,16,17].

Factors associated with poor adherence include the high cost of medications, prolonged treatment duration, adverse drug effects (such as fatigue, nausea, vomiting, psychological distress, and itching), sociocultural influences, substance use, limited health literacy, personal beliefs, and the availability and affordability of insulin. Non-persistence with treatment is also a major concern [18–20]. Studies conducted in middle-income countries like Egypt [21] and Tunisia [22], using the adherence to refills and medications scale (ARMS), have reported low adherence rates due in part to medication costs and sociocultural constraints. These findings underscore similar challenges to those expected in Morocco. However, the general medication adherence scale (GMAS) offers a more tailored assessment by incorporating socioeconomic and behavioral factors specific to the Moroccan context.

HIGHLIGHTS

- A high rate of medication adherence (96.2%) was observed among Moroccan patients with type 2 diabetes.
- The use of multiple oral antidiabetic drugs and a diabetes duration of 11 to 15 years were significantly associated with lower adherence.
- Targeted strategies are essential to improve adherence in these high-risk groups.

Globally, medication adherence in diabetes is influenced by various factors, including gender [23], education level [24,25], knowledge about diabetes [24,25], disease duration [25,26], and perceived severity of the disease [23], among others. Medication adherence refers to the extent to which a patient's behavior aligns with agreed-upon recommendations from the prescriber [27]. It is an active commitment that requires collaboration with the healthcare provider, as well as a personal and motivated decision aimed at maintaining effective management of one's health condition [27]. Non-adherence manifests as missed doses, irregular medication intake, or discontinuation of the treatment regimen [27]. According to extensive research, approximately half of patients with chronic diseases do not adhere to their medication regimens, and around half of these individuals discontinue treatment within 1 year [28]. Non-adherence remains a major challenge among patients with chronic diseases, particularly those with T2DM. This lack of adherence contributes to disease progression and increased healthcare costs, underscoring the urgent need for improved management strategies.

Several tools are available to assess medication adherence, including the Shea scale [29], ARMS [30], brief medication questionnaire [31], and Morisky medication adherence scale [32]. Although widely used, these tools are not always suitable for specific contexts, such as Morocco. Addressing this gap, the GMAS was developed in Urdu in 2017 [33] and was subsequently validated in Moroccan Arabic in 2023 [6]. This scale represented a major advancement in assessing

medication adherence by focusing on 3 fundamental aspects: behavioral non-adherence, non-adherence associated with the additional burden of disease and medications, and cost-related non-adherence (CRNA).

Despite the scale's potential utility and relevance, few studies have explored medication adherence in Morocco along these dimensions [7,34]. To date, no systematic research has evaluated the impact of socioeconomic and clinical factors on treatment adherence using this locally validated tool, highlighting a clear need for such investigation. Therefore, this study aims to assess medication adherence levels and identify associated factors among Moroccan patients with T2DM, using the GMAS within the framework of a multicenter study.

Materials and Methods

Study Design and Setting

This multicenter cross-sectional study was conducted from February to June 2024 and included 584 patients with T2DM. Participants were randomly selected from the patient registers of primary healthcare facilities in the Casablanca-Settat and Rabat-Sale regions of Morocco.

Participants

Inclusion criteria

Participants were required to: (1) be diagnosed with T2DM, (2) have received antidiabetic treatment for at least 3 months prior to the study, and (3) be at least 18 years old. T2DM diagnosis was confirmed using patients' medical histories, antidiabetic treatments, and records from the primary healthcare facilities.

Exclusion criteria

Individuals were excluded if they: (1) had type 1 DM, (2) had gestational diabetes, (3) were severely ill and required urgent medical care, or (4) refused to participate or failed to complete the survey. Participation was voluntary, with informed consent obtained through a signed consent form.

Data Collection

Data were collected using a self-administered questionnaire comprising 2 main sections: (1) sociodemographic and disease-related data, and (2) the 11-item GMAS tool for assessing medication adherence.

Sample Size

The necessary sample size was determined based on a diabetes prevalence rate of 12.4%, as reported in previous research [4]. The initial calculation estimated a minimum of 167 patients, assuming a 95% confidence interval (CI) and 5% precision. However, 584 patients were included, significantly exceeding the initial target. This larger sample size increases statistical robustness, improves representativeness of the diabetic population studied, and facilitates detailed analyses and the identification of significant associations. Furthermore, a larger sample yields a study with greater statistical power, aiding the detection of meaningful differences concerning medication adherence among patients with T2DM.

General Medication Adherence Scale

The GMAS, developed in 2017 [33] to assess medication adherence through self-reporting, was translated and validated in Moroccan patients with T2DM in 2023 [6]. This scale is divided into 3 sections: patient behavior-related non-adherence, assessed by 5 items (questions 1–5); non-adherence related to additional disease and pill burden (ADPB), covered by 4 items (questions 6–9); and CRNA, assessed by 2 items (questions 10 and 11). These sections thoroughly evaluate various factors impacting medication adherence among patients with T2DM. Participants respond to these 11 items on a 4-point Likert scale, ranging from 0 (always) to 3 (never). The total score ranges from 0 to 33, with higher scores indicating better adherence.

Adherence levels are categorized based on GMAS total score: scores of 10 or less indicate poor adherence, 11 to 16 indicate low adherence, 17 to 26 reflect partial adherence, 27 to 29 represent good adherence, and 30 to 33 indicate high adherence. These categories provide a clear framework for assessing the degree of medication adherence among patients with T2DM. A total score of 27 or higher classifies patients as adherent, while a score of 26 or lower classifies them as non-adherent. The GMAS has been transculturally validated in multiple countries, including Saudi Arabia [28,35], Jordan [36], Sudan [37], Nepal [38], China [39], and the United Arab Emirates [40].

Statistical Analysis

We performed statistical analysis using IBM SPSS ver. 23.0 (IBM Corp.). Data were anonymized to ensure participant confidentiality. The sociodemographic characteristics of participants are presented as frequencies, percentages, means, and standard deviations.

To evaluate statistically significant associations between variables, cross-tabulations with the Pearson chi-square test were used. For counts less than 5, the Fisher exact test was applied. A multivariate binary logistic regression model was employed to explore significant associations between explanatory and outcome variables. The independent variables included in the model were gender, age group, area

https://doi.org/10.24171/j.phrp.2025.0014

of residence, marital status, employment status, education level, health insurance status, type of health insurance, prescribed treatment, prevalence of combination therapy, diabetes-related comorbidities, duration of the disease, and patient education regarding their condition. A *p*-value less than 0.05 was considered to indicate statistical significance.

Ethics Approval

Data collection for the study was approved by IRB00012973 of the Moroccan Association for Research and Ethics, IRB #1 [09/REC/22], in accordance with the ethical principles outlined in the Declaration of Helsinki and relevant local ethical regulations. Written informed consent was obtained from all participants prior to their inclusion in the study. Participants were fully informed about the purpose of the study, the procedures involved, and their right to withdraw at any time without consequences to their medical care. This informed consent process was also approved by IRB #1 [09/REC/22].

Results

Figure 1 illustrates the steps of participant recruitment from the 4 healthcare facilities involved in the study, outlining the selection process based on the inclusion and exclusion criteria.

Association between Sociodemographic Characteristics and Medication Adherence

Medication adherence was observed in 96.2% of participants (*n*=562/584), as shown in Figure 2. Table 1 presents the

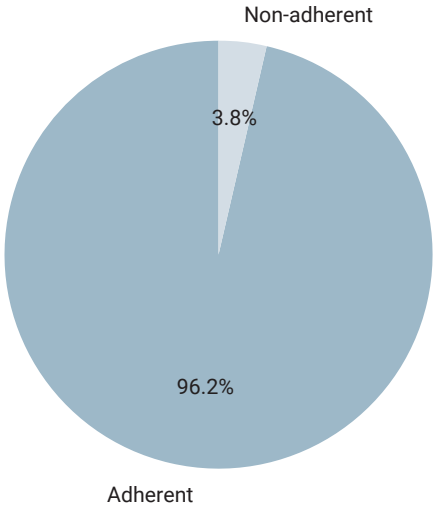


Figure 2. Proportion of medication adherence among patients with type 2 diabetes (*n*=584).



Figure 1. Organogram of the participant recruitment process.
T2DM, type 2 diabetes mellitus; T1DM, type 1 diabetes mellitus.

Table 1. Cross-tabulations between sociodemographic characteristics of the sample and adherence category

Variable	Total population ($n=584$)		General medication adherence scale		X^2	p
	Category	Value	Adherent	Non-adherent		
Area of residence	Rural	343 (58.7)	331 (96.5)	12 (3.5)	0.14	0.90
	Urban	241 (41.3)	233 (96.7)	8 (3.3)		
Sex	Male	237 (40.6)	230 (97.0)	7 (3.0)	268	0.60
	Female	347 (59.4)	343 (98.8)	13 (3.7)		
Age (y)	29–49	110 (18.8)	108 (98.2)	2 (1.8)	3.48	0.17
	50–79	438 (75.0)	423 (96.6)	15 (3.4)		
	80–91	36 (6.2)	33 (91.7)	3 (8.3)		
Marital status	Single	67 (11.5)	64 (95.5)	3 (4.5)	1.48	0.68
	Married	398 (68.2)	384 (96.5)	14 (3.5)		
	Widowed	84 (14.4)	81 (96.4)	3 (3.6)		
	Divorced	35 (6.0)	35 (100.0)	0 (0)		
Education level	Illiterate	401 (68.7)	384 (95.8)	17 (4.2)	3.49	0.47
	Primary	74 (12.7)	73 (98.6)	1 (1.4)		
	Middle school	48 (8.2)	47 (97.9)	1 (2.1)		
	High school	39 (6.7)	39 (100.0)	0 (0)		
	University	22 (3.8)	21 (95.5)	1 (4.5)		
Employment status	Unemployed	469 (80.3)	450 (95.9)	19 (4.1)	3.16	0.2
	Retired	65 (11.1)	65 (100.0)	0 (0)		
	Employed	50 (8.6)	49 (98.0)	1 (2.0)		
Health insurance status	Insured	384 (65.8)	366 (95.3)	18 (4.7)	5.40	0.02*
	Uninsured	200 (34.2)	198 (99.0)	2 (1.0)		
Type of insurance	Uninsured	190 (32.5)	188 (98.9)	2 (1.1)	7.13	0.06
	CNOPS	53 (9.1)	52 (98.1)	1 (1.9)		
	CNSS	67 (11.5)	65 (97.0)	2 (3.0)		
	AMO	274 (46.9)	259 (94.5)	15 (5.5)		

Data are presented as *n* (%).
CNOPS, National Fund of Social Welfare Organizations; CNSS, National Social Security Fund; AMO, Mandatory Health Insurance.
*$p<0.05$.

associations between various sociodemographic characteristics and medication adherence, assessed using the GMAS. The mean age of participants was 60.53 ±12.47 years (range, 29–91 years). Most participants lived in rural areas ($n=343$, 58.7%), and adherence rates were similar between rural (96.5%, $n=331$) and urban residents (96.7%, $n=233$; $\chi^2=0.14$, $p=0.90$). Women comprised 59.4% of the sample ($n=347$) and exhibited an adherence rate comparable to men (96.3% vs. 97.1%), again without any significant difference ($\chi^2=0.60$, $p=0.60$). Participants aged 29 to 49 years ($n=110$) had the highest adherence rate (98.2%, $n=108$), while those aged 80 to 91 years ($n=36$) had the highest non-adherence rate (8.3%, $n=3$); however, this difference was not statistically significant ($\chi^2=3.48$, $p=0.17$). Marital status was not significantly associated with adherence ($\chi^2=1.48$, $p=0.68$). Single participants ($n=67$) exhibited a non-adherence rate of 4.5% ($n=3$), while divorced individuals ($n=35$) displayed full adherence (100%). Regarding education level, illiterate patients ($n=401$, 68.7%) had a slightly higher non-adherence rate of 4.2% ($n=17$) compared to those with primary (1.4%, $n=1/74$) or middle school education (2.1%, $n=1/48$). Patients with high school education ($n=39$) reported 100% adherence, while university-educated participants ($n=22$) had an adherence rate of 95.5% ($n=21$). These differences were not statistically significant ($\chi^2=3.49$, $p=0.47$). Similarly, employment status was not significantly associated with adherence ($\chi^2=3.16$, $p=0.20$). Unemployed individuals ($n=469$, 80.3%) had a 95.9% adherence rate ($n=450$), retired participants ($n=65$) showed full adherence (100%), and employed patients ($n=50$) had slightly higher adherence than unemployed patients (98.0%, $n=49$). Notably, health insurance status exhibited a significant association ($\chi^2=5.40$, $p=0.02$); uninsured patients ($n=200$) had higher adherence (99.0%, $n=198$) than insured patients (95.3%, $n=366$). Although type of insurance did not reach statistical significance ($\chi^2=7.13$, $p=0.06$), participants covered by Mandatory Health Insurance (AMO; $n=274$) displayed a higher non-adherence rate (5.5%, $n=15$) than those insured through the National Fund of Social Welfare Organizations (1.9%, $n=1/53$) or the National Social Security Fund (3.0%, $n=2/67$). Overall, while most sociodemographic

https://doi.org/10.24171/j.phrp.2025.0014

factors were not significantly associated with medication adherence, being uninsured was significantly linked to better adherence in this population.

Association between Clinical Variables and Medication Adherence

Table 2 presents the associations between clinical variables and medication adherence among the 584 participants. Patients with diabetes for less than 1 year (n=47, 8.0%) exhibited a high adherence rate of 97.9% (n=46/47), compared to 95.2% for 1 to 5 years (n=158/166), 96.6% for 6 to 10 years (n=172/178), and 98.8% for 11 to 15 years (n=80/81). However, the association between disease duration and adherence was not statistically significant (χ^2=2.4, p=0.79). Regarding body mass index, adherence rates were 100% among underweight patients (n=18/18), 96.7% for those

Table 2. Cross-tabulations between diabetes-related clinical characteristics of the sample and adherence category

Variable	Total population (n=584)		General medication adherence scale		X^2	p
	Category	Value	Adherent	Non-adherent		
Duration of disease (y)	<1	47 (8.0)	46 (97.9)	1 (2.1)	2.4	0.79
	1–5	166 (28.4)	158 (95.2)	8 (4.8)		
	6–10	178 (30.5)	172 (96.6)	6 (3.4)		
	11–15	81 (13.9)	80 (98.8)	1 (1.2)		
	16–20	55 (9.4)	53 (96.4)	2 (3.6)		
	≥21	57 (9.8)	55 (96.5)	2 (3.5)		
BMI	Underweight (BMI <18.5 kg/m^2)	18 (3.1)	18 (100.0)	0 (0)	3.10	0.37
	Normal weight (BMI 18.5–24.9 kg/m^2)	390 (66.8)	377 (96.7)	13 (3.3)		
	Overweight (BMI 25–29.9 kg/m^2)	96 (16.4)	94 (97.9)	2 (2.1)		
	Obesity (BMI ≥30 kg/m^2)	80 (13.7)	75 (93.8)	5 (6.3)		
Self-monitoring of blood glucose	Yes	419 (71.7)	410 (97.9)	9 (2.1)	7.30	0.007*
	No	165 (28.3)	154 (93.3)	11 (6.7)		
Patient's knowledge about their condition	Informed patient	333 (57.0)	324 (97.3)	9 (2.7)	1.22	0.26
	Uninformed patient	251 (43.0)	240 (95.6)	11 (4.4)		
Treatment	Single OAD	163 (27.9)	156 (95.7)	7 (4.3)	5.24	0.15
	OAD+insulin	68 (11.6)	65 (95.6)	3 (4.4)		
	Two OADs or more	195 (33.4)	193 (99.0)	2 (1.0)		
	Insulin only	158 (27.1)	150 (94.9)	8 (0.98)		
OAD used	Diamicron (gliclazide)	91 (15.6)	90 (98.9)	1 (5.1)	18.32	0.01*
	Geprid (glipizide)	18 (3.1)	18 (100.0)	0 (0)		
	Acol (acarbose)	116 (19.9)	111 (95.7)	5 (4.3)		
	Diamicron (gliclazide)+Acol (acarbose)	155 (26.5)	155 (100.0)	0 (0)		
	Geprid (glipizide)+Acol (acarbose)	33 (5.7)	30 (90.9)	3 (9.1)		
	Diaformine (metformin)+glycan (glimepiride)	5 (0.9)	5 (100.0)	0 (0)		
	Glycophage (metformin)	6 (1.0)	5 (83.3)	1 (16.7)		
	No OAD	160 (27.4)	150 (93.8)	10 (6.3)		
Use of combination therapy	Monotherapy	252 (43.2)	241 (95.6)	11 (4.4)	1.38	0.49
	Dual therapy	211 (36.1)	206 (97.6)	5 (2.4)		
	Triple therapy or more	121 (20.7)	117 (96.7)	4 (3.3)		
Associated diseases	HBP	211 (36.1)	205 (97.2)	6 (2.8)	8.99	0.17
	Dyslipidemia	22 (3.8)	22 (100.0)	0 (0)		
	Other conditions	58 (9.9)	53 (91.4)	5 (8.6)		
	No condition	192 (32.9)	187 (97.4)	5 (2.6)		
	HBP+dyslipidemia	27 (4.6)	25 (92.6)	2 (7.4)		
	HBP+dyslipidemia+other	35 (6.0)	35 (100.0)	0 (0)		
	HBP+other	39 (6.7)	37 (94.9)	2 (5.1)		

Data are presented as n (%).
BMI, body mass index; OAD, oral antidiabetic drug; HBP, high blood pressure.
*p<0.05.

with normal weight (n=377/390), 97.9% for the overweight group (n=94/96), and 93.8% for those with obesity (n=75/80). These differences were not statistically significant (χ^2=3.10, p=0.37). Self-monitoring of blood glucose was reported by 71.7% of participants (n=419/584), with significantly higher adherence (97.9%, n=410/419) compared to patients who did not self-monitor (93.3%, n=154/165; χ^2=7.30, p=0.007); this finding suggests a positive impact of self-monitoring on adherence. Patients considered to be informed regarding their condition (n=333, 57.0%) exhibited a slightly higher adherence rate (97.3%, n=324/333) than uninformed patients (95.6%, n=240/251), although this difference was statistically insignificant (χ^2=1.22, p=0.26). We also examined the type of pharmacological treatment. Patients taking 2 or more oral antidiabetic drugs (OADs; n=195) exhibited the highest adherence (99.0%, n=193/195), followed by those receiving an OAD plus insulin (95.6%, n=65/68), an OAD alone (95.7%, n=156/163), and insulin alone (94.9%, n=150/158). These differences were not statistically significant (χ^2=5.24, p=0.15). However, analysis of specific OADs did reveal a significant difference in adherence rates (χ^2=18.32, p=0.01). Patients treated with combined gliclazide and acarbose (n=155), glipizide (n=18), or combined metformin and glimepiride (n=5) showed perfect adherence (100%). Lower adherence occurred with metformin alone (83.3%, n=5/6) and combined gliclazide, acarbose, and glipizide (90.9%, n=30/33). Combination therapy was prevalent, with adherence rates of 97.6% for dual therapy (n=206/211), 96.7% for triple therapy or more (n=117/121), and 95.6% for monotherapy (n=241/252). However, number of medications was not significantly associated with adherence (χ^2=1.38, p=0.49). Regarding comorbidities, high adherence rates were noted among patients with hypertension (97.2%, n=205/211), dyslipidemia (100%, n=22/22), and other conditions (91.4%, n=53/58), as well as those without comorbidities (97.4%, n=187/192). The overall association between comorbidities and adherence was not statistically significant (χ^2=8.99, p=0.17). Overall, while most clinical variables were not significantly associated with adherence, self-monitoring of blood glucose emerged as a significant determinant, highlighting its potential as an intervention target.

Medication Adherence Patterns Based on GMAS Items

Table 3 presents data on treatment adherence based on participant responses to the Moroccan Arabic version of

Table 3. Description of responses provided by participants to the Moroccan Arabic version of the GMAS

Construct	Study item	Frequency (n, %)			
		Always	Mostly	Sometimes	Never (n=584)
PBNA	GMAS1: Do you have difficulty remembering to take your medications?	2 (0.3)	13 (2.2)	62 (10.6)	507 (86.8)
	GMAS2: Do you forget to take your medication due to your busy schedule, traveling, meetings, events at home, parties, marriage, religious celebrations, etc.?	2 (0.3)	12 (2.1)	60 (10.3)	510 (87.3)
	GMAS3: Do you discontinue your medication when you feel well?	1 (0.2)	1 (0.2)	29 (5.0)	553 (94.7)
	GMAS4: Do you stop taking medications when you feel adverse effects such as gastric discomfort, etc.?	1 (0.2)	1 (0.2)	23 (3.9)	559 (95.7)
	GMAS5: Do you stop taking medications without informing your doctor?	2 (0.3)	1 (0.2)	30 (5.1)	551 (94.3)
ADPB	GMAS6: Do you discontinue your medicines due to other medicines that you have to take for additional disease?	1 (0.2)	0 (0)	16 (2.7)	567 (97.1)
	GMAS7: Do you find it to be a hassle to remember your medications due to medication regimen complexity?	1 (0.2)	7 (1.2)	49 (8.4)	527 (90.2)
	GMAS8: During the last month, did you ever miss your medicines due to disease progression and addition of new medicines?	2 (0.3)	5 (0.9)	48 (8.2)	529 (90.6)
	GMAS9: Do you alter medication regimen, dose, and frequency by yourself?	0 (0)	7 (1.2)	31 (5.3)	546 (93.5)
CRNA	GMAS10: Do you discontinue medications because they are not worth the money you spent on them?	1 (0.2)	8 (1.4)	38 (6.5)	537 (92.0)
	GMAS11: Do you find it difficult to buy your medicines because they are expensive?	6 (1.0)	23 (3.9)	61 (10.4)	494 (84.6)

GMAS, general medication adherence scale; PBNA, patient behavior-related non-adherence; ADPB, non-adherence related to additional disease and pill burden; CRNA, cost-related non-adherence.

https://doi.org/10.24171/j.phrp.2025.0014

the GMAS. The results, representing self-reported answers, indicate that 86.8% of patients (n = 507) never had difficulty remembering to take their medications (GMAS1), while 87.3% (n = 510) never missed doses due to busy schedules or unexpected events (GMAS2). Regarding intentional non-adherence, 94.7% (n = 553) reported continuing their treatment even when feeling well (GMAS3), while 95.7% (n = 559) did not stop medication despite experiencing side effects (GMAS4). Additionally, 94.3% (n = 551) reported never discontinuing treatment without informing their doctor (GMAS5). In the ADPB domain, 97.1% of respondents (n = 567) did not discontinue medication due to concurrent treatments (GMAS6). Moreover, 90.2% (n = 527) did not find their treatment regimen complex or difficult to manage (GMAS7), and 90.6% (n = 529) did not miss medications due to the addition of new drugs (GMAS8). A large majority (93.5%, n = 546) indicated that they never modified their dosage or treatment schedule without medical advice (GMAS9).

Concerning CRNA, 92.0% of patients (n = 537) stated that they do not stop treatment due to the perceived value of the medication (GMAS10). However, 10.4% (n = 61) reported difficulty purchasing medications due to their cost (GMAS11), although 84.6% (n = 494) indicated an absence of financial barriers. These findings reflect a high overall adherence rate among the study population, although minor challenges remain regarding treatment complexity and medication affordability.

Probabilities of Medication Adherence

Table 4 summarizes the results of the multivariate logistic regression examining associations of sociodemographic and clinical factors with medication adherence among patients with T2DM in Morocco. The outcome variable is binary, distinguishing between adherence and non-adherence. Patients in rural areas exhibited a lower probability of adherence compared to urban residents (adjusted odds ratio [aOR], 0.245; 95% CI, 0.053–1.123; p = 0.070), although this finding was not statistically significant (p > 0.05). Men displayed a slightly lower probability of adherence compared to women, while the marital status categories of "single," "married," and "widowed"—relative to the reference category of "divorced"—displayed high odds ratios with wide CIs and high p-values (0.997 for all), suggesting a lack of statistical power or limited variability within these groups. Education level did not exhibit a significant association with adherence; for instance, illiterate participants had a high odds ratio without statistical significance (aOR, 1.429; 95% CI, 0.033–62.133; p = 0.853). Similarly, professional status did not significantly affect adherence, as exemplified by the finding regarding retirees (aOR, 0.000; p = 0.996). The chi-square test revealed significantly lower non-adherence among uninsured patients (1%) compared to insured patients (4.7%; p = 0.02). However, logistic regression adjusting for age, sex, residence, and other variables showed that insured patients had a higher probability of adherence (aOR, 2.585), although this difference was statistically insignificant (p = 0.827). Patients covered by AMO showed a slightly elevated, although not significant, probability of adherence (aOR, 1.248; 95% CI, 0.114–13.66; p = 0.856) compared to those without insurance. Patients receiving combined treatment with 2 or more OADs displayed significantly better adherence compared to those on a single treatment (aOR, 0.026; 95% CI, 0.001–0.642; p = 0.026). Patients with a diabetes duration of 11 to 15 years had significantly lower odds of adherence (aOR, 0.037; 95% CI, 0.001–0.956; p = 0.047), possibly reflecting disease duration-related burnout or a decline in motivation to follow treatment guidelines. Lastly, being informed about their illness had no significant impact on adherence in our study population (aOR, 0.552; 95% CI, 0.121–2.527; p = 0.444).

Figure 3 illustrates the distribution of medication adherence levels among patients with T2DM, showing that 89.4% of participants exhibited high adherence, while 7.2% had good adherence. Notably, no patients demonstrated low or moderate adherence (0.0%). These results highlight positive adherence to treatment overall, possibly reflecting effective awareness and diabetes management strategies in primary healthcare facilities in Morocco.

Figure 4 details medication adherence levels across dimensions of the GMAS, including patient behavior (intentional and non-intentional), comorbidities, polypharmacy, and financial constraints. The results indicate that 93.0% of patients exhibited high behavior-related adherence. Additionally, 91.5% of patients demonstrated good adherence despite comorbidities and a significant medication burden, with no cases of low or poor adherence. Finally, 81.5% of patients maintained good adherence despite financial constraints, with only 0.3% showing poor adherence for this reason.

Discussion

The assessment of medication adherence is complex, as adherence depends on both intentional and unintentional patient behaviors [41]. Adherence to antidiabetic regimens is essential for optimal blood glucose control, while non-adherence can lead to serious complications. Our study found high adherence levels among patients with T2DM, aligning with findings from Naqvi et al. [35] and Khardali et al. [42], who also reported satisfactory adherence rates. Notably, however, our observed rate of 96.2% is significantly higher than those reported by Naqvi et al. [35] and Khardali

Table 4. Probability of medication adherence based on sociodemographic characteristics and disease-related data

Determinant	Category	Adjusted odds ratio	95% CI	*p*-value
Area of residence	Rural	0.245	0.053–1.123	0.070
	Urban	Ref.		
Sex	Male	0.515	0.137–1.938	0.327
	Female	Ref.		
Marital status	Single	2.87×10^{10}	0.000–not estimable	0.997
	Married	4.16×10^{10}	0.000–not estimable	0.997
	Widowed	2.05×10^{10}	0.000–not estimable	0.997
	Divorced	Ref.		
Education level	Illiterate	1.429	0.033–62.133	0.853
	Primary	1.019	0.011–94.331	0.994
	Middle school	0.286	0.003–30.171	0.598
	High school	0.000	0.000–not estimable	0.996
	University	Ref.		
Employment status	Unemployed	0.819	0.023–29.357	0.913
	Retired	0.000	0.000–not estimable	0.996
	Employed	Ref.		
Health insurance status	Insured	2.585	0.001–12.67	0.827
	Uninsured	Ref.		
Type of insurance	CNOPS	0.087	0.00–466.96	0.578
	CNSS	0.109	0.002–51.99	0.261
	AMO	1.248	0.114–13.66	0.856
	Uninsured	Ref.		
Treatment	One OAD	0.300	0.001–642	0.295
	OAD+insulin	0.168	0.012–2.275	0.179
	Two or more OADs	0.026	0.001–0.642	0.026*
	Insulin only	Ref.		
Use of combination therapy	Monotherapy	0.292	0.063–1.347	0.115
	Dual therapy	0.209	0.021–2.117	0.185
	Triple therapy	Ref.		
Associated diseases	HBP	3.81×10^{10}	0.000–not estimable	0.997
	Dyslipidemia	0.923	0.000–not estimable	1.000
	Other diseases	1.05×10^{11}	0.000–not estimable	0.997
	No associated disease	6.19×10^{9}	0.000–not estimable	0.997
	HBP+dyslipidemia	1.23×10^{11}	0.000–not estimable	0.997
	HBP+other	5.66×10^{11}	0.000–not estimable	0.996
	HBP+dyslipidemia+other	Ref.		
Duration of disease (y)	<1	0.367	0.012–11.440	0.568
	1–5	0.620	0.083–4.656	0.642
	6–10	0.291	0.033–2.562	0.266
	11–15	0.037	0.001–0.956	0.047*
	16–20	0.368	0.023–5.909	0.480
	≥21	Ref.		
Patient's knowledge about their condition	Informed patient	0.552	0.121–2.527	0.444
	Uninformed patient	Ref.		

The variables included in the multivariable binary logistic regression model were residence, sex, marital status, education level, employment status, health insurance status, type of insurance, prescribed treatment, use of combination therapy, comorbidities associated with diabetes, disease duration, and patient knowledge about their diabetes. Medication adherence represented the dependent variable.

CI, confidence interval; ref, reference; CNOPS, National Fund of Social Welfare Organizations; CNSS, National Social Security Fund; AMO, Mandatory Health Insurance; OAD, oral antidiabetic drug; HBP, high blood pressure.

*$p<0.05$.

https://doi.org/10.24171/j.phrp.2025.0014

et al. [42] (61.7% and 66%, respectively). These differences might reflect regional variations, despite the use of the same GMAS-based definition of adherence (with scores of ≥27 categorized as adherence and ≤26 as non-adherence).

Previous studies conducted in Saudi Arabia have reported high rates of non-adherence, reaching 67.9% in the Al Hasa district [43]. Additionally, high rates of non-adherence have been observed in various Middle Eastern countries, including Jordan (58%) [44,45], Palestine (52.7%) [46], the United Arab Emirates (61.7%) [47], and Qatar (73%) [48]. These findings contrast with ours, underscoring the importance of regional context.

The data from our study corroborate the results of a previous investigation we conducted with 284 patients [7]. However, expanding the sample size to 584 patients, distributed across 2 major regions, and examining factors associated with adherence provide a more comprehensive perspective. A complementary qualitative study is planned to better understand the reasons for the high adherence rate in Morocco and to identify additional influencing factors.

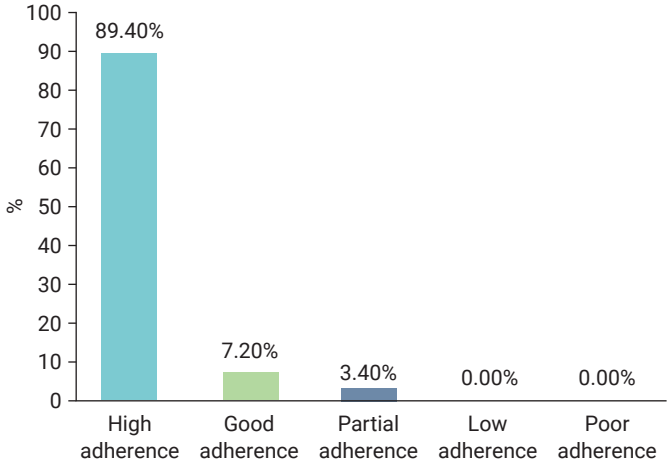


Figure 3. Distribution of medication adherence levels among patients with type 2 diabetes in primary healthcare facilities in Morocco.

Public health policies in Morocco, such as initiatives from the Ministry of Health and Social Protection, could play a key role in improving medication adherence. These initiatives aim to promote economic and health prosperity by reducing health disparities among patients with chronic diseases [49]. Strategies include improving access to treatments and preventive care services and providing free basic medical care to all Moroccan citizens, including those with non-communicable diseases. Other noteworthy measures include increasing the budget for antidiabetic medications, raising awareness about diabetes, and collaborating with the private sector and civil society regarding therapeutic education [49]. Expanding health coverage may also improve adherence.

We identified a significant association between treatment adherence and the absence of health insurance, suggesting that uninsured patients might be more mindful of medical costs and more diligent in adhering to treatment. This observation contrasts with findings from Naqvi et al. [35], Khardali et al. [42], Allaham et al. [40], and Shakya et al. [9], who reported no significant association between health insurance and adherence. However, our results may be compared with an Ethiopian study [50] that indicated lower adherence among uninsured patients paying out-of-pocket for medications. Our unexpected finding may stem from Morocco's cultural and economic context. Uninsured Moroccan patients might view healthcare costs as a

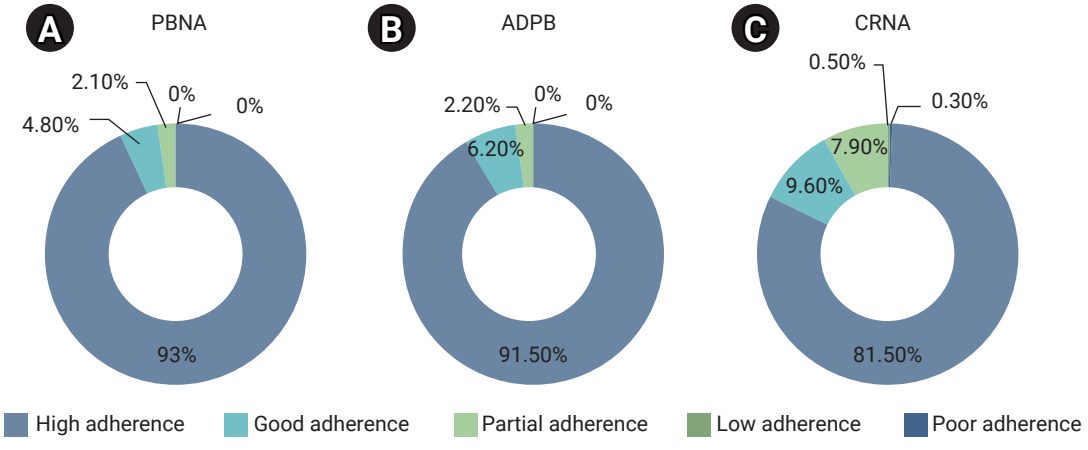


Figure 4. Factors related to adherence. (A) PBNA, patient behavior-related non-adherence, (B) ADPB, non-adherence related to additional disease and pill burden, (C) CRNA, cost-related non-adherence.

substantial financial burden, prompting stricter treatment management to avoid costly medical consultations or hospitalizations.

Furthermore, individuals from lower socioeconomic backgrounds may adopt stricter health behaviors, driven by the need to maximize the effectiveness of self-funded treatments. Psychological factors, such as fear of diabetes-related complications or cultural emphasis on individual responsibility in managing illness, might also influence this behavior. Additionally, strong community support systems in rural areas of Morocco could reinforce adherence among uninsured patients through informal advice and social solidarity. A lack of health coverage may also foster a heightened sense of personal accountability, motivating patients to avoid expenses related to complications from poor adherence. Nonetheless, we must acknowledge that unmeasured variables, including cultural beliefs about illness or psychological factors such as anxiety over future medical costs, might also influence this association. Future research should explore these hypotheses to clarify the mechanisms driving this unanticipated finding.

A significant association was observed between treatment adherence and self-monitoring of blood glucose. Self-monitoring enables patients to regularly track glucose levels, detect variations, and adjust their treatment accordingly, thereby fostering a better understanding of diabetes and improving disease management. Thus, this practice might be expected to increase adherence to medical recommendations, aligning with Sendekie et al. [50]. However, some studies have not found a significant relationship between adherence and self-monitoring [51,52].

Combinations of specific antidiabetic medications, such as gliclazide and acarbose, were associated with relatively high adherence levels. This significant association suggests that medication choice might influence treatment compliance. Conversely, our study found no significant links between adherence and factors such as sex, marital status, education level, employment status, or comorbidities, although other studies have identified such associations [9,35,42,51].

Logistic regression analysis revealed that patients on 2 or more OADs had significantly lower adherence, consistent with studies from the United Arab Emirates and Ethiopia [40,50]. This reduced adherence may result from the complexity and higher risk of errors associated with managing multiple medications.

Finally, longer disease duration, specifically 11 to 15 years, was associated with lower medication adherence, a finding consistent with a study conducted in Nepal [51]. Despite developing a better understanding of their condition, patients may experience fatigue or apathy towards treatment, undermining rigorous adherence.

Although our study primarily analyzed socioeconomic and clinical factors associated with medication adherence, cultural beliefs, lifestyle, and patient education can also play critical roles. In Morocco, traditional practices involving medicinal plants and cultural perceptions of diabetes might influence patient attitudes toward medication adherence. Additionally, lifestyle factors, such as dietary habits and physical activity, affect diabetes management and treatment motivation. Furthermore, a low level of health education could limit patient understanding of medical recommendations and compromise adherence, as observed in other studies of chronic diseases in low-resource settings.

In this context, our findings also highlight the positive impact of Moroccan public health policies, such as free access to care and increased resources for treatment. However, certain limitations of the study should be acknowledged. Its focus on 2 regions of the country may limit the generalizability of the results. Additionally, certain key variables were not captured by the GMAS tool used. Psychological factors such as depression and anxiety, as well as cultural beliefs, may also influence adherence but were not explored in this study. These dimensions warrant consideration in future research to gain a more comprehensive understanding of determinants of adherence and to enhance therapeutic strategies. A broader and more diverse approach, incorporating these factors, will help strengthen the effectiveness of interventions for patients with T2DM.

This study has several notable strengths. First, it includes a large, multicenter sample of 584 patients from 2 major regions of Morocco, thus providing nationally representative results. Additionally, its use of the GMAS, specifically validated for the Moroccan context, facilitates an accurate assessment of adherence by considering local socioeconomic and cultural factors. Furthermore, the study adopts a multidimensional approach, exploring a variety of socioeconomic and clinical factors associated with medication adherence; as such, it offers an in-depth understanding of the underlying determinants. Finally, the results of this study complement existing research while providing new and relevant insights to improve public health policies and psychosocial support programs in Morocco.

In conclusion, while our study reveals good medication adherence among patients with T2DM, implementing personalized strategies to strengthen this adherence is essential, especially for groups identified as at-risk. To address the lower adherence rates observed among these patients, several public health interventions can be considered.

https://doi.org/10.24171/j.phrp.2025.0014

Education and awareness programs are necessary to inform patients about diabetes management and emphasize the importance of adherence to treatment. Furthermore, personalized follow-up combined with the use of mobile applications could facilitate treatment monitoring. Additionally, psychosocial support through support groups and counselors with lived experience can increase patient motivation. Involving family members in the care process and proposing cost-reduction strategies for medications could also contribute to improving therapeutic adherence.

Finally, additional limitations of our study should be acknowledged. The cross-sectional design limits the ability to establish causal relationships between sociodemographic factors and medication adherence. Furthermore, the inclusion criteria may have influenced the sample by excluding certain vulnerable groups, such as newly diagnosed patients or those with acute conditions. Although reasonably large, the sample was limited to 2 regions of Morocco, which may not fully represent the diverse socioeconomic and cultural realities across the country. Furthermore, the GMAS, while reliable, does not account for psychosocial and cultural factors potentially influencing adherence, such as personal beliefs or family support systems.

Future research should aim for a broader and more diverse analysis to better identify the factors influencing treatment adherence and to optimize T2DM management strategies.

Implications and Directions for Future Research

In Morocco, public health reforms are needed to optimize healthcare spending and improve outcomes for citizens and residents. Increasing medication adherence for T2DM could significantly impact health economics, patient clinical outcomes, and medical practices. Strategies such as simplifying treatment regimens, actively involving healthcare professionals in medication management, and educating patients are often recommended. Healthcare professionals must also be encouraged to proactively foster treatment adherence.

The results of this study suggest the need for further research to better understand the factors contributing to the high levels of adherence observed. Qualitative surveys could provide valuable insights by exploring the experiences and perceptions of patients in detail. Additionally, future efforts should examine the barriers to adherence among patients in rural areas and identify facilitating factors for adherence among patients with long disease duration. These approaches will help develop more effective strategies to improve health outcomes and reduce the economic burden associated with medication non-adherence in the Moroccan healthcare system.

Conclusion

This study highlights a high medication adherence rate of 96.2% among patients with T2DM in Morocco. Adherence is significantly associated with regular self-monitoring of blood glucose and the use of certain combined oral antidiabetic treatments. Conversely, lower adherence was observed among patients undergoing dual therapy or polypharmacy and those with a longer disease duration. Interestingly, the absence of health insurance was linked to better adherence, suggesting that uninsured patients may adopt more diligent medication practices to avoid additional healthcare costs. These findings provide valuable insights into determinants of adherence and underscore the need for targeted interventions to support long-term treatment adherence, particularly among high-risk patient groups.

Notes

Ethics Approval

Data collection for the study was approved by IRB00012973 of the Moroccan Association for Research and Ethics, IRB #1 [09/REC/22], in accordance with the ethical principles outlined in the Declaration of Helsinki and relevant local ethical regulations. Written informed consent was obtained from all participants prior to their inclusion in the study. Participants were fully informed about the purpose of the study, the procedures involved, and their right to withdraw at any time without consequences to their medical care. This informed consent process was also approved by IRB #1 [09/REC/22].

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

None.

Availability of Data

The datasets are not publicly available but can be obtained from the corresponding author upon reasonable request.

Authors' Contributions

Conceptualization: MA, NAW; Data curation: MC; Formal analysis: MA; Funding acquisition: MC; Investigation: MA, KB, YI; Methodology: MA, YI, KB; Project administration: MC; Resources: MA, NAW; Validation: MA, FZB; Visualization: MC; Writing–original draft: MA, YI, KB, MC; Writing–review & editing: MA, NAW, YI, FZB, MC. All authors read and approved the final manuscript.

References

1. Wilson D, Diji AK, Marfo R, et al. Dietary adherence among persons with type 2 diabetes: a concurrent mixed methods study. PLoS One 2024;19:e0302914.
2. World Health Organization (WHO) (CH). Diabetes [Internet]. WHO;

2024 [cited 2024 Feb 11]. Available from: https://www.who.int/news-room/fact-sheets/detail/diabetes.
3. International Diabetes Federation (IDF) (BE). IDF diabetes atlas. IDF; 2021.
4. Arraji M, Al Wachami N, Boumendil K, et al. Ethnobotanical survey on herbal remedies for the management of type 2 diabetes in the Casablanca-Settat region, Morocco. BMC Complement Med Ther 2024;24:160.
5. World Health Organization (CH). Morocco: health system review [Internet]. WHO Regional Office for the Eastern Mediterranean; 2023 [cited 2024 Sep 17]. Available from: https://www.emro.who.int/publications/.
6. Maryem A, Younes I, Yassmine M, et al. Translation, cultural adaptation and validation of the General Medication Adherence Scale (GMAS) in moroccan patients with type-2 diabetes. BMC Nurs 2023;22:302.
7. Maryem A, Nadia AW, Younes I, et al. Measurement of medication adherence among type II diabetes patients attending primary healthcare facilities in Morocco. F1000Res 2023;12:1316.
8. Huang J, Ding S, Xiong S, et al. Medication adherence and associated factors in patients with type 2 diabetes: a structural equation model. Front Public Health 2021;9:730845.
9. Shakya P, Shrestha A, Karmacharya BM, et al. Factors associated with medication adherence among patients with type 2 diabetes mellitus: a hospital-based cross-sectional study in Nepal. Int J Environ Res Public Health 2023;20:1537.
10. Kang Y, Hur Y. Medication adherence and its associated factors in laotians with type 2 diabetes mellitus. Clin Nurs Res 2020;29:331–8.
11. Murwanashyaka JD, Ndagijimana A, Biracyaza E, et al. Non-adherence to medication and associated factors among type 2 diabetes patients at Clinique Medicale Fraternite, Rwanda: a cross-sectional study. BMC Endocr Disord 2022;22:219.
12. Cramer JA. A systematic review of adherence with medications for diabetes. Diabetes Care 2004;27:1218–24.
13. Blackburn DF, Swidrovich J, Lemstra M. Non-adherence in type 2 diabetes: practical considerations for interpreting the literature. Patient Prefer Adherence 2013;7:183–9.
14. Kassahun A, Gashe F, Mulisa E, et al. Nonadherence and factors affecting adherence of diabetic patients to anti-diabetic medication in Assela General Hospital, Oromia Region, Ethiopia. J Pharm Bioallied Sci 2016;8:124–9.
15. Fischer MA, Stedman MR, Lii J, et al. Primary medication non-adherence: analysis of 195,930 electronic prescriptions. J Gen Intern Med 2010;25:284–90.
16. Waari GK. Assessment of medication non-adherence and associated factors among type 2 diabetes mellitus patients attending the diabetic clinic at Kenyatta National Hospital [dissertation]. Jomo Kenyatta University; 2019.
17. Kalyango JN, Owino E, Nambuya AP. Non-adherence to diabetes treatment at Mulago Hospital in Uganda: prevalence and associated factors. Afr Health Sci 2008;8:67–73.
18. Mirahmadizadeh A, Khorshidsavar H, Seif M, et al. Adherence to medication, diet and physical activity and the associated factors amongst patients with type 2 diabetes. Diabetes Ther 2020;11:479–94.
19. Rwegerera GM. Adherence to anti-diabetic drugs among patients with type 2 diabetes mellitus at Muhimbili National Hospital, Dar es Salaam, Tanzania: a cross-sectional study. Pan Afr Med J 2014;17:252.
20. Aminde LN, Tindong M, Ngwasiri CA, et al. Adherence to antidiabetic medication and factors associated with non-adherence among patients with type-2 diabetes mellitus in two regional hospitals in Cameroon. BMC Endocr Disord 2019;19:35.
21. Kamel AM, Khatiwada AP, Qian J. EPH101 Medication adherence in Egypt: a systematic review. Value Health 2023;26(6 Supplement):S182.
22. Alammari G, Alhazzani H, AlRajhi N, et al. Validation of an Arabic version of the Adherence to Refills and Medications Scale (ARMS). Healthcare (Basel) 2021;9:1430.
23. Elsous A, Radwan M, Al-Sharif H, et al. Medications adherence and associated factors among patients with type 2 diabetes mellitus in the Gaza Strip, Palestine. Front Endocrinol (Lausanne) 2017;8:100.
24. Atekha BO. Factors associated with adherence to diabetes medication among individuals with type 2 diabetes in Cambridge, Ontario, Canada [dissertation]. Walden University; 2018.
25. Abebaw M, Messele A, Hailu M, et al. Adherence and associated factors towards antidiabetic medication among type II diabetic patients on follow-up at University of Gondar Hospital, Northwest Ethiopia. Adv Nurs 2016;2016:8579157.
26. Feldman BS, Cohen-Stavi CJ, Leibowitz M, et al. Defining the role of medication adherence in poor glycemic control among a general adult population with diabetes. PLoS One 2014;9:e108145.
27. Abate TW. Medication non-adherence and associated factors among diabetes patients in Felege Hiwot Referral Hospital, Bahir Dar city administration, Northwest Ethiopia. BMC Res Notes 2019;12:175.
28. AlQarni K, AlQarni EA, Naqvi AA, et al. Assessment of medication adherence in Saudi patients with type II diabetes mellitus in Khobar City, Saudi Arabia. Front Pharmacol 2019;10:1306.
29. Dunbar-Jacob J, Sereika SM, Houze M, et al. Accuracy of measures of medication adherence in a cholesterol-lowering regimen. West J Nurs Res 2012;34:578–97.
30. Nordstrom BL, Simeone JC, Zhao Z, et al. Adherence and persistence with prasugrel following acute coronary syndrome with percutaneous coronary intervention. Am J Cardiovasc Drugs 2013;13:263–71.
31. Nori AY, Ali MS, Ibrahim B, et al. PW269 Modifying the Morisky Medication Adherence Scale by adapting two items from the brief medication questionnaire to fit assessing the overall adherence to polypharmacy regimen of cardiac patients. Glob Heart 2014;9(1 Supplement):e313.
32. Shalansky SJ, Levy AR, Ignaszewski AP. Self-reported Morisky score for identifying nonadherence with cardiovascular medications. Ann Pharmacother 2004;38:1363–8.
33. Naqvi AA, Hassali MA, Rizvi M, et al. Development and validation of

https://doi.org/10.24171/j.phrp.2025.0014

a novel General Medication Adherence Scale (GMAS) for chronic illness patients in Pakistan. Front Pharmacol 2018;9:1124.

34. Houguig K, Rkha S, Rayadi M, et al. Factors influencing therapeutic observance in diabetic subjects in the province of Essaouira (Morocco): a cross-sectional study. Ethiop J Health Sci 2022;32:747–54.
35. Naqvi AA, Mahmoud MA, AlShayban DM, et al. Translation and validation of the Arabic version of the General Medication Adherence Scale (GMAS) in Saudi patients with chronic illnesses. Saudi Pharm J 2020;28:1055–61.
36. Islam MA, El-Dahiyat F, Nouri A, et al. Validation of the Arabic version of the general medication adherence scale in patients with type 2 diabetes mellitus in Jordan. Front Pharmacol 2023;14:1194672.
37. Mahmoud MA, Islam MA, Ahmed M, et al. Validation of the Arabic version of General Medication Adherence Scale (GMAS) in Sudanese patients with diabetes mellitus. Risk Manag Healthc Policy 2021; 14:4235–41.
38. Shrestha R, Sapkota B, Khatiwada AP, et al. Translation, cultural adaptation and validation of General Medication Adherence Scale (GMAS) into the Nepalese language. Patient Prefer Adherence 2021; 15:1873–85.
39. Wang Y, Wang X, Wang X, et al. Translation and validation of the Chinese version of the general medication adherence scale (GMAS) in patients with chronic illness. Curr Med Res Opin 2021;37:829–37.
40. Allaham KK, Feyasa MB, Govender RD, et al. Medication adherence among patients with multimorbidity in the United Arab Emirates. Patient Prefer Adherence 2022;16:1187–200.
41. Osterberg L, Blaschke T. Adherence to medication. N Engl J Med 2005;353:487–49.
42. Khardali A, Kashan Syed N, Alqahtani SS, et al. Assessing medication adherence and their associated factors amongst type-2 diabetes mellitus patients of Jazan Province, Saudi Arabia: a single-center, cross-sectional study. Saudi Pharm J 2024;32:101896.
43. Khan AR, Al-Abdul Lateef ZN, Al Aithan MA, et al. Factors contributing to non-compliance among diabetics attending primary health centers in the Al Hasa district of Saudi Arabia. J Family Community Med 2012;19:26–32.
44. Jarab AS, Almrayat R, Alqudah S, et al. Predictors of non-adherence to pharmacotherapy in patients with type 2 diabetes. Int J Clin Pharm 2014;36:725–33.
45. Al-Qerem W, Jarab AS, Badinjki M, et al. Factors associated with glycemic control among patients with type 2 diabetes: a cross-sectional study. Eur Rev Med Pharmacol Sci 2022;26:2415–21.
46. Almadhoun R, Kadadha M, Alhemeiri M, et al. A user authentication scheme of IoT devices using blockchain-enabled fog nodes. Proc IEEE/ACS Int Conf Comput Syst Appl 2018;1–8.
47. Asheq A, Ashames A, Al-Tabakha M, et al. Medication adherence in type 2 diabetes mellitus patients during COVID-19 pandemic: a cross-sectional study from the United Arab Emirates. F1000Res 2021; 10:435.
48. Jaam M, Mohamed Ibrahim MI, Kheir N, et al. Assessing prevalence of and barriers to medication adherence in patients with uncontrolled diabetes attending primary healthcare clinics in Qatar. Prim Care Diabetes 2018;12:116–25.
49. World Bank (US). New program in Morocco supports improved access to quality healthcare for all [Internet]. World Bank; 2023 [cited 2024 Sep 17]. Available from: https://www.worldbank.org/en/news/press-release/2023/06/19/new-program-in-morocco-supports-improved-access-to-quality-healthcare-for-all.
50. Sendekie AK, Netere AK, Kasahun AE, et al. Medication adherence and its impact on glycemic control in type 2 diabetes mellitus patients with comorbidity: a multicenter cross-sectional study in Northwest Ethiopia. PLoS One 2022;17:e0274971.
51. Sah BK, Basyal D, Gaire A. Medication non-adherence among type-II diabetes mellitus out-patients attending at tertiary care hospital, Nepal. Clinical Pharmacol Biopharm 2021;10:1–7.
52. Kafle NK, Poudel RR, Shrestha SM. Noncompliance to diet and medication among patients with type 2 diabetes mellitus in selected hospitals of Kathmandu, Nepal. J Soc Health Diabetes 2018;6:90–5.

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2024.0310
Osong Public Health Res Perspect 2025;16(4):406-414

Original Article

Risk of tuberculosis in individuals with type 2 diabetes mellitus based on the tuberculosis predictive index score: a case-control study in Indonesia

Dea P. Audina[1], Rachel S. Aritonang[1], Muhammad I. Mokoagow[2]

[1]Department of Pulmonology and Respiratory Medicine, Persahabatan Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[2]Fatmawati General Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

ABSTRACT

Objectives: The co-occurrence of tuberculosis (TB) and diabetes mellitus presents a significant global health challenge, marked by a bidirectional relationship. This study aims to evaluate the effectiveness of the tuberculosis predictive index (TPI) score, developed by Isfandiari et al., in predicting TB occurrence among individuals living with type 2 diabetes mellitus.

Methods: A case-control study was conducted using primary data collected through questionnaires administered to individuals with type 2 diabetes mellitus, with and without pulmonary TB, at the internal medicine outpatient clinic of Fatmawati General Hospital from June to August 2024. The study compared TPI scores between those with type 2 diabetes mellitus who had TB and those who did not.

Results: TPI scores were significantly associated with TB risk. Individuals with both type 2 diabetes mellitus and TB had a 6.8-fold higher risk (95% confidence interval [CI], 2.6–17.6; $p < 0.001$) than those without TB. Further chi-square analysis identified three significant risk factors: individuals with type 2 diabetes mellitus exhibiting TB-like symptoms had a 13.3-fold increased TB risk (95% CI, 5.1–34.3; $p < 0.001$); those with a body mass index below 18.5 kg/m² had a 3.3-fold higher risk (95% CI, 1.0–11.0; $p = 0.039$); and those living in poorly ventilated homes (ventilation ≤10%) had a 3.2-fold higher risk (95% CI, 1.0–9.8; $p = 0.035$).

Conclusion: This study demonstrates that individuals with type 2 diabetes mellitus who developed TB had significantly higher TPI scores, corresponding to a 6.8-fold increased risk compared to their counterparts without TB. The TPI score may serve as a valuable tool for predicting TB risk among populations living with type 2 diabetes mellitus.

Keywords: Diabetes mellitus type 2; Risk assessment; Tuberculosis pulmonary

Received: November 5, 2024
Revised: April 30, 2025
Accepted: May 11, 2025

Corresponding author:
Dea P. Audina
Department of Pulmonology and Respiratory Medicine, Persahabatan Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
E-mail: drdeaputriaudina@gmail.com

Introduction

Tuberculosis (TB) remains a major global health concern, affecting approximately one-third of

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Korea Disease Control and Prevention Agency and Korea National Institute of Health.

© 2025 Korea Disease Control and Prevention Agency.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

the world's population and causing around 2 million deaths annually due to *Mycobacterium tuberculosis* infection [1]. In 2019 alone, global TB cases were estimated between 8.9 and 11 million, with Indonesia ranking second-highest in the world [2]. TB risk factors are categorized as intrinsic, such as diabetes mellitus, malnutrition, and malignancy, and extrinsic, including high population density, smoking, and alcohol consumption [3].

The prevalence of diabetes mellitus has risen dramatically, with the World Health Organization (WHO) reporting an increase from 4.7% in 1980 to 8.5% in 2014. Diabetes mellitus has become a leading global non-communicable disease, projected to affect approximately 700 million individuals by 2045 [3]. Patients diagnosed with both diabetes mellitus and TB experience notably worse outcomes, including higher risks of severe clinical presentations, treatment failure, relapse, and mortality [4,5]. Epidemiological studies report that between 5% and 30% of TB patients also have comorbid diabetes mellitus, and individuals with diabetes mellitus face a threefold increased risk of developing TB compared to those without diabetes mellitus [6]. Diabetes mellitus adversely impacts immune function and influences TB progression by elevating bacterial load and altering the absorption of anti-TB medications, leading to increased treatment complexity [7].

Emerging evidence suggests chronic inflammation significantly contributes to the TB-diabetes mellitus relationship. Type 2 diabetes mellitus (T2DM) is characterized by persistent systemic inflammation, which is integral to its complications. Studies utilizing inflammatory marker scores indicate that patients with T2DM exhibit elevated systemic inflammatory responses compared to healthy individuals [8–10]. Likewise, TB induces excessive immune activation, predominantly driven by neutrophilic responses, leading to lung tissue damage and worsened clinical outcomes. The overlapping inflammatory mechanisms between diabetes mellitus and TB imply that systemic inflammation may underpin the heightened susceptibility to TB observed in patients with diabetes [11].

Despite the well-established association between TB and diabetes mellitus, existing screening methods remain insufficient, frequently failing to identify patients with diabetes who are at high risk. Many patients with diabetes mellitus and TB present atypically, contributing to higher rates of treatment failure, relapse, and latent TB infections [12,13]. Conventional diagnostic tools—such as sputum smear microscopy, nucleic acid amplification tests, and chest X-rays—have limitations, particularly in identifying latent or early-stage TB among patients with diabetes who often exhibit altered immune responses [14]. Moreover, poor glycemic control complicates TB risk prediction and management, adding complexity to clinical outcomes [15].

To address this gap, Isfandiari et al. [16] developed the tuberculosis predictive index (TPI) score, a novel predictive tool incorporating biological, social, and environmental risk factors to estimate TB risk among patients with diabetes. This scoring system provides a structured method for identifying high-risk individuals, potentially improving early detection and targeted screening, particularly in TB-endemic regions like Indonesia. By evaluating the TPI score's effectiveness, the present study aims to enhance risk stratification and inform prevention strategies for TB in populations with diabetes mellitus.

HIGHLIGHTS

- The tuberculosis predictive index (TPI) score effectively predicts tuberculosis (TB) risk in patients with type 2 diabetes mellitus.
- Patients with diabetes and TB have a 6.8-fold higher TPI score risk than those without TB.
- TB-like symptoms increase TB risk by 13.3-fold in patients with diabetes.
- Low body mass index (body mass index <18.5 kg/m^2) and poor housing ventilation further increase TB risk in patients with diabetes.
- The TPI score can serve as a practical tool to assess TB risk among populations with diabetes.

Materials and Methods

Study Design and Setting

This study used an observational case-control design. Data collection took place at Fatmawati General Hospital from May to August 2024. Medical records from the Internal Medicine Outpatient Clinic at Fatmawati General Hospital, spanning from 2021 to 2024, were reviewed. Additionally, primary data were collected directly from patients through questionnaires administered during follow-up visits in July and August 2024.

Participants

The study targeted adult patients (aged over 18 years) diagnosed with diabetes mellitus, with or without pulmonary TB, who attended the Internal Medicine Outpatient Department at Fatmawati General Hospital between 2021 and 2024. Participants were selected using a

consecutive sampling method.

The inclusion criteria were: (1) adult patients aged over 18 years, (2) diagnosis of T2DM, and (3) presence or absence of clinically or bacteriologically confirmed pulmonary TB. Exclusion criteria included: (1) patients with immunocompromised conditions or autoimmune diseases receiving post-transplant care, chemotherapy, or immunosuppressive therapy, and (2) patients with incomplete medical records. Continuous follow-up monitoring was conducted for eligible patients throughout the study period.

Variables

The primary independent variable was the TPI score of patients from the Internal Medicine Outpatient Department at Fatmawati General Hospital. Dependent variables included age, sex, body mass index (BMI), history of contact with TB patients, duration of diabetes mellitus, symptoms resembling pulmonary TB, hemoglobin A1c (HbA1c) levels, housing environment, and psychological well-being.

The TPI score was calculated using the following formula [16]: −3.218+0.867×age (≤55 years)+1.339×sex (male)+1.493×contact with previous TB patients (yes)+1.089×glycemic control (HbA1c >7%)+1.622×clinical complaints resemble TB symptoms (>3 symptom)+1.183×BMI (≤18.5 kg/m^2)+0.891×duration of T2DM (≤10 years)+0.454×house ventilation width (≤10% house floor width)+0.583×psychological well-being (poor).

Clinical symptoms resembling TB included at least 3 of the following: cough lasting over 2 weeks, hemoptysis, fever, night sweats, unintended weight loss, and reduced appetite. A TPI score below 0 indicated low TB risk among patients with T2DM, whereas a score of 0 or higher indicated higher TB risk.

Diabetes mellitus was diagnosed according to the American Diabetes Association 2024 criteria [17], defined as fasting plasma glucose ≥126 mg/dL, 2-hour plasma glucose ≥200 mg/dL in an oral glucose tolerance test, HbA1c ≥6.5%, or random plasma glucose ≥200 mg/dL in the presence of hyperglycemic symptoms. Glycemic control was classified as good (HbA1c ≤7%) or poor (HbA1c >7%).

History of TB contact was determined by exposure to an individual diagnosed with TB within the previous 2 years. Psychological well-being was assessed using the WHO-5 Well-Being Index, with scores ≤50 indicating poor well-being and scores >50 indicating good well-being. Age was recorded at data collection, while sex referred to biological sex. BMI was calculated by dividing weight (kg) by height squared (m^2). The duration of T2DM reflected the period from the diagnosis to the interview date.

Data Measurement

Data were collected from medical records and patient interviews. In cases of discrepancies between self-reported data and medical records, priority was given to medical records for objective measures such as diabetes status, HbA1c levels, BMI, and TB contact history. For subjective variables like TB-like symptoms and psychological well-being, patient-reported data were accepted unless inconsistencies indicated potential recall bias, in which case validation was conducted through physician confirmation.

Individuals were classified based on diabetes status, recorded as controlled diabetes (0) or uncontrolled diabetes (1). History of TB contact was determined from medical records and patient history, categorized as prior contact with a TB patient (1) or no contact (0). Presence of TB-like symptoms was assessed via medical records and patient history, classified as the presence of 3 or more symptoms (1) or fewer symptoms (0). Random blood glucose levels were measured in mg/dL, and HbA1c was categorized as ≤7% (0) or >7% (1).

Age was documented from medical records and grouped as ≤55 years (1) or >55 years (0). Sex was categorized as male (1) or female (0). BMI, calculated from weight and height using the Asia-Pacific classification, was categorized as ≤18.5 kg/m^2 (1) or >18.5 kg/m^2 (0). Housing conditions were evaluated using the Indonesian Healthy House Card criteria, categorizing ventilation as ≤10% of the floor area (1) or >10% (0). Psychological well-being, assessed using the WHO-5 Index, was categorized as poor (≤50; score=1) or good (>50; score=0). Diabetes duration, determined from medical records, was categorized as ≤10 years (1) or >10 years (0).

Bias

Potential bias arose from interviewing patients with both diabetes and TB regarding their TB-related symptoms 2 months after initiating anti-TB treatment, at which point symptoms may have resolved, affecting reporting accuracy. To minimize this bias, symptom evaluation was explicitly limited to the period preceding initiation of anti-TB therapy. This ensured that reported symptoms accurately represented patients' pre-treatment conditions.

Study Size

The sample size calculation aimed to determine the proportion of TB among patients with diabetes mellitus using a formula for unpaired numerical data (Figure 1). Given the global prevalence of diabetes among TB patients is approximately 13.73% [9], with a 95% confidence interval (CI) and a 5% type I error rate, the necessary sample size

https://doi.org/10.24171/j.phrp.2024.0310

$$n_1 = n_2 = 2\left(\frac{(Z_a+Z_b)S^2}{X_1-X_2}\right)^2$$

Figure 1. Sample size formula for unpaired numerical data.

was calculated using a combined standard deviation of 40. Using this formula, the minimum sample size required was determined to be 69 subjects per group.

Quantitative Variables

Quantitative variables analyzed in this study included age, BMI, blood glucose levels, and HbA1c. Each variable was categorized and measured specifically to evaluate their associations with TB risk. To assess the effect of age on TB risk, patients were categorized into 2 groups: older than 55 years, and 55 years or younger. BMI was classified according to Asia-Pacific guidelines into 2 categories: greater than 18.5 kg/m² and 18.5 kg/m² or less, to determine the relationship between weight status and TB risk. Blood glucose levels were measured in milligrams per deciliter, while HbA1c values were reported as percentages and categorized as either less than 7% or 7% or higher to evaluate long-term glycemic control. These categorizations were selected based on established clinical thresholds to ensure relevance and consistency when analyzing the impact of these quantitative variables on TB risk.

Statistical Methods

All baseline data and study outcomes were stored electronically for statistical analysis. Data were analyzed using SPSS version 25. Descriptive statistics were employed to determine frequency distributions, percentages, means, and ranges of observed variables. Bivariate analysis was performed to explore the distribution and associations between independent and dependent variables. Chi-square tests were applied to assess differences in TPI scores between the groups with and without TB.

To estimate the association between TB risk and predictor variables, logistic regression analysis was conducted. Both the composite TPI score and individual components—including age, sex, TB contact history, glycemic control, presence of TB-like symptoms, BMI, duration of diabetes, housing ventilation, and psychological well-being—were analyzed as predictors of TB risk. Odds ratios (ORs) with 95% CIs were calculated to quantify the strength of these associations. Cases with missing data were excluded from analyses to maintain statistical accuracy.

Ethics Statement

This study received approval from the Director of Human Resources, Education, and Research of Fatmawati General Hospital (approval number: DP.04.03/DXXI.2/7392/2024) on July 19, 2024. The research adhered to bioethical principles, including autonomy, beneficence, nonmaleficence, and justice. Written informed consent was obtained from all participants for publication of the study results.

Results

Participants

The minimum required sample size calculated for each group was 69 subjects. Initially, 70 control subjects and 79 case subjects were recruited. However, following a review of medical records, there was an insufficient number of eligible cases, prompting an adjustment in the case-to-control ratio to approximately 1:2. As a result, the final analysis included 70 controls (patients with diabetes) and 39 cases (patients with both diabetes and TB), totaling 109 participants. The study flow is detailed in Figure 2.

Descriptive Data

Participants' characteristics, including age, sex, TB contact history, presence of TB-like symptoms, HbA1c levels, duration of diabetes, BMI, housing ventilation quality, psychological well-being, and overall TPI scores, are summarized in Table 1. Among the 39 patients with diabetes and TB, 41% were aged ≤55 years, compared to 74.3% among those without TB. There was a higher proportion

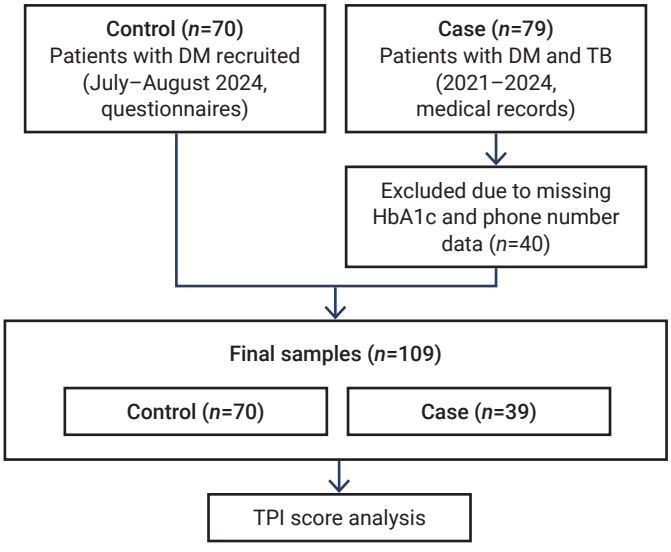


Figure 2. Research flow.
DM, diabetes mellitus, TB, tuberculosis; HbA1c, hemoglobin A1c; TPI, tuberculosis predictive index.

of men among patients with diabetes and TB (53.8%) compared to patients with diabetes but not TB, who were predominantly female (64.3%). Both groups had a majority without a history of TB contact (56.4% in the TB group and 67.1% in the non-TB group). Notably, TB-like symptoms were much more frequent among patients with diabetes and TB (76.9%) compared to those without TB (20%).

HbA1c levels ≥7% were similarly common in both groups, present in 66.7% of patients with TB and 60% of patients without TB. Most patients had diabetes for more than 10 years (59% of patients with TB, 52.9% of patients without TB). Most participants in both groups had a BMI >18.5 kg/m² (79.5% of patients with TB, 92.9% of patients without TB). Good housing ventilation (>10%) was more common among patients with diabetes but not TB (91.4%) compared to those with TB (76.9%). Psychological well-being scores were positive (good) for 53.8% of patients with TB and 57.1% of patients without TB. Significantly, 82.1% of patients with diabetes and TB had high TPI scores, whereas 60% of patients with diabetes but not TB had low TPI scores.

Table 1. Demographic data

Variable	Type 2 DM	
	TB (*n* = 39)	Non-TB (*n* = 70)
Age (y)		
≤55	16 (41.0)	18 (25.7)
>55	23 (59.0)	52 (74.3)
Sex		
Male	21 (53.8)	25 (35.7)
Female	18 (46.2)	45 (64.3)
History of TB contact		
Yes	17 (43.6)	23 (32.9)
No	22 (56.4)	47 (67.1)
TB-like symptoms		
Present	30 (76.9)	14 (20.0)
Absent	9 (23.1)	56 (80.0)
HbA1c		
≥7%	26 (66.7)	42 (60.0)
<7%	13 (33.3)	28 (40.0)
Duration of type 2 DM		
≤10 y	16 (41.0)	33 (47.1)
>10 y	23 (59.0)	37 (52.9)
Body mass index (kg/m^2)		
≤18.5	8 (20.5)	5 (7.1)
>18.5	31 (79.5)	65 (92.9)
Housing ventilation		
≤10%	9 (23.1)	6 (8.6)
>10%	30 (76.9)	64 (91.4)
Mental health		
Poor	18 (46.2)	30 (42.9)
Good	21 (53.8)	40 (57.1)
TPI score		
High risk	32 (82.1)	28 (40.0)
Low risk	7 (17.9)	42 (60.0)

Data are presented as *n* (%).
DM, diabetes mellitus; TB, tuberculosis; HbA1c, hemoglobin A1c; TPI, tuberculosis predictive index.

TB Risk Based on TPI Score

The distribution of TB risk according to TPI scores is summarized in Table 2. Patients were categorized into high- and low-risk groups based on their TPI scores. Among patients with diabetes and TB, 32 (82.1%) had high TPI scores, compared to only 28 of patients (40.0%) with diabetes but not TB. Conversely, low TPI scores were found in 7 patients (17.9%) with diabetes and TB and in 42 patients (60.0%) with diabetes but not TB. The association between high TPI scores and TB was statistically significant (OR, 6.8; 95% CI, 2.6–17.6; $p < 0.001$), confirming that patients with diabetes and TB were substantially more likely to have high TPI scores compared to those without TB. This finding aligns with prior evidence that diabetes significantly increases the risk of active TB [9].

TB Risk Based on TPI Score Variables

All variables analyzed in Table 3—age, sex, TB contact history, glycemic control (HbA1c), presence of TB-like symptoms, BMI, diabetes duration, housing ventilation, and psychological well-being—are components of the original TPI formula [16]. Thus, the strong associations observed in this study are anticipated outcomes. For example,

Table 2. Association between TB status and TPI score in patients with diabetes

	TPI score (*n*, %)		OR (95% CI)	*p*
	High (*n* = 60)	Low (*n* = 49)		
DM			6.8 (2.6–17.6)	<0.001***
TB	32 (53.3)	7 (14.3)		
Non-TB	28 (46.7)	42 (85.7)		

TB, tuberculosis; TPI, tuberculosis predictive index; OR, odds ratio; CI, confidence interval; DM, diabetes mellitus.
***$p < 0.001$.

https://doi.org/10.24171/j.phrp.2024.0310

Table 3. Association between patient characteristics and TPI scores

	TPI score (*n*, %)		OR (95% CI)	*p*
	High (*n* = 60)	Low (*n* = 49)		
Age (y)			4.9 (1.9–12.6)	< 0.001***
≤ 55	27 (45.0)	7 (14.3)		
> 55	33 (55.0)	42 (85.7)		
Sex			4.0 (1.7–9.2)	< 0.001***
Male	34 (56.7)	12 (24.5)		
Female	26 (43.3)	37 (75.5)		
History of TB contact			3.2 (1.4–7.5)	0.005**
Yes	29 (48.3)	11 (22.4)		
No	31 (51.7)	38 (77.6)		
TB-like symptoms			2.8 (1.2–6.3)	0.009**
Present	44 (73.3)	24 (49.0)		
Absent	16 (26.7)	25 (51.0)		
HbA1c			22.5 (7.0–71.0)	< 0.001***
≥ 7%	40 (66.7)	4 (8.2)		
< 7%	20 (33.3)	45 (91.8)		
Duration of type 2 DM (y)			0.5 (0.4–0.6)	< 0.001***
≤ 10	13 (21.7)	0 (0)		
> 10	47 (78.3)	49 (100.0)		
Body mass index (kg/m^2)			2.5 (1.1–5.5)	0.02*
≤ 18.5	33 (55.0)	16 (32.7)		
> 18.5	27 (45.0)	33 (67.3)		
Housing ventilation			6.5 (1.4–30.4)	0.008**
≤ 10%	13 (21.7)	2 (4.1)		
> 10%	47 (78.3)	47 (95.9)		
Mental health			1.7 (0.8–3.2)	0.165
Poor	30 (50.0)	18 (36.7)		
Good	30 (50.0)	31 (63.3)		

TPI, tuberculosis predictive index; OR, odds ratio; CI, confidence interval; TB, tuberculosis; HbA1c, hemoglobin A1c; DM, diabetes mellitus.
*p < 0.05, **p < 0.01, ***p < 0.001.

patients aged 55 or younger had an OR of 4.9 compared to those older than 55, and males had an OR of 4.0, reflecting the weights assigned to younger age and male sex in the original TPI calculation [16]. Similarly, the very high odds ratio (OR) observed for TB-like symptoms (OR, 22.5; 95% confidence interval [CI], 7.0–71.0) and the elevated OR for poor glycemic control (HbA1c >7%) (OR, 2.8; 95% CI, 1.2–6.3) are consistent with their large coefficients in the predictive index formula [16]. Additionally, shorter diabetes duration (≤ 10 years) was associated with a higher TB risk (OR, 2.5; 95% CI, 1.1–5.9), and poor housing ventilation (≤ 10% of floor area) showed a substantial association (OR, 6.5; 95% CI, 1.4–30.4), confirming their roles as significant predictors in the TPI model. Conversely, psychological distress was not significantly associated with increased TB risk (OR, 1.7; 95% CI, 0.8–3.2; p = 0.165), which aligns with its relatively low weighting in the TPI score. These findings confirm that each component of the TPI score behaves in accordance with its intended predictive role, thereby validating the overall design of the index rather than identifying novel independent risk factors [16].

TB Risk Based Among Patients with Diabetes

Among individuals living with diabetes (Table 4), three key factors were significantly associated with increased TB risk. Firstly, individuals reporting TB-like symptoms had markedly higher odds of TB (OR, 13.3; 95% CI, 5.1–34.3; p < 0.001) compared to those without symptoms. This strong association highlights the clinical value of symptom screening in detecting TB among people with diabetes, though it does not imply that symptoms cause TB. Indeed, clinical guidelines advocate vigilant monitoring of TB symptoms in this population as an essential case-finding strategy [17–20]. Secondly, undernutrition was a significant risk factor. Individuals with a body mass index (BMI) below 18.5 kg/m² had significantly increased odds of TB (OR, 3.3; 95% CI, 1.0–11.0; p = 0.039) compared to those with normal or higher BMI. This finding is consistent with systematic

Table 4. Association between TB risk factors and TB status among patients with diabetes

	DM patients (*n*, %)		OR (95% CI)	*p*
	TB (*n* = 39)	Non-TB (*n* = 70)		
TB-like symptoms (minimum 3)			13.3 (5.1–34.3)	< 0.001***
Present	30 (76.9)	14 (20.0)		
Absent	9 (23.1)	56 (80.0)		
Body mass index (kg/m^2)			3.3 (1.0–11.0)	0.039*
≤ 18.5	8 (20.5)	5 (7.1)		
> 18.5	31 (79.5)	65 (92.9)		
Housing ventilation			3.2 (1.0–9.8)	0.035*
≤ 10%	9 (23.1)	6 (8.6)		
> 10%	30 (76.9)	64 (91.4)		

TB, tuberculosis; DM, diabetes mellitus; OR, odds ratio; CI, confidence interval.
*p < 0.05, ***p < 0.001.

reviews showing that undernutrition can nearly double TB risk [21–23]. Thirdly, inadequate housing ventilation (≤ 10% of floor area) was associated with higher TB risk (OR, 3.2; 95% CI, 1.4–9.8; p = 0.008), reaffirming established relationships between substandard housing and airborne TB transmission [24–26]. Finally, poor housing ventilation (≤ 10%) was also associated with higher TB risk (OR, 3.2; 95% CI, 1.4–9.8; p = 0.008), reflecting established relationships between inadequate housing conditions and increased TB transmission [9,27,28]. Collectively, these results emphasize the importance of prompt TB evaluation in patients with diabetes presenting with respiratory symptoms, and highlight the significance of addressing modifiable risk factors such as nutrition and living conditions for effective TB prevention within this vulnerable population.

Discussion

This study demonstrates that the TPI score is an effective and practical tool for assessing TB risk in patients with T2DM. Our findings confirm that the TPI score reliably differentiates high-risk patients with diabetes from those at lower risk, offering a valuable mechanism for early screening and timely intervention. Patients with diabetes diagnosed with TB had significantly higher TPI scores, corresponding to a 6.8-fold increase in the odds of TB compared to patients with diabetes but not TB. This underscores the potential clinical utility of the TPI score as a proactive screening tool, particularly among patients with diabetes who may not yet present overt TB symptoms but remain at considerable risk.

Standard TB screening among patients with diabetes generally depends on symptom-based assessments or bacteriological confirmation, potentially delaying diagnosis, especially in subclinical or early-stage cases. The TPI score overcomes this limitation by integrating multiple established risk factors—biological characteristics (e.g., age and sex), metabolic factors (e.g., glycemic control and BMI), and environmental conditions (e.g., housing ventilation)—into a comprehensive predictive model [16]. Utilizing the TPI score in clinical practice could enhance TB detection among populations with diabetes, allowing more targeted investigations and earlier therapeutic interventions.

All variables used in the TPI calculation—including younger age (≤ 55 years), male sex, poor glycemic control, low BMI, TB-like symptoms, short diabetes duration, inadequate housing ventilation, and psychological distress—are integral, weighted components of the original predictive index [16]. Thus, their significant associations with TB in this study confirm the validity of the TPI score rather than introducing new independent risk factors. Among these variables, the strongest association observed was with the presence of TB-like symptoms. However, this association likely indicates reverse causation, in which the presence of TB itself results in symptom manifestation rather than symptoms independently predicting future TB risk. Nevertheless, this symptom-based association remains clinically significant for effective case detection [18,19]. Our data reveal that 76.9% of patients with diabetes and TB presented with 3 or more TB-like symptoms, compared to only 20% of patients with diabetes but not TB. This highlights the clinical importance of symptom-based triage but also supports employing multifactorial tools like the TPI score for comprehensive screening to avoid missing asymptomatic or early cases [18–20].

Metabolic factors such as elevated HbA1c (> 7%) and low BMI (≤ 18.5 kg/m²) were also significantly associated with increased TB risk. These findings align with previous research indicating hyperglycemia impairs immune responses and undernutrition increases susceptibility to TB [17,20]. Nonetheless, since these factors are integral to the

https://doi.org/10.24171/j.phrp.2024.0310

TPI score itself, the associations observed here reinforce the score's validity rather than identify novel independent risk factors.

Duration of diabetes was also a contributing factor. Patients with diabetes for ≤10 years exhibited lower odds of TB, though some of these individuals still scored highly on the TPI, suggesting early metabolic derangements in a subset of patients. This finding emphasizes the importance of proactive TB risk assessment early in the diabetes disease course [22,23].

Lastly, environmental conditions such as inadequate housing ventilation were strongly associated with TB, consistent with prior findings linking housing quality to TB transmission [20,24]. In contrast, psychological well-being, although included in the TPI score, did not show a significant association in this sample. This suggests it may have less predictive utility in similar clinical settings.

Environmental factors, particularly housing ventilation, significantly impacted TB risk. Patients with diabetes living in poorly ventilated homes (ventilation area ≤10% of floor area) had notably higher odds of TB, with an OR of 6.5 based on TPI score stratification. Within our study, 23.1% of patients with TB lived in poorly ventilated homes, compared to 8.6% of patients without TB (OR, 3.2; 95% CI, 1.0–9.8; $p=0.035$). These findings are consistent with previous studies linking inadequate ventilation to increased airborne TB transmission [27], underscoring the need to integrate environmental assessments into TB screening and preventive strategies.

A history of TB contact was also significantly associated with TB risk in patients with diabetes; those reporting previous TB exposure exhibited a 3.2-fold increased risk. While prior TB contact is an established risk factor, our findings suggest that metabolic and environmental factors contribute more substantially to TB susceptibility among patients with diabetes. Conversely, psychological well-being did not significantly correlate with TB risk in this study, despite its recognized role in chronic disease management. This finding suggests that mental health alone might have limited predictive utility regarding TB susceptibility in similar clinical populations.

Our study highlights the potential clinical value of implementing the TPI score. Routinely incorporating the TPI score in diabetes management could help healthcare providers efficiently identify patients at elevated TB risk who would benefit from closer monitoring, earlier diagnostic testing, or preventive interventions. If validated by future research, the TPI score could become an accessible and practical tool to optimize early TB detection, improve patient outcomes, and enhance preventive measures within diabetes care.

Despite the promising results, this study has limitations. First, data collection relied partially on self-reported questionnaires, introducing potential recall bias, especially concerning TB-like symptoms, previous TB exposure, and housing conditions. While attempts were made to mitigate bias through medical record cross-referencing, self-reporting remains susceptible to individual perception and recall issues. Second, as a case-control design, this study establishes associations but cannot demonstrate causality; longitudinal or cohort studies would be necessary to determine whether elevated TPI scores directly predict future TB incidence.

Additionally, the single-center setting limits generalizability. Although Fatmawati General Hospital serves a diverse patient population, multi-center validation studies are essential to confirm the TPI score's applicability across various clinical and geographic contexts. Further, the TPI scoring system itself is novel and unvalidated externally; additional research is required to confirm its reliability and validity beyond the initial study setting. Finally, constraints such as limited sample size—particularly of patients with both diabetes and TB—and time limitations necessitated adjusting the study design to a 1:2 case-to-control ratio, which may influence the findings' robustness.

Conclusion

In conclusion, the TPI score effectively predicts TB risk in patients with T2DM. As such, the TPI score holds significant promise as an early screening tool to identify patients with diabetes at high risk of developing TB, facilitating timely clinical interventions and potentially reducing TB-related morbidity within this vulnerable population.

Notes

Ethics Approval

This study was approved by the Director of Human Resources, Education, and Research of Fatmawati General Hospital (No. DP.04.03/DXXI.2/7392/2024) on 2024 July 19. Written informed consent was obtained for publication of this study.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

None.

Availability of Data

All data generated or analyzed during this study are included in this published article. For other data, these may be requested through the corresponding author.

Authors' Contributions

Conceptualization: MIM; Data curation: DPA, RSA; Formal analysis: DPA; Funding acquisition: MIM; Investigation: DPA, RSA; Methodology:

RSA; Project administration: RSA; Resources: MIM; Software: DPA; Supervision: MIM; Validation: MIM; Visualization: DPA; Writing–original draft: DPA; Writing–review & editing: all authors. All authors read and approved the final manuscript.

Additional Contributions
Kevin Aristyo and Fia Mustika (Universitas Indonesia, Jakarta, Indonesia) provided guidance and technical support during the research.

References

1. Steffanus M, Fodianto AP, Hadiyanto JN. Correlation between type 2 diabetes mellitus and pulmonary tuberculosis at Atma Jaya Hospital. Jurnal Respirologi Indones 2021;41:170–3.
2. Isbaniah F, Burhan E, Sinaga YB, et al. Tuberculosis: guidelines for diagnosis and management in Indonesia. Indonesian Society of Respirology; 2021. Indonesian.
3. Krishna S, Jacob JJ. A service of the National Library of Medicine. National Institutes of Health; 2000.
4. Foe-Essomba JR, Kenmoe S, Tchatchouang S, et al. Diabetes mellitus and tuberculosis, a systematic review and meta-analysis with sensitivity analysis for studies comparable for confounders. PLoS One 2021;16:e0261246.
5. Davis K, Richard S, William W. Tuberculosis and diabetes mellitus interaction: a current clinical perspective. Infect Dis Clin Pract 2013; 21:355–358.
6. Nugraha IB, Gotera W, Yustin WE. Diabetes mellitus as a risk factor for tuberculosis. J Kdokt Meditek 2021;27:273–81. Indonesian.
7. Restrepo BI. Diabetes and tuberculosis. Microbiol Spectr 2016;4.
8. Xu G, Hu X, Lian Y, et al. Diabetes mellitus affects the treatment outcomes of drug-resistant tuberculosis: a systematic review and meta-analysis. BMC Infect Dis 2023;23:813.
9. Aktas G. Association between the prognostic nutritional index and chronic microvascular complications in patients with type 2 diabetes mellitus. J Clin Med 2023;12:5952.
10. Aktas G, Duman TT, Atak Tel B. Controlling Nutritional Status (CONUT) score is a novel marker of type 2 diabetes mellitus and diabetic microvascular complications. Postgrad Med 2024;136:496–503.
11. Muefong CN, Sutherland JS. Neutrophils in tuberculosis-associated inflammation and lung pathology. Front Immunol 2020;11:962.
12. Dousa KM, Hamad A, Albirair M, et al. Impact of diabetes mellitus on the presentation and response to treatment of adults with pulmonary tuberculosis in Qatar. Open Forum Infect Dis 2018;6: ofy335.
13. Mukherjee S, Perveen S, Negi A, et al. Evolution of tuberculosis diagnostics: from molecular strategies to nanodiagnostics. Tuberculosis (Edinb) 2023;140:102340.
14. Lee MR, Huang YP, Kuo YT, et al. Diabetes mellitus and latent tuberculosis infection: a systematic review and metaanalysis. Clin Infect Dis 2017;64:719–27.
15. Zhao L, Gao F, Zheng C, et al. The impact of optimal glycemic control on tuberculosis treatment outcomes in patients with diabetes mellitus: systematic review and meta-analysis. JMIR Public Health Surveill 2024;10:e53948.
16. Isfandiari MA, Wahyuni CU, Pranoto A. Tuberculosis predictive index for type 2 diabetes mellitus patients based on biological, social, housing environment, and psychological well-being factors. Healthcare (Basel) 2022;10:872.
17. American Diabetes Association Professional Practice Committee. 2. Diagnosis and classification of diabetes: standards of care in diabetes-2024. Diabetes Care 2024;47(Suppl 1):S20–42.
18. Bouton TC, Jacobson KR. Symptom screens are not sufficient: the fight against tuberculosis needs better weapons. Clin Infect Dis 2021;73:121–3.
19. Htet KK, Chongsuvivatwong V, Aung ST. Sensitivity and specificity of tuberculosis signs and symptoms screening and adjunct role of social pathology characteristics in predicting bacteriologically confirmed tuberculosis in Myanmar. Trop Med Health 2021;49:3.
20. Cheng J, Sun YN, Zhang CY, et al. Incidence and risk factors of tuberculosis among the elderly population in China: a prospective cohort study. Infect Dis Poverty 2020;9:13.
21. Setyaningrum R, Zubaedah T. Correlation between gender, age, education level, and working status with anti-tuberculosis drug uses (OATS) in patients with lung TB in Indonesia 2013. Int J Chem Mater Sci 2018;1:7–13.
22. Marcoa R, Ribeiro AI, Zao I, et al. Tuberculosis and gender: factors influencing the risk of tuberculosis among men and women by age group. Pulmonology 2018;24:199–202.
23. Alturki S, Al Amad M, Mahyoub E, et al. Prevalence of diabetes mellitus among patients with tuberculosis and its associated factors in Sana'a, Yemen, 2021. Epidemiologia (Basel) 2023;4:202–11.
24. Sajjadi S, Rad MA, Hejazi S, et al. The relationship between diabetes mellitus and pulmonary diseases: a systematic review. J Cardio-Thorac Med 2018;6:274–81.
25. Schluger NW. The pathogenesis of tuberculosis: the first one hundred (and twenty-three) years. Am J Respir Cell Mol Biol 2005; 32:251–6.
26. Zhao Q, Laukkanen JA, Li Q, et al. Body mass index is associated with type 2 diabetes mellitus in Chinese elderly. Clin Interv Aging 2017;12:745–52.
27. Deol AK, Shaikh N, Middelkoop K, et al. Importance of ventilation and occupancy to Mycobacterium tuberculosis transmission rates in congregate settings. BMC Public Health 2022;22:1772.
28. Bhargava A, Benedetti A, Oxlade O, et al. Undernutrition and the incidence of tuberculosis in India: national and subnational estimates of the population-attributable fraction related to undernutrition. Natl Med J India 2014;27:128–33.

https://doi.org/10.24171/j.phrp.2024.0310

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2025.0104
Osong Public Health Res Perspect 2025;16(4):415-422

Original Article

Drug use intentions among young adults in the Republic of Korea: a cross-sectional study applying the extended theory of planned behavior with emphasis on impulsive behavior and sensation seeking

Aeree Sohn

Department of Public Health, Sahmyook University, Seoul, Republic of Korea

ABSTRACT

Objectives: The increasing prevalence of drug use in the Republic of Korea has emerged as a significant social concern. This study applied the extended theory of planned behavior to investigate the factors influencing intentions to use drugs among young adults (aged 20–30 years) in the Republic of Korea. The study integrated personal traits—specifically, impulsivity, sensation seeking, and self-efficacy—into 2 core theory of planned behavior constructs: attitudes and subjective norms. The principal aim was to improve the prediction of drug use intentions by incorporating these variables.

Methods: Data were obtained from the 2023 National Survey of Drug Harm Perception, which sampled 1,500 individuals aged 19 to 39 years. Hierarchical regression analysis was employed to assess the influence of psychological and social factors on intentions to use drugs.

Results: In the primary model, age emerged as a significant predictor of drug use intentions ($R^2 = 0.01$). The secondary model showed that positive attitudes toward drugs, subjective norms, and lower self-efficacy significantly increased drug use intentions ($R^2 = 0.23$). In the final tertiary model, the addition of sensation seeking and impulsivity further amplified these intentions ($R^2 = 0.25$).

Conclusion: The findings underscore the pivotal roles of attitudes, subjective norms, and self-efficacy in shaping intentions to use drugs. Sensation seeking and impulsivity were found to further elevate vulnerability. Effective prevention efforts must address both psychological traits and social influences. Future research should examine the long-term behavioral outcomes associated with these factors.

Keywords: Drug use; Extended theory of planned behavior; Impulsive behavior; Sensation seeking

Received: April 3, 2025
Revised: May 26, 2025
Accepted: June 29, 2025

Corresponding author:
Aeree Sohn
Department of Public Health, Sahmyook University, 815 Hwarang-ro, Nowon-gu, Seoul 01795, Republic of Korea
E-mail: aeree@syu.ac.kr

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Korea Disease Control and Prevention Agency and Korea National Institute of Health.

© 2025 Korea Disease Control and Prevention Agency.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

The Republic of Korea has witnessed a substantial rise in illicit drug use since the coronavirus disease 2019 pandemic, encompassing narcotics, psychotropic drugs, and cannabis [1]. The Psychotropic Drug Control Act classifies these substances as highly dangerous and strictly regulates their use. Despite rigorous enforcement, both the incidence of drug-related offenses and the prevalence of drug-related criminal activities continue to climb [2].

Recent data from the National Police Agency and the Supreme Prosecutors' Office [3] indicate a marked increase in arrests for drug-related crimes. Specifically, the number of drug offenders has more than tripled, rising from fewer than 9,000 in 2010 to over 27,000 in 2023. Individuals aged 20 to 30 years accounted for more than half of these cases, with 8,368 offenders (30.3%) in their 20s and 6,683 (24.2%) in their 30s in 2023 [4]. This highlights the urgent need for targeted prevention strategies for this high-risk demographic. Furthermore, the National Bureau of Investigation reported an alarming 172.7% increase in drug-related deaths over a decade, with fatalities climbing from 205 in 2011 to 559 in 2021. These figures starkly illustrate the severe and growing public health crisis posed by drug abuse in the Republic of Korea. Additionally, drug distribution has shifted increasingly from offline to online platforms, facilitated by the Internet, social media, and cryptocurrency transactions [5].

It is evident that intensifying regulatory measures alone is inadequate for combating drug abuse in the Republic of Korea. The development of prevention strategies that consider both social and psychological factors is imperative. Currently, there is a lack of research analyzing the psychological and social determinants of drug use among Korean users. Most existing studies focus on strengthening legal regulations and imposing harsher penalties. Moreover, the stigmatization of drug users complicates prevention efforts by reducing their willingness to participate in research, thereby impeding the collection of reliable data. There is thus a pressing need for research that thoroughly examines the psychological and social influences on drug use in the Republic of Korea, enabling the design of more effective and targeted prevention strategies.

The theory of planned behavior (TPB) provides a valuable theoretical framework for understanding drug use intentions. It posits that attitudes, subjective norms, and perceived behavioral control shape intentions to engage in behaviors such as drug use. The extended TPB (ETPB) builds upon this model by incorporating personal traits—including impulsivity, sensation seeking, and self-efficacy—which enhance the accuracy of predictions regarding drug use intentions [6–9].

HIGHLIGHTS

- A cross-sectional study applying the extended theory of planned behavior analyzed a nationally representative sample of individuals aged 19–39 to explore psychological and social factors influencing drug use intentions.
- Hierarchical regression analysis revealed that peer subjective norms and self-efficacy were strongly associated with reduced intentions, while sensation seeking and impulsivity were significantly linked to increased intentions. The findings highlight the need for multidimensional prevention strategies that address both individual traits and perceived social influences.

Individuals with high impulsivity may be more vulnerable to drug use due to diminished behavioral control. In contrast, those with elevated sensation-seeking tendencies may be more likely to experiment with drugs in pursuit of novel experiences [10–14]. Individuals with higher self-efficacy are better equipped to regulate their drug use. These individual differences underscore the necessity of an extended model to provide a more comprehensive account of drug use intentions than the standard TPB.

This study aimed to apply the ETPB to identify factors influencing drug use intentions among young adults (aged 20–30 years) in the Republic of Korea. Personal traits—specifically impulsivity, sensation seeking, and self-efficacy—were incorporated into the core TPB components of attitudes and subjective norms. The primary objective was to enhance the prediction of drug use intentions by including these factors. The findings are expected to inform the development of practical, effective prevention and intervention strategies. By taking into account individual psychological and behavioral characteristics, these approaches will address the limitations of current prevention efforts that focus predominantly on legal deterrents.

Materials and Methods

Study Design and Participants

This study was conducted in 2023, utilizing data from a total of 3,000 participants aged 19 to 59 years [4]. To better understand drug use intentions among younger adults, a subset of 1,500 participants aged 19 to 39 years was selected for secondary analysis. The demographic and other characteristics of these 1,500 participants are presented in Table 1. The original 2023 survey employed a cross-sectional design with self-reported data, using stratified sampling

https://doi.org/10.24171/j.phrp.2025.0104

Table 1. Demographic characteristics of participants

Variable	Total (*n* = 1,500)	Man (*n* = 750)	Woman (*n* = 750)
Age (y)			
19–29	750 (50.0)	375 (50.0)	375 (50.0)
30–39	750 (50.0)	375 (50.0)	375 (50.0)
Education level			
Less than a high school diploma	324 (21.6)	186 (24.8)	138 (18.4)
High school diploma or higher	1176 (78.4)	564 (75.2)	612 (81.6)
Occupation			
Professional/business/management	757 (50.5)	358 (47.7)	399 (53.2)
Labor/production/transportation/self-employed/sales	207 (13.8)	137 (18.3)	70 (9.3)
Student/unemployed/other	536 (35.7)	255 (34.0)	281 (37.5)
Monthly household income (million KRW)			
Less than 2	175 (11.7)	93 (12.4)	82 (10.9)
2–4	546 (36.4)	282 (37.6)	264 (35.2)
4–6	328 (21.9)	177 (23.6)	151 (20.1)
More than 6	451 (30.1)	198 (26.4)	253 (33.7)

Data are presented as *n* (%).
KRW, Korean won.

based on gender, age, and geographical region.

Variables

This study employed the ETPB to examine associations between psychological and social factors and drug use intentions. To ensure reliability and validity, all study variables were measured using scales previously validated in the literature. All items were scored on a 5-point Likert scale (1 = not at all, 5 = very much). For each variable, scores were calculated by summing the relevant item scores, with higher values indicating greater expression of the given psychological or social factor.

Demographic characteristics

Demographic variables served as control variables: gender (male/female), age in years (continuous variable), education level (2-level categorical variable), monthly household income (4 income quintiles), and occupation (3-level categorical variable). Gender was included to capture differences in drug use patterns between men and women [14], while age was included to account for varying risks of experimental drug use across the life span [15]. Education level and monthly household income were considered due to their established impact on drug accessibility and availability [10,11]. Occupation was categorized into 3 groups: (1) professional/business/management; (2) labor/production/transportation/self-employed/sales; and (3) student/unemployed/other. Including this variable enabled assessment of potential differences in social structure, economic stability, and daily routine. Prior research indicates that employment status significantly influences substance use and risk perception. Individuals who are unemployed or working irregular jobs may experience greater psychological stress, social isolation, or exposure to high-risk environments—factors associated with heightened vulnerability to drug use [16].

Dependent variable

The dependent variable, drug use intention (i.e., likelihood of future drug use), was assessed by summing scores from 4 items measuring the likelihood of drug use in the following year. The reliability coefficient (Cronbach's α) was 0.66.

Independent variables

TPB constructs

Attitude variables (positive drug attitudes, negative drug attitudes) were measured using a scale evaluating both positive and negative attitudes toward drug use. Positive drug attitudes were measured by summing 3 items that assessed respondents' beliefs regarding whether drug use would help them recover from fatigue, lose weight, or feel more alert (Cronbach's α = 0.77). Negative drug attitudes were measured by summing 3 items that gauged perceptions of the probability of adverse consequences from drug use, such as sleep disturbances, digestive problems, or physical alterations like damaged teeth (Cronbach's α = 0.89). Higher scores reflected stronger positive or negative attitudes, respectively.

Subjective norms were evaluated with 2 subscales: public subjective norms and peer subjective norms. Public subjective norms assessed perceptions of societal approval of drug use, while peer subjective norms measured perceived expectations of close friends regarding drug use. Each subscale consisted

of 3 items. Both subscales demonstrated acceptable internal consistency (Cronbach's α=0.87 for peer subjective norms; α=0.81 for public subjective norms). Higher scores indicated greater perceived social pressure to use drugs. Because respondents might be uncertain about how drug use is perceived by the general public or their peers, a "don't know" option was included for all subjective norms items. These responses were excluded from mean score calculations. This option was not included for other variables.

Self-efficacy was measured by summing 3 items assessing an individual's confidence in their ability to regulate drug use (Cronbach's α=0.79), with higher scores indicating greater self-efficacy.

Propensity

Impulsivity was measured with 4 items from the Barratt impulsiveness scale (BIS-11), assessing the tendency to act impulsively (Cronbach's α=0.63). Higher scores indicated greater impulsivity [11].

Sensation seeking was measured using 3 items from Zuckerman [17]'s sensation seeking scale, which assesses the tendency to seek novel or risky stimuli (Cronbach's α=0.76). Higher scores reflected greater sensation-seeking propensity.

Statistical Methods

All analyses were conducted using IBM SPSS Statistics for Windows ver. 25.0 (IBM Corp.). Descriptive statistics were calculated to summarize participant characteristics. Pearson correlation coefficients were used to examine the relationships between independent variables (e.g., positive and negative drug attitudes, subjective norms) and the dependent variable (drug use intentions). Cronbach's α coefficients were calculated to evaluate the internal consistency of the study's scales. Multiple linear regression was used to examine the influence of independent variables on drug use intention, which was analyzed as a continuous variable. The analysis included assessments for multicollinearity using variance inflation factors, and checked the assumptions of independence.

Ethics Statement

This study, which was conducted in 2023, received approval from the Institutional Review Board of Sahmyook University (approval number: SYU 2023-05-008-001).

Results

Participants

Among the total sample of respondents (n=1,500), gender distribution was balanced, with 750 men and 750 women. Regarding age, 50% (n=750) of participants were between 19 and 29 years old, while 25% (n=375) were aged 30 to 39 years. In terms of education, 78.4% (n=1,176) held a high school diploma or higher, comprising 75.2% (n=564) of men and 81.6% (n=612) of women. The remaining 21.6% (n=324) reported having less than a high school diploma.

Professional, business, and management positions constituted the largest proportion of occupations (50.5%), followed by the student/unemployed/other category (35.7%, n=536). The labor/production/transportation/self-employed/sales category accounted for a relatively small proportion (13.8%, n=207) of the total sample.

Monthly household income most commonly fell within the 2 to 4 million Korean won (KRW) range (36.4%), with little difference between men (37.6%) and women (35.2%). Many respondents reported a monthly household income exceeding 6 million KRW (30.1%). Incomes between 4 to 6 million KRW were slightly more prevalent among men (23.6%) than women (20.1%). A minority of participants (11.7%) reported monthly household incomes below 2 million KRW.

Descriptive Statistics and Normality Analysis

Descriptive statistics are summarized in Table 2. The mean score for positive drug attitudes was 2.09±0.96 out of 5, indicating that respondents generally did not hold favorable perceptions of drugs. In contrast, the mean score for negative drug attitudes was high (3.97±1.03), reflecting a strong recognition of the harmful effects of drug use.

For subjective norms, respondents reported perceiving robust social norms against drug use. The mean perceived peer disapproval of drug use was 4.71±0.55 out of 5, while the mean response for public disapproval was 4.44±0.72.

Young adults in the sample showed a greater propensity for impulsivity than for sensation seeking. The mean sensation seeking score was 1.67±0.62, and the mean impulsivity score was 2.22±0.51 [11].

Most participants indicated no intention to use drugs, as reflected by a mean drug use intentions score of 0.03±0.08, ranging from a minimum of 0 to a maximum of 0.51. This resulted in a right-skewed distribution with a pronounced long tail for drug use intentions, suggesting that the normality assumption may not have been met; log transformation was thus performed to achieve normality.

Skewness and kurtosis were analyzed to assess the normality of all variables. Skewness measures distribution asymmetry, and kurtosis assesses the peakedness or concentration. Normality is considered violated when the absolute value of skewness exceeds 3 or kurtosis exceeds 10 [18]. For all study variables, skewness and kurtosis were

https://doi.org/10.24171/j.phrp.2025.0104

Table 2. Descriptive statistics and normality analysis

Variable	Name	Min–max	Mean ± SD	Skewness
Theory of planned behavior	Positive drug attitudes	1–5	2.09 ± 0.96	0.57
	Negative drug attitudes	1–5	3.97 ± 1.03	−1.26
	Peer subjective norms	1.3–5	4.71 ± 0.55	−1.99
	Public subjective norms	1.4–5	4.44 ± 0.72	−1.36
	Self-efficacy	1–5	4.38 ± 0.74	−1.47
Propensity	Sensation seeking	1–4	1.67 ± 0.62	0.94
	Impulsivity	1–4	2.22 ± 0.51	−0.13
	Log drug use intention	0–0.51	0.03 ± 0.08	2.94

Min, minimum; Max, maximum; SD, standard deviation.

within the established thresholds, as shown in Table 2, confirming that normality assumptions were met.

Correlation Analysis

Table 3 presents the results of the correlation analysis for all respondents. The Pearson correlation coefficient between positive drug attitudes and drug use intentions was r = 0.20 ($p < 0.01$), indicating that stronger positive attitudes toward drugs corresponded with higher intentions to use drugs. The correlation coefficient between negative drug attitudes and drug use intentions was r = −0.08 ($p < 0.01$), suggesting that stronger negative perceptions of drugs tended to decrease drug use intentions, though the effect was relatively weak. The correlation coefficients between peer subjective norms and drug use intentions, and between public subjective norms and drug use intentions, were r = −0.45 ($p < 0.01$) and r = −0.33 ($p < 0.01$), respectively. These findings indicate that stronger subjective perceptions of social norms against drug use—both from peers and the general public—were associated with lower intentions to use drugs. Thus, individuals who perceive negative attitudes toward drug use from their peers and the broader society are less likely to intend to use drugs. The correlation coefficient between self-efficacy and drug use intentions was r = −0.27 ($p < 0.01$), indicating that higher self-efficacy tended to be associated with lower intentions to use drugs. This underscores the pivotal role of self-control, defined as the capacity to refuse drug use, in inhibiting drug use intentions. Furthermore, the analysis showed a correlation coefficient of r = 0.27 ($p < 0.01$) for the association between sensation seeking and drug use intentions. A similarly significant correlation was observed between impulsivity and drug use intentions (r = 0.20; $p < 0.01$), suggesting that impulsivity also influences the intention to use drugs.

Factors Influencing Drug Use Intentions

Table 4 presents the results of the hierarchical regression analysis, which was conducted to identify the main factors influencing the intention to use drugs. Peer subjective norms had the highest standardized beta value (β) across all analyses, indicating that this variable exerted the greatest impact on drug use intentions in this study. The secondary analysis coefficient (β = −0.34, $p < 0.001$) and the tertiary analysis coefficient (β = −0.32, $p < 0.001$) were both significant, demonstrating that stronger peer subjective perceptions of social norms against drug use were associated with lower intentions to use drugs. These findings suggest that peer social norms and pressures may deter drug use.

Secondary and tertiary analyses revealed a negative relationship between self-efficacy and drug use intentions (β = −0.12 and β = −0.08, respectively; $p < 0.001$), indicating that higher self-efficacy was associated with a reduced intention to use drugs. Positive drug attitudes showed a significant positive coefficient in both the secondary (β = 0.08, $p < 0.01$) and tertiary analyses (β = 0.05, $p < 0.05$), confirming that positive attitudes toward drugs were associated with an increased intention to use drugs. Additionally, the tertiary analysis indicated that impulsivity (β = 0.11, $p < 0.001$) and sensation seeking (β = 0.07, $p < 0.01$) had significant effects on drug use intentions.

Furthermore, gender was found to have a significant effect on drug use intentions in the tertiary analysis only (β = 0.05, $p < 0.05$). This suggests that gender differences may influence intentions to use drugs, but their impact is relatively modest compared to other key variables. Age, education level, occupation, monthly household income, and negative drug attitudes did not have significant effects on drug use intentions. In the primary analysis, the age coefficient was significant (β = −0.06, $p < 0.05$), but it became nonsignificant in subsequent analyses. Education level, occupation, and monthly household income were also nonsignificant, with standardized coefficients close to zero and p-values exceeding 0.1 in all analyses.

The model's explanatory power (R^2) was 0.01 in the primary analysis, indicating that demographic control variables contributed minimally to explaining drug use intentions.

Table 3. Correlation analysis of study variables for all participants (n = 1,500)

Variable	1	2	3	4	5	6	7	8
1. Positive drug attitudes	-							
2. Negative drug attitudes	−0.02	-						
3. Peer subjective norms	−0.23***	0.13***	-					
4. Public subjective norms	−0.20***	0.07*	0.53***	-				
5. Self-efficacy	−0.24***	0.26***	0.32***	0.18***	-			
6. Sensation seeking	0.22***	−0.14***	−0.32***	−0.19***	−0.33***	-		
7. Impulsivity	0.19***	−0.05	−0.15***	−0.17***	−0.27***	0.27***	-	
8. Drug use intentions	0.20***	−0.08**	−0.45***	−0.33***	−0.27***	0.27***	0.20***	-

*p < 0.05, **p < 0.01, ***p < 0.001.

Table 4. Hierarchical regression analysis of factors influencing drug use intentions

	Primary			Secondary			Tertiary		
	β	SE	p	β	SE	p	β	SE	p
Gender	0.00	0.00	0.920	0.03	0.00	0.269	0.05	0.00	0.030
Age	−0.06	0.00	0.039	−0.04	0.00	0.149	−0.03	0.00	0.191
Education level	−0.02	0.00	0.548	−0.02	0.00	0.391	−0.02	0.00	0.379
Occupation	0.00	0.00	0.880	0.01	0.00	0.568	0.01	0.00	0.690
Monthly household income	−0.05	0.00	0.059	−0.03	0.00	0.135	−0.03	0.00	0.148
Positive drug attitudes				0.08	0.00	0.001	0.05	0.00	0.023
Negative drug attitudes				0.00	0.00	0.963	0.00	0.00	0.843
Peer subjective norms				−0.34	0.00	0.000	−0.32	0.00	0.000
Public subjective norms				−0.10	0.00	0.000	−0.09	0.00	0.001
Self-efficacy				−0.12	0.00	0.000	−0.08	0.00	0.002
Sensation seeking							0.11	0.00	0.000
Impulsivity							0.07	0.00	0.004
R_{adj}^2	0.01			0.23			0.25		
Change in R_{adj}^2				0.22			0.02		

SE, standard error.

In the secondary analysis, R^2 increased to 0.23, suggesting that the TPB variables (positive drug attitudes, negative drug attitudes, subjective norms, and self-efficacy) played a substantial role in predicting drug use intentions. In the tertiary analysis, R^2 further increased to 0.25, indicating that the addition of propensity variables (sensation seeking and impulsivity) provided a modest improvement in the model's explanatory power.

Discussion

In recent years, increasing drug use among young adults has become a prominent social issue in the Republic of Korea, raising widespread concern about its broader implications. This phenomenon can be attributed to a complex interplay between psychological (individual) and social factors. Greater access to drugs and evolving social attitudes toward drug use have shaped young adults' intentions to use drugs. Against this backdrop, the present study empirically identified and analyzed key determinants of young adults' drug use intentions to provide a multidimensional understanding of this issue. Hierarchical regression analysis revealed that subjective norms, self-efficacy, sensation seeking, positive attitudes toward drugs, and impulsivity were all significant predictors of drug use intentions. In contrast, demographic factors—including age, education, occupation, and monthly income—did not significantly influence drug use intentions. These findings indicate that individual psychological and behavioral characteristics, along with the social environment, play a more substantial role in shaping drug use intentions than demographic characteristics [19].

Our findings demonstrate that positive attitudes toward drug use significantly increase intentions to use drugs, whereas negative attitudes exerted no significant effect. This is consistent with previous research by McMillan and Conner [20], who found that positive attitudes are a major predictor of drug use intentions, but negative attitudes do not necessarily reduce intentions. Young people are therefore

https://doi.org/10.24171/j.phrp.2025.0104

more likely to be influenced by the perceived benefits of drug use, such as stimulation, weight loss, and stress relief. As a result, prevention strategies that simply reinforce negative perceptions are unlikely to be sufficiently effective [21,22]. One plausible explanation is that young adults may cognitively discount or normalize potential harms, especially when the perceived benefits are immediate or personally relevant. This asymmetry in influence suggests that health communication strategies focusing only on negative consequences are inadequate. Instead, effective prevention efforts should challenge and reframe perceived benefits of drug use, and offer realistic alternatives that fulfill similar psychological or emotional needs (e.g., stress management or energy enhancement). Interventions should therefore prioritize reducing positive expectations and emphasize long-term negative consequences to maximize deterrence.

Subjective norms emerged as the most robust predictor of drug use intentions in this study. Stronger perceptions of social norms against drug use were associated with significantly lower intentions to use drugs, consistent with the TPB, which posits that social norms and others' expectations moderate behavioral intentions [6]. Kam et al. [9] similarly found that subjective norms reduce intentions to use drugs, and that social norms can indirectly influence intentions by interacting with attitudes and perceived behavioral control. When young adults perceive drug use as socially unacceptable, their intentions to use drugs are diminished. Accordingly, interventions that reinforce social norms—such as media campaigns or peer-based programs—may be effective in reducing drug use intentions.

Self-efficacy was also identified as an important inhibitor of drug use intentions. This study introduced and analyzed self-efficacy as a concept closely related to perceived behavioral control in the TPB framework. Higher self-efficacy was associated with a lower likelihood of intending to use drugs. This finding aligns with Bandura's theory, which holds that individuals with greater self-efficacy are better able to regulate their behavior and resist external pressures [23]. Enhancing self-efficacy may thus be a powerful component of prevention education, enabling young adults to exercise greater control over their actions despite environmental influences [24].

Sensation seeking was shown to be a significant predictor of intentions to use drugs. Individuals with high sensation-seeking tendencies are more inclined to seek out novel stimuli, which may, in turn, increase their interest in drug use [17]. Several studies have similarly reported that young adults with high sensation-seeking tendencies are more likely to engage in substance use and exhibit a greater propensity for drug use [15,25]. These individuals may also develop more positive attitudes toward drug use, as the pursuit of stimulating experiences can increase their intentions to use drugs. Consequently, it is essential to develop prevention strategies that target and reduce positive attitudes toward drug use among young adults. This may be accomplished by providing alternative activities—such as extreme sports or creative hobbies—that can satisfy sensation-seeking tendencies in healthier ways .

This study also identified impulsivity as a significant predictor of drug use intentions. Moshier and Otto [13] found that impulsivity can widen the gap between intentions to use drugs and actual drug use behavior. Highly impulsive young adults may lack cognitive inhibition, thereby increasing the likelihood of acting on their intentions, such as using drugs. This highlights impulse control training as a potentially effective prevention strategy, particularly for young adults. By helping young adults improve impulse regulation and self-control, it may be possible to reduce the likelihood of drug use.

Demographic characteristics—including age, education level, occupation, and monthly household income—did not have a significant impact on drug use intentions in this study. While age was significant in the primary analysis, its effect became nonsignificant in later analyses. Thus, drug use intentions appear to be influenced more strongly by psychological and social factors than by basic demographic characteristics.

Variables derived from the TPB were robust predictors of drug use intentions, underscoring the importance of a multifaceted approach that incorporates both individual characteristics and social influences. Our findings suggest that interventions designed to reduce drug use would be more effective if tailored to psychological and social factors, rather than demographic variables alone. This study therefore emphasizes the need to design comprehensive prevention education and intervention programs that reflect the diverse characteristics and social environments of young people.

Conclusion

The findings of this study underscore the necessity of a multifaceted approach to reducing drug use among young adults. Specifically, there is a clear need for interventions that address a range of factors, including subjective norms, self-efficacy, sensation seeking, and impulsivity. The results indicate that prevention strategies focusing on reinforcing social norms against drug use, enhancing self-efficacy, and providing alternative activities to manage sensation-seeking and impulsive tendencies may be more effective than approaches relying solely on stricter regulation or harsher

punishment. Future research should consider the impact of varying cultural contexts and environmental factors on drug use intentions to further refine prevention strategies.

Notes

Ethics Approval

This study was conducted per the Declaration of Helsinki and was approved by the Institutional Review Board of Sahmyook University (approval number: SYU 2023-05-008-001). Informed consent was obtained from all participants as the study involved the general population and was conducted through an online survey. All authors have reviewed the manuscript and provided consent for publication.

Conflicts of Interest

Aeree Sohn serves as an Editorial Board member of *Osong Public Health and Research Perspectives*, but had no role in the decision to publish this article. Except for that, no potential conflict of interest relevant to this article was reported.

Funding

None.

Availability of Data

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Sohn A, Kim MH, Yang JY, et al. A national survey of the attitudes of drug in South Korea. Ministry of Food and Drug Safety; 2023. Korean.
2. Kim JH. Status of drug offenders and countermeasures. Korean Assoc Addctn Crim Rev 2005;7:243–50.
3. Supreme Prosecutors' Office Republic of Korea. SPOotRo. Chapter 3: investigation and prosecution of drug offenses. White paper on narcotics crime. Supreme Prosecutors' Office Republic of Korea; 2023. Korean.
4. Sohn A, Yang JY, Kim M, et al. A survey of drug harm perception. Ministry of Food and Drug Safety; 2023. Korean.
5. Yang JY, Kim M, Sohn A. Development and validation of a questionnaire for assessing drug use motives in the general population in South Korea. Healthcare (Basel) 2023;12:86.
6. Ajzen I. The theory of planned behavior. Organ Behav Hum Decis Process 1991;50:179–211.
7. Booth BM, Stewart KE, Curran GM, et al. Beliefs and attitudes regarding drug treatment: application of the theory of planned behavior in African-American cocaine users. Addict Behav 2014;39:1441–6.
8. Conner M, Armitage CJ. Extending the theory of planned behavior: a review and avenues for further research. J Appl Soc Psychol 1998;28:1429–64.
9. Kam JA, Matsunaga M, Hecht ML, et al. Extending the theory of planned behavior to predict alcohol, tobacco, and marijuana use among youth of Mexican heritage. Prev Sci 2009;10:41–53.
10. Brajkovic L, Jelincic K, Kopilas V. Relationship between dark triad, coping styles, sensation seeking and substance use among youth. Open J Psychol 2022;15.
11. Evans SL, Norbury R. Associations between diurnal preference, impulsivity and substance use in a young-adult student sample. Chronobiol Int 2021;38:79–89.
12. Molero Jurado MD, Perez-Fuentes MD, Simon Marquez MD, et al. Relationship between impulsivity, sensation-seeking, and drug use in aggressors and victims of violence. Front Psychol 2020;11:600055.
13. Moshier SJ, Ewen M, Otto MW. Impulsivity as a moderator of the intention-behavior relationship for illicit drug use in patients undergoing treatment. Addict Behav 2013;38:1651–5.
14. Wasserman AM, Mathias CW, Hill-Kapturczak N, et al. The development of impulsivity and sensation seeking: associations with substance use among at-risk adolescents. J Res Adolesc 2020;30:1051–66.
15. Woicik PA, Stewart SH, Pihl RO, et al. The substance use risk profile scale: a scale measuring traits linked to reinforcement-specific substance use profiles. Addict Behav 2009;34:1042–55.
16. Assari S, Barsha RA, Egboluche C, et al. The CEASE tobacco cessation controlled trial for low-income racial and ethnic minority participants: key predictors of success. Glob J Cardiovasc Dis 2025;4:1246.
17. Zuckerman M. Behavioral expressions and biosocial bases of sensation seeking. Cambridge University Press; 1994.
18. Kline RB. Principles and practice of structural equation modeling. Guilford Publications; 2023.
19. Kin TW, Rahamat S, Yusri M. Consumer's intention to purchase healthy foods and beverages from vending machines in a healthcare institution: an application of theory of planned behavior. Malays J Med Health Sci 2024;20:42–51.
20. McMillan B, Conner M. Applying an extended version of the Theory of Planned Behavior to illicit drug use among students. J Appl Soc Psychol 2003;33:1662–83.
21. Slater MD, Kelly KJ, Lawrence FR, et al. Assessing media campaigns linking marijuana non-use with autonomy and aspirations: "Be under your own influence" and ONDCP's "Above the influence". Prev Sci 2011;12:12–22.
22. Arnold ER, Liddelow C, Vella SA. Exploring a mother's engagement in team sport: an application of an extended theory of planned behaviour. J Sci Med Sport 2024;27:786–92.
23. Griffin KW, Botvin GJ, Nichols TR, et al. Effectiveness of a universal drug abuse prevention approach for youth at high risk for substance use initiation. Prev Med 2003;36:1–7.
24. Yusuf A, Pervin N, Roman-Gonzalez M. Generative AI and the future of higher education: a threat to academic integrity or reformation?: evidence from multicultural perspectives. Int J Educ Technol High Educ 2024;21:21.
25. Leeman RF, Hoff RA, Krishnan-Sarin S, et al. Impulsivity, sensation-seeking, and part-time job status in relation to substance use and gambling in adolescents. J Adolesc Health 2014;54:460–6.

https://doi.org/10.24171/j.phrp.2025.0104

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

Instruction for authors

Enacted: January 1, 2010
Last revision: June 1, 2024
Partial revision: December 4, 2024

Osong Public Health and Research Perspectives (PHRP) is the international bimonthly (published at the end of February, April, June, August, October, and December) journal founded in 2010 by the Korea Disease Control and Prevention Agency (KDCA). With the mission of the KDCA, to create a disease-free world, PHRP encourages sharing medical information and knowledge in the areas of public health. PHRP publishes original articles, review articles, guidelines, data profiles (including cohort profiles), special articles, short communications, viewpoints, editorials and correspondence, with a focus on the following areas of expertise: emerging infectious diseases, vaccinology, zoonotic diseases, non-communicable diseases, intractable and rare diseases, and human genomics.

Before submitting a manuscript, authors should carefully read and follow the instructions for writing an article for PHRP. For issues not addressed in these instructions, authors should refer to the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (http://www.icmje.org/recommendations/) from the International Committee of Medical Journal Editors (ICMJE). Manuscripts submitted to PHRP that do not follow these instructions will be returned to the authors without further review.

Contact Us

Editorial Office: Korea Disease Control and Prevention Agency
National Center for Medical Information and Knowledge, 202 Osongsengmyung 2nd street, Osong-eup, Heungdeok-gu, Cheongju 28159, Korea
E-mail: ophrp@korea.kr

Table of Contents

ARTICLE PROCESSING CHARGES
RESEARCH AND PUBLICATION ETHICS
EDITORIAL POLICY
SUBMISSION & PEER REVIEW PROCESS
MANUSCRIPT PREPARATION
FINAL PREPARATION FOR PUBLICATION

ARTICLE PROCESSING CHARGES

The author does not have pay publication charges for open access. The KDCA will pay to make the article open access.

RESEARCH AND PUBLICATION ETHICS

The journal adheres to the guidelines and best practices published by professional organizations, including the ICMJE Recommendations and the Principles of Transparency and Best Practice in Scholarly Publishing (joint statement by the Committee on Publication Ethics [COPE], Directory of Open Access Journals [DOAJ], World Association of Medical Editors [WAME], and Open Access Scholarly Publishers Association [OASPA]; https://doaj.org/bestpractice). Furthermore, all processes of handling research and publication misconduct shall follow the applicable COPE guidances (https://publicationethics.org/)

Human and Animal Rights

Clinical research should be conducted in accordance with the World Medical Association's Declaration of Helsinki (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/) and approved by the Institutional Review Board (IRB) of the institution where the experiment was performed. Animal experiments should also be reviewed by an appropriate committee (Institutional Animal Care and Use Committee [IACUC]) for the care and use of animals. Studies involving pathogens requiring a high degree of biosafety should pass review of a relevant committee (Institutional Biosafety Committee [IBC]). Clinical studies that do not meet the Helsinki Declaration will not be considered for publication.

Statement of Informed Consent and Institutional Approval

The editor of PHRP may request submission of copies of informed consent forms from human subjects in all studies and IRB approval documents. For articles involving human subjects who can be identified through descriptions, photographs, or pedigrees, a signed informed consent statement must be provided. This consent must be obtained from each identifiable participant, or from a parent or legal guardian if the participant is unable to provide consent. The statement should explicitly permit the publication of the relevant descriptions, photographs, and pedigrees in both print and online formats. Articles describing the use of animals in experiments must be approved by the relevant authorities.

© 2025 Korea Disease Control and Prevention Agency

Protection of privacy and confidentiality

Patients have a right to privacy that must not be violated without informed consent. Identifying information, such as names, initials, or hospital numbers, should not be published unless essential for scientific purposes and with written informed consent from the patient (or parent or guardian). Nonessential identifying details should be omitted. If there is any doubt about maintaining anonymity, informed consent is necessary, as masking the eye region in photographs is inadequate. If identifying characteristics are deidentified, authors must assure, and editors must confirm, that these changes do not distort scientific meaning.

Originality

Manuscripts are considered with the understanding that no part of the work has been published previously in print or electronic format and the paper is not under consideration by another publication or electronic medium.

Secondary Publication

It is possible to republish manuscripts if the manuscripts satisfy the conditions for secondary publication as described in the ICMJE Recommendations (http://www.icmje.org/icmje-recommendations.pdf).

Plagiarism and Duplicate Publication

Attempting to publish substantially similar work more than once without attribution of the original source(s) is considered a redundant publication. The definition of "substantially similar" is as follows: (1) at least one of the authors is common to all reports (it is likely to be plagiarism if there are no common authors); (2) the subject or study populations are the same or similar; (3) the methodology is typically identical or nearly so and; (4) the results and interpretation vary little or not at all.

If all or part of the subject population has been reported previously, it should be declared in the Materials and Methods and must be appropriately referenced. In cases where authors are concerned with any potential overlap with published manuscripts or manuscripts being reviewed, the authors must include a letter explaining how the manuscript submitted to PHRP significantly differs from other materials. For more information, please refer to ICMJE Recommendation (available at: http://www.icmje.org/recommendations/).

Authorship and the Author's Responsibilities

Authorship credit must be based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; (3) final approval of the version to be published; and (4) agreeing to be accountable for all aspects of the work in ensuring that the questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The authors should meet these 4 conditions. If the number of authors exceeds 3, the specific role(s) of authors should be described at the end of the main text.

- Correction of authorship: Any requests for changes in authorship (adding author(s), removing author(s), or re-arranging the order of authors) after the initial manuscript submission and before publication should be explained in writing to the editor in a letter or e-mail from all authors. This letter must be signed by all authors of the paper. A copyright assignment must be completed by every author.
- Role of the corresponding author: The corresponding author takes primary responsibility for communication with the journal during the manuscript submission, peer review, and publication process. The corresponding author typically ensures that all of the journal's administrative requirements, such as providing the details of authorship, ethics committee approval, clinical trial registration documentation, and conflict of interest forms and statements, are properly completed, although these duties may be delegated to one or more coauthors. The corresponding author should be available throughout the submission and peer review process to respond to editorial queries in a timely manner, and after publication, should be available to respond to critiques of the work and cooperate with any requests from the journal for data or additional information or questions about the article.
- Contributors: Any researcher who does not meet all 4 ICMJE criteria for authorship discussed above but contributes substantively to the study in terms of idea development, manuscript writing, conducting research, data analysis, and financial support should have their contributions listed in the Notes section of the article.
- Recommendations for working with people with personal connections: Authors who intend to include minors (under the age of 19) or their family members (such as spouse, children, and relatives) in their research, including when publishing or presenting papers jointly with them, should clearly indicate this in the cover letter. For further information, please refer to the "Guidelines

for Preventing Illegitimate Authorship" by the National Research Foundation of Korea (https://www.nrf.re.kr/eng/).

Conflict of Interest Statement

The corresponding author must inform the editor of any potential conflicts of interest that could influence the authors' interpretation of the data. Potential conflicts should be reported even if the authors believe they were not influenced in preparing the manuscript. All authors must disclose i.e., (1) financial relationships (such as employment, consultancies, stock ownership, honoraria, paid expert testimony), (2) personal relationship, (3) academic competition, and (4) intellectual passion. These disclosures should be included on the title page.

Role of the Funding Source

The author is requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor (s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement, then this should be stated.

Inclusive Language

Authors are encouraged to use inclusive language that acknowledges diversity, respects all individuals, and promotes equal opportunities. Avoid assumptions about the reader's beliefs or identities, and refrain from language that implies superiority based on factors such as age, gender, race, ethnicity, culture, sexual orientation, disability, or health condition. Writing should be free of bias, stereotypes, and cultural assumptions. Gender neutrality is encouraged by using plural nouns (e.g., "patients") instead of gendered pronouns ("he/she"). Descriptors of personal attributes should only be used if relevant and valid.

Process for Managing Research and Publication Misconduct

When the journal faces suspected cases of research and publication misconduct such as redundant (duplicate) publication, plagiarism, fraudulent or fabricated data, changes in authorship, an undisclosed conflict of interest, ethical problems with a submitted manuscript, a reviewer who has appropriated an author's idea or data, complaints against editors, and so on, the resolution process will follow the guidances provided by the COPE (https://publicationethics.org/guidance). The editorial boards of PHRP will carry out the discussion and decision for suspected cases. We will not hesitate to publish errata, corrigenda, clarifications, retractions, and apologies when needed.

Complaints and Appeals Policy

The policies of PHRP are principally intended to protect the authors, reviewers, editors, and the publisher of the journal. The process of handling complaints and appeals follows the guidances of COPE (https://publicationethics.org/guidance).

Editorial Responsibilities

The Editorial Board will continuously work to monitor and safeguard publication ethics, including guidelines for retracting articles; maintenance of the integrity of the academic record; preclusion of business needs from compromising intellectual and ethical standards; publishing corrections, clarifications, retractions, and apologies when needed; and excluding plagiarism and fraudulent data. The editors maintain the following responsibilities: responsibility and authority to reject and accept articles; avoiding any conflict of interest with respect to articles they reject or accept; promoting publication of corrections or retractions when errors are found; and preservation of the anonymity of reviewers.

EDITORIAL POLICY

Copyright

The Korea Disease Control and Prevention Agency (publisher) holds the copyright on all submitted materials and the right to publish, transmit, sell, and distribute them in the journal or other media. The publisher applies the Creative Commons Attribution license to works it publishes. Under this license, although the publisher retains ownership of the copyright for content, it allows anyone to download, reuse, reprint, distribute, and/or copy the content for non-commercial purposes.

Open Access License

Every article appearing in this journal will be published as open-access. Articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derives (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. Author(s) do not need to

permission to use tables or figures published in PHRP in other journals, books, or media for scholarly and educational purposes.

Article Sharing (Author Self-Archiving) Policy

PHRP is an open access journal, which means that authors who publish with us are able to freely share their research in various ways, including preprint servers, social media platforms, conferences, and educational materials, in accordance with our open access policy.

• Accepted version
The accepted version, which incorporates all peer review amendments prior to final publication, may be shared on non-commercial websites and repositories. Authors who previously uploaded their article to a preprint server should update the preprint with the accepted manuscript following peer review.

• Published version
The final, published version can be shared immediately upon publication. Under the CC BY-NC 4.0 license, this version permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Data Sharing Policy

To foster transparency, we encourage authors to state the availability of their data in your submission.
Subject to ethical and legal considerations, authors are encouraged to:

- Upload research data during the submission process; otherwise, share research data in a relevant public data repository with DOI for the data location.
- Include a data availability statement linking to the data. If it is not possible to share the data, use the statement to confirm why it cannot be shared.

Clinical Trials: PHRP accepts the ICMJE Recommendations for data sharing statement policies. Authors may refer to the editorial, "Data Sharing Statements for Clinical Trials: A Requirement of the International Committee of Medical Journal Editors," in the Journal of Korean Medical Science (https://dx.doi.org/10.3346/jkms.2017.32.7.1051).

Archiving Policy

PHRP provides electronic backup and preservation of access to the journal content in the event the journal is no longer published by archiving the journal content in National Library of Korea and PubMed Central (https://www.ncbi.nlm.nih.gov/pmc/journals/2151/) from the first volume, 2010.

Preprint Policy

A preprint can be defined as a version of a scholarly paper that precedes formal peer review and publication in a peer-reviewed scholarly journal. PHRP allows authors to submit the preprint to the journal. It is not treated as duplicate submission or duplicate publication. PHRP recommend authors to disclose it with DOI in the letter to the editor during the submission process. Otherwise, it may be screened from the plagiarism check program—Similarity Check (Crosscheck) or Copy Killer. Preprint submission will be processed through the same peer-review process with a usual submission. If the preprint is accepted for publication, authors are recommended to update the info at the preprint with a link to the published article in PHRP, including DOI at PHRP. It is strongly recommended that authors cite the article in PHRP instead of the preprint at their next submission to journals.

Use of Artificial Intelligence (AI)-Assisted Technologies

The journal has adopted policies, as specified by the ICMJE, regarding the use of AI in the preparation of materials intended for publication in the journal. Generative AI, including language models, chatbots, image creators, machine learning, or similar technologies, may be employed to enhance readability and language accuracy in scientific writing. However, chatbots or other AI-assisted technologies cannot be listed as authors.

Generative AI images: We do not permit the use of generative AI or AI-assisted tools for creating or altering images in submitted manuscripts. Adjustments like brightness, contrast, or color balance are acceptable if they don't obscure original information. Exceptions apply if AI is integral to the research design or methods, in which case details must be provided in the methods section. Authors should adhere to AI software policies and may be asked for pre-AI-adjusted versions of images for editorial review.

Declaration of AI and AI-assisted technologies in the writing process: Authors are required to disclose whether AI-assisted technologies were used in the production of the submitted work at the time of manuscript submission.

It must be clearly reported in a dedicated section of the Methods, or in the Acknowledgements section for article types lacking a Methods section. This disclosure should provide details about the specific tools used, including the model name, version, and manufacturer, along with an explanation of the capacity in which they were utilized. Authors should affirm that there is no plagiarism of text or images in materials produced by AI. It is not acceptable to cite AI-generated material as a primary source.

Peer Review Policy

All papers, including those invited by the editor, are subject to peer review. PHRP has adopted a double-blind peer review policy, where the author identities remain anonymous to the reviewers, and vice versa, and the identities of the reviewers and authors are visible to (decision-making) the editor throughout the peer review process. The Editorial Board selects reviewers based on expertise, publication history, and past reviews. During the peer review process, reviewers can interact directly or exchange information (e.g., via submission systems or email) with only an editor, which is known as "independent review." An initial decision will normally be made within 4–6 weeks after the reviewers agree to review a manuscript. No information about the review process or editorial decision process is published on the article page.

- AI use by peer reviewers: Generative AI tools can lack up-to-date knowledge and may produce nonsensical, biased or false information. Manuscripts may also include sensitive or proprietary information that should not be shared outside the peer review process. For these reasons we ask that, while the journal explores providing our peer reviewers with access to safe AI tools, peer reviewers do not upload manuscripts into generative AI tools. If any part of the evaluation of the claims made in the manuscript was in any way supported by an AI tool, we ask peer reviewers to declare the use of such tools transparently in the peer review report.

SUBMISSION & PEER REVIEW PROCESS

Online Submission

All manuscripts should be submitted online at https://mc04.manuscriptcentral.com/osongphrp (PHRP online submission system: ScholarOne). The entire process of manuscript submission, peer-review, and resubmission to PHRP is done through the online system.

Manuscripts submitted to PHRP will be preliminarily reviewed by the Editorial Office. Manuscripts not conforming to the instructions will be returned to the corresponding authors without being considered for publication. Submitted manuscripts are also screened for possible plagiarism or duplicate publication using Crossref Similarity Check. If a paper that might be regarded as duplicate or redundant had already been published in another journal or submitted for publication, the author should notify the fact in advance at the time of submission.

Any inquiry concerning manuscript submission should be directed to the editorial office at ophrp@korea.kr.

Peer Review Process

This journal operates a **double-blind** review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of 2 independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. The detailed review process is as follows.

- The Editorial Office of PHRP receives and reviews all submitted manuscripts, and all submitted manuscripts are considered confidential. The submitted manuscripts are initially screened for formatting. Once the manuscript is provisionally accepted, it is sent to the 2 most relevant reviewers for review.
- The reviewers are selected by the editor from the Editorial Board's database or the board members' recommendation. The reviewers are then requested to evaluate the manuscript based on originality, validity, presentation, and importance and interest, and, when considered necessary, statistics.
- Acceptance of a manuscript depends on the evaluation, critiques, and recommended decision made by the reviewers. A reviewer may recommend "accept," "minor revision," "major revision," and "reject." If there are conflicting decisions between reviewers, or between the author and reviewer(s), the Editor-in-Chief has the full right to decide whether the manuscript will be published in the journal. Three repeated decisions of "major revisions" are equivalent to rejection, and rejected papers will not be considered further.
- The reviewed manuscript with comments, recommendations, and revisions is returned to the corresponding author. The corresponding author is to submit the revised manuscript

accompanied by point-to-point replies to the comments given by the editor and how the revisions have been made. There should be a reasonable explanation for any noncompliance with the recommendations. In cases where references, tables, or figures are moved, added, or deleted during the revision process, renumbering must be done so that all references, tables, and figures are cited in numeric order. If the revised paper is not received within 2 months of decision, the manuscript is considered to have been withdrawn.

- When the final decision on the acceptance of the manuscript is made, the Editorial Office notifies the corresponding author. The peer-review process takes approximately 8–12 weeks.

Submission by Editors

Final decisions regarding manuscript publication are made by the editor-in-chief or a designated editor who does not have any relevant conflicts of interest. In the event that an editor has a conflict of interest with a submitted manuscript or with the authors, the manuscript will be handled by one of the other editors who does not have a conflict with the review and who is not at the same institution as the submitting editor. In such circumstances, full masking of the process will be ensured so that the anonymity of the peer reviewers is maintained.

MANUSCRIPT PREPARATION

General Requirements

- All manuscripts must be in grammatically correct English and should be created using MS Word. The manuscript must be double-spaced and written in an A4 page format. Do not leave a space between paragraphs. Only a single font (preferably Times New Roman) should be used in 11 point with margins of 2.5 cm.
- All pages should be paginated consecutively.
- All numbers should be written in Arabic numerals throughout the manuscript except for the first word of the sentence. Texts should be justified on both sides and not hyphenated and headings should be in bold letters, aligned in the center. If possible, avoid using abbreviated words at the beginning of sentences.
- Abbreviations: Where a term/definition is repeatedly referred to (i.e., 3 times in the text), it is written in full when it first appears, followed by the subsequent abbreviation in parentheses (even if it was previously defined in the abstract); thereafter, the abbreviation is used.
- Gene nomenclature: Current standard international nomenclature for genes should be adhered to. Genes should be typed in italic font and include the accession number. For human genes, use the genetic notation and symbols approved by the HUGO Gene Nomenclature Committee (http://www.genenames.org/) or refer to PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez).
- Units: Système International (SI) units must be used, with the exception of blood pressure values, which are to be reported in mmHg. Please use the metric system for expressions of length, area, mass, and volume. There should be a space between the numerals and the unit symbol. When indicating time, the 24-hour system is to be used.
- Math formulae: Present simple formulae in the line of normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by "exp." Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Reporting Guidelines for Specific Study Designs

For specific study designs, such as randomized control studies, studies of diagnostic accuracy, meta-analyses, observational studies, and non-randomized studies, authors should adhere the reporting guidelines relevant to their specific research design and submit a checklist. A good source of reporting guidelines is the EQUATOR Network (https://www.equator-network.org/) and NLM (https://www.nlm.nih.gov/services/research_report_guide.html).

Manuscript Types

PHPR publishes editorials, original articles, review articles, guidelines, data profiles (including cohort profiles), special articles, short communications, viewpoints, editorials, commentaries, and correspondence, and book reviews.

- **Original articles** are papers containing results of basic and clinical investigations, which are sufficiently well documented to be acceptable to critical readers. These articles should be written in the following format: title page; abstract and keywords; main body (introduction, materials and methods, results, discussion, conclusion [if any]); references; and tables and figure legends. Manuscript limitations are 5,000 words, excluding the abstract,

references, and tables and figure legends.

- **Review articles** provide concise reviews of subjects important to medical researchers, and can be written by an invited medical expert. These have the same format as original articles, but the details may be more flexible depending on the content. Manuscript limitations are 6,500 words from introduction to conclusion, 100 references, 10 figures and 10 tables. The abstract should not exceed 200 words, and must be written as one unstructured paragraph.
- **Systematic reviews** should adhere to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The template for systematic reviews can be downloaded from https://ophrp.org/file/PHRP_Systematic%20Review_PRISMA.docx.
- **Guidelines** are similar to original articles, but provide evidence-based recommendations expected to impact clinical research and practice. This category can include consensus-based statements of reporting standards or clinical practice guidelines.
- **Data Profiles (including Cohort Profiles)** present large data sets from specific populations that could be analyzed in epidemiological studies. Data Profiles should be structured with the following headings in the main text: Introduction, Collection, Data Resource Use, Strengths and Weaknesses, and Access. Cohort Profiles present up-to-date information about large population-based cohorts for which long-term data collection is planned. Data Profiles should be structured with the following headings in the main text: Introduction, Study Participants, Measurements, Key Findings, Strengths and Weaknesses, and Access. The main text of Data and Cohort Profiles is limited to 4,000 words, with an unstructured abstract of up to 200 words, a maximum of 7 tables and figures, and no more than 40 references.
- **Special Articles** deal with topics or issues that are relevant to public health, but without following a traditional study format. For example, articles in this category may address scientific methodology, wide-ranging ethical and social issues, scientific methodology, or other scholarly topics. Reports from consensus committees and working groups can be published as Special Articles. This category has a main text limit of 3,500 words, with an unstructured abstract of no more than 200 words, a maximum of 7 tables and figures, and no more than 40 references.
- **Brief reports** deal with issues of importance to biomedical researchers and follow the general guidelines for original articles. The manuscript should not exceed 2,000 words, excluding references, tables and figures, and must include a structured abstract of no more than 250 words.
- **Short communications** follow the general guidelines for original articles but present original research and/or preliminary findings in a more concise format with fewer details. The manuscript should not exceed 3,000 words, excluding references, tables, and figures, and must include a structured abstract of no more than 250 words.
- **Viewpoints** may deal with almost any topic deemed to be important in the fields of public health, ethics, health law, prevention, or health policy, and are not typically written in response to a specific article. Viewpoints should have a clear focus and present material in a well-organized and scholarly manner, but should not contain novel research findings or previously unpublished data. Although we welcome unsolicited viewpoint contributions, we request that authors contact the Editorial Office (ophrp@korea.kr) prior to submission to confirm that the proposed topic is suitable for the journal. The main text of Viewpoints is limited to 3,000 words, with an unstructured abstract of up to 150 words, a maximum of 4 tables and figures, and no more than 30 references.
- **Editorials** provide invited perspective on an area of PHRP, dealing with very active fields of research, current interests, fresh insights, and debates. An abstract is not required and a brief unstructured text should be prepared. Although editorials are normally invited or written by an editor, unsolicited editorials may be submitted. Manuscript limitations are 1,000 words and 20 references.
- **Commentaries** are brief articles with a narrow focus. The journal commissions most commentaries, but unsolicited commentaries will also be considered. Commentaries may undergo peer review. The length of commentaries should be limited to 500 words, 10 references, and 2 figure or small table.
- **Correspondence** is a comment from readers regarding a published article with a reply from the authors of the article. Manuscript limitations are 500 words, 2 tables/figures, and 5 references.
- **Book reviews** may be published. Please dispatch a book to the editorial office if you think the book is essential to public health personnel.

Key features and limits of articles are summarized in Table 1 below.

Table 1. Key features and limits of articles

Type of article	Abstract (words)	Text (words)*	References	Tables and figures
Original article	Structured, 250	5,000	-	-
Review article	200	6,500	100	10 Tables 10 Figures
Data profile	200	4,000	40	7
Special article	200	3,500	40	7
Brief report	Structured, 250	2,000	-	-
Short communication	Structured, 250	3,000	-	-
Viewpoint	150	3,000	30	4
Editorial	Not required	1,000	20	-
Commentary	Not required	500	10	2
Correspondence	Not required	500	-	2

*Excluding abstract, references, tables, and figure legends.

Title Page

Title page should include (1) the title of the article (less than 50 words); (2) name of the authors (first name, middle initial, last name in capitals) and institutional affiliation including the name of department(s) and institution(s) of each author; (3) name, full address (including the postal code) of the institutional affiliation, telephone and e-mail address of the corresponding author; (4) a running title of 50 characters or less including blank spaces; and (5) notes (disclaimers). Notes include ethics approval and consent to participate, conflict of interest, funding, availability of data, authors' contributions according to the CRediT taxonomy (https://credit.niso.org/), additional contributions, and ORCID of all authors. All contributors who do not meet the criteria for authorship as defined above should be listed in an additional contribution section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, or a department chair who provided only general support. Authors should disclose whether they had any writing assistance and identify the entity that paid for this assistance.

Abstract and Keywords

An abstract and 3–6 relevant keywords (in alphabetical order) are required. Abstracts should be no more than 250 words in length. Abstracts should be structured, with the following section headings: Objectives, Methods, Results, Conclusion. For selecting keywords, refer to the MeSH browser (http://www.ncbi.nlm.nih.gov/mesh).

Highlights

All papers must include 3–5 short sentences presenting short summary or findings in the next of title page. The highlight section should be no more than 100 words, including spaces.

Main Body

- **Introduction** should provide concise yet sufficient background information about the study to provide the readers with a better understanding of the study, avoiding a detailed literature survey or a summary of the results.
- **Materials and methods** should contain detailed procedures of the study or experiment including investigation period, methods of subject selection, and information on subjects such as age, sex or gender, and other significant features, in order to enable the experiment to be repeated. A procedure that has been already published or standardized should be described only briefly using literature citations. Clinical trials or experiments involving laboratory animals or pathogens must elaborate on the animal care and use and experimental protocols, in addition to mentioning approval from the relevant committees. The sources of special equipment and chemicals must be stated with the name of the manufacturer. All statistical procedures used in the study and criteria for determining significance levels must be described. Ensure correct use of the terms "sex" (when reporting biological factors) and "gender" (identity, psychosocial or cultural factors). Unless inappropriate, report the sex and/or gender of study participants, the sex of animals or cells, and describe the methods used to determine sex or gender. If the study involved an exclusive population (only one sex, for example), authors should justify why, except in obvious cases (e.g., prostate cancer). Authors should define how they determined race or ethnicity, and justify its relevance. Institutional Review Board approval and informed consent procedures can be described as follows: The study protocol was approved by the Institutional Review Board of OOO (IRB No: OO-OO-OO). Informed consent was obtained by all participants (or the participant's legal guardian) / Informed consent was waived by the IRB.
- **Results** should be presented in logical sequence. Only the most important observations should be emphasized or summarized, and the main or the most important findings should be mentioned first. Tables and figures must be numbered in the order they are cited in the text, kept to a minimum, and should not be repeated. Supplementary materials and other details can be separately presented in an appendix. The authors

should state the statistical method used to analyze the results (statistical significance of differences) with the probability values given in parentheses.
- **Discussion** should contain an interpretation and explanation of the results and important aspects of the study, followed by the conclusions drawn from them. Information already mentioned in the Introduction or Results sections should not be repeated and the main conclusions of the study may be presented in the discussion.
- **Conclusion** (if any) must be linked with the purpose of the study stated in the abstract, and clearly supported by the data produced in the study. New hypotheses may be stated when warranted, but must be clearly labeled.

References

Authors are responsible for the accuracy and completeness of their references and for correct text citations.

- References are presented with [ ] following a surname in the main text, such as Kim [1] and Kim et al. [2]. When a reference is cited within the content, it is shown as [3] or [4,5] at the end. References should be searchable online.
- The last names and initials of all the authors (up to 3) should be included. For articles with more than 3 authors, list the first 3 authors only followed by "et al."
- When citing organizations that are national bodies such as government agencies, if a nationality is not part of the name, place the country in parentheses after the name, using the two-letter ISO country code.
- References cited in tables or figure legends should be included in sequence at the point where the table or figure is first mentioned in the main text.
- Do not cite abstracts unless they are the only available reference to an important concept.
- Uncompleted work or work that has not yet been accepted for publication (i.e., an "unpublished observation" or "personal communication" should not be cited as a reference). In the references list, references should be limited to those cited in the text and listed in the order in which they appear in the text. The journals should be abbreviated according to the style used in the list of journals indexed in the NLM Journal Catalog (http://www.ncbi.nlm.nih.gov/nlmcatalog/journals).
- Use of DOI is highly encouraged. Note that missing data will be highlighted at the proof stage for the author to correct.
- Other types of references not described below should follow the ICMJE Recommendations (https://www.nlm.nih.gov/bsd/uniform_requirements.html).

Please refer to the following examples.

- **Journal articles**
  1. Park AK, Kim IH, Kim J, et al. Genomic surveillance of SARS-CoV-2: distribution of clades in the Republic of Korea in 2020. Osong Public Health Res Perspect 2021; 12:37-43.
  2. Hyun J, Lee JH, Park Y, et al. Interim epidemiological and clinical characteristic of COVID-19 28 cases in South Korea. Public Health Wkly Rep 2020;13:464-74. Korean.
  3. Gultekin V, Allmer J. Novel perspectives for SARS-CoV-2 genome browsing. J Integr Bioinform 2021 Mar 15 [Epub]. https://doi.org/10.1515/jib-2021-0001.
- **Books**
  1. Riffenburgh RH, Gillen DL. Statistics in medicine. 4th ed. Academic Press; 2020.
  2. Miller DD. Minerals. In: Damodaran S, Parkin KL, editors. Fennema's food chemistry. 5th ed. CRC Press; 2017. p. 627-80.
  3. Ministry of Employment and Labor (KR). Statistics on occupational injuries and illnesses, 2008. Ministry of Employment and Labor; 2009. Korean.
- **Websites**
  1. World Health Organization (WHO) (CH). COVID-19 vaccines [Internet]. WHO; 2021 [cited 2021 Mar 15]. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/covid-19-vaccines.
- **Conference papers**
  1. Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, IE. Springer; 2002. p. 182-91.
- **Dissertation**
  1. Park HY. The role of the thrombomodulin gene in the development of myocardial infarction [dissertation]. Yonsei University; 2000.

Tables and Figures

Tables should be simple, self-explanatory, and supplemental, and should not duplicate the text or figures. Each table must be on a separate page, not exceeding 1 page when printed, and have a concise and informative title. The tables should be numbered with Arabic numerals in consecutive order. Each column should be appropriately headed with units in parentheses if numerical measures are given. All units of measurements and concentrations must be indicated. Footnotes are followed by the source notes, other general notes, abbreviation, notes on specific parts of the table ([a], [b], [c],

[d]...), and notes on level of probability (*, **, *** for *p*).

Figures should be numbered with Arabic numerals consecutively in figure legends. The figures must not be interfered and must be clearly seen. The legend for each light microscopic image should include name of the stain and magnification. Electron microscopic images should contain an internal scale marker. All figures may be altered in size by the editor. The legends should briefly describe the data shown, explain abbreviations or reference points, and identify all units, mathematical expressions, abscissas, ordinates, and symbols.

Figures that are drawn or photographed professionally should be sent as JPG or PPT files. However, if an article receives approval for publication, files must be submitted as .tiff or .pdf. Each figure must have a caption explaining the figure. The preferred size of the images is 8 × 8 cm but 16.5 cm in width × 8 cm in length is also acceptable. It is authors' full responsibility to submit images of sufficient quality for accurate reproduction and to approve the final color galley proof. All images must be correctly exposed, sharply focused, and prepared in files of 500 dpi or more.

When tables and figures are mentioned together in the text, they should be presented in parentheses as follows: (Table 1; Figure 1), (Tables 1, 2; Figures 1–3).

Appendix and Supplemental Data

If any materials are not enough to be included in the main text such as questionnaires, they can be listed in the Appendix. Any supplementary materials that help the understanding of readers or contain too great an amount of data to be included in the main text may be placed as supplementary data. Not only a recording of the abstract, text, audio or video files, but also data files should be added here.

FINAL PREPARATION FOR PUBLICATION

Final Version

After the paper has been accepted for publication, the author(s) should submit the final version of the manuscript. The names and affiliations of the authors should be double-checked, and if the originally submitted image files were of poor resolution, higher-resolution image files should be submitted at this time. Symbols (e.g., circles, triangles, squares), letters (e.g., words, abbreviations), and numbers should be large enough to be legible on reduction to the journal's column widths. All symbols must be defined in the figure caption. If references, tables, or figures are moved, added, or deleted during the revision process, renumber them to reflect such changes so that all tables, references, and figures are cited in numeric order.

Manuscript Corrections

Before publication, the manuscript editor will correct the manuscript such that it meets the standard publication format. The author (s) must respond within 48 hours when the manuscript editor contacts the corresponding author for revisions. If the response is delayed, the manuscript's publication may be postponed to the next issue.

Proofs and Reprints

The author(s) will receive the final version of the manuscript as a PDF file. Upon receipt, the author(s) must notify the editorial office of any errors found in the file within 48 hours. Any errors found after this time are the responsibility of the author(s) and will have to be corrected as an erratum.

Correction

To correct errors in published articles, the corresponding author should contact the journal's editorial office with a detailed description of the proposed correction. Corrections that profoundly affect the interpretation or conclusions of the article will be reviewed by the editors. Corrections will be published as author correction or publisher correction in a later issue of the journal.

Minor errors will be corrected directly in the online version of the article. An indication of the correction, along with the date it was made, will be added to the article information in both the HTML and PDF versions. A separate correction note will not be published.

NOTICE: These recently revised instructions for authors will be applied beginning with the January 2025 issue.

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

Author's checklist

General Requirements

- ▫ The corresponding author (or the representative author of the co-corresponding authors) is the submitter of this manuscript.
- ▫ All manuscripts should be written in English.
- ▫ The main document with manuscript text and tables should be prepared in an MS Word (docx) or RTF file format.
- ▫ Manuscripts should be double-spaced in A4-size pages.
- ▫ Manuscripts should include line numbers.
- ▫ All pages should be numbered consecutively, starting with the abstract.

Title Page

- ▫ The title page and the rest of the manuscript text are prepared separately in two files (not combined together).
- ▫ The title page is arranged in the following order: article title, authors' full name(s), affiliation(s), and corresponding author's information, running title (less than 50 characters), notes.
- ▫ The notes section including (1) ethics approval and consent to participate, (2) conflicts of interest, (3) funding, (4) availability of data, (5) author contributions, (6) additional contributions, and ORCID is in title page, not in the manuscript.

Abstract

- ▫ The abstract does not exceed 250 words (Objectives, Methods, Results, Conclusion) for original articles and 200 words for reviews. Up to 3–6 keywords are listed at the bottom of the abstract.

Main Text

- ▫ The manuscript is organized according to following sequence: Title page, Abstract and keywords, Main text, References, Tables, and Figure legends.

Tables and Figures

- ▫ All tables and figures are numbered in the order of their appearance in a main text.
- ▫ Tables are included at the end of the manuscript as editable text and not as images.
- ▫ Figures are as separate files, in jpg, ppt, tiff, or pdf format.

References

- ▫ References are listed in proper format.
- ▫ All references listed in the reference section are cited in the text and vice versa.

© 2025 Korea Disease Control and Prevention Agency

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

Copyright Transfer and Conflict of Interest Disclosure Form

Title of the paper: _____

_____

I. Copyright Transfer Form

The authors hereby transfer all copyrights in and to the manuscript named above in all forms and media, now or hereafter known, to the Korea Disease Control and Prevention Agency effective if and when the paper is accepted for publication in the *Osong Public Health and Research Perspectives*. The authors reserve all proprietary right other than copyright, such as patent rights.
Everyone who is listed as an author in this article should have made a substantial, direct, intellectual contribution to the work and should take public responsibility for it.
This paper contains works that have not previously published or not under consideration for publication in other journals.

II. Conflict of Interest Disclosure Form

All authors are responsible for recognizing any conflict of interest that could bias their work in the acknowledgments, disclosing all financial support and any other personal connections.

Please check the appropriate box below:

- ▫ No author of this paper has a conflict of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included in this manuscript.

OR

- ▫ The authors certify that all conflicts of interest, as applicable to each author, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials are disclosed in the manuscript.
  (Please describe in detail about these interests.)

_____

_____

_____

These interests may include one or more of the following: employment; consultancy within the past two years; ownership interests – including stock options – in a start-up company, the stock of which is not publicly traded; ownership interest - including stock options but excluding indirect investments through mutual funds and the like - in a publicly traded company; research funding; honoraria directly received from an entity; paid expert testimony within the past two years; any other financial relationship (e.g., receiving royalties); membership on another entity's Board of Directors or its advisory committees (whether for profit or not for profit).

- ▫ All authors certify that the work followed the research ethics and have approved the submission of the manuscript for publication.

© 2025 Korea Disease Control and Prevention Agency

phrp
OSONG PUBLIC HEALTH
AND RESEARCH PERSPECTIVES

	Name	Signature	Date
Corresponding author:			
Authors:			

(This form must be signed by all authors in an order as appeared in the article, and should be returned to the editorial office.)

© 2025 Korea Disease Control and Prevention Agency