



# SMIJ

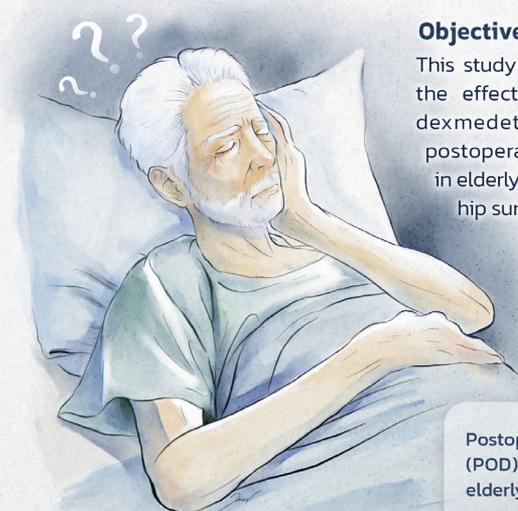
## Siriraj Medical Journal

The world-leading biomedical science of Thailand

MONTHLY

ORIGINAL ARTICLE REVIEW ARTICLE

### Effect of a Single-dose Dexmedetomidine on Postoperative Delirium and Intraoperative Hemodynamic Outcomes in Elderly Hip Surgery; A Randomized Controlled Trial Dexmedetomidine for Postoperative Delirium



#### Objective :

This study aims to investigate the effect of a single-dose dexmedetomidine (DEX) on postoperative delirium (POD) in elderly patients undergoing hip surgery.

Postoperative delirium (POD) is common in elderly patients.

#### Materials and Methods:

This prospective, randomized, double-blinded trial enrolled patients aged over 65 years who underwent hip surgery under spinal anesthesia.

#### Patients were assigned to either

Dexmedetomidine (DEX)



0.3-0.5 µg/kg IV

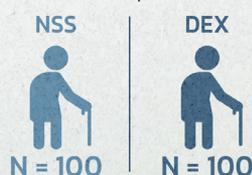
Normal saline solution (NSS)



Delirium assessments were conducted at  
PACU 24 hr 48 hr 72 hr  
postoperatively using the Confusion Assessment Method (CAM).

#### Results:

A total of 200 patients were randomized.



The incidence of **POD was significantly lower in the DEX** compared to the NSS (P < 0.001, RR 0.45, 95%CI: 0.28, 0.73)

This difference remained significant at each postoperative assessment time point.



There was no significant difference in sedation score and perioperative hemodynamics.

Additionally, the length of stay after surgery in the DEX was significantly shorter compare to the NSS (P=0.006).

#### Conclusion:

A single-dose dexmedetomidine can reduce the incidence of POD within 72 hours postoperatively in elderly patients undergoing hip surgery without compromising intraoperative hemodynamic stability.



SMIJ

SIRIRAJ  
MEDICAL  
JOURNAL

Copyright Notice: Copyright ©T Vorawanakul. All rights reserved.

C Choovongkomol et al. *Siriraj Med J* 2024;76:80-89.

Indexed by

Scopus®



<https://he02.tci-thaijo.org/index.php/sirirajmedj/index>  
E-mail: [sijournal92@gmail.com](mailto:sijournal92@gmail.com)

THAILAND SECTION  
1954

## ORIGINAL ARTICLE

- 40** Enhancing Adolescent Self-Esteem: A Pilot Randomized Controlled Trial of the Online Mindfulness-Based Intervention Program (MBSI Online)  
*Tuksina Krobtrakulchai, Tidarat Puranachaikere, Wanlop Atsariyasing, Natee Viravan, Kanthip Thongchoi, Pennapa Prommin*
- 
- 52** Retrospective Analysis of Inpatient Dermatologic Consultations in a Residency Training Program  
*Pantaree Kobkurkul, Chanakarn Pisankikitti, Jidapa Rueangkaew, Nattha Angkoolpakdeekul, Supenya Varothai, Sumanas Bunyaratavej, Narumol Silpa-archa*
- 
- 61** The Effect of Diabetes Self-management Education Provided by Certified Diabetes Educator Compared to Usual Diabetes Education on Glycemic Level and Stage of Behavior Change in Adult with Types 2 Diabetes Mellitus  
*Kanyarat Wongmuan, Narinnad Thanaboonsutti, Wilawan Ketpan, Sarawoot Uprarat, Varisara Lapinee, Lukana Preechasuk*
- 
- 69** Real-world data on the Immunity Response to the COVID-19 Vaccine among Patients with Central Nervous System Immunological Diseases  
*Punchika Kosiyakul, Jiraporn Jitprapaikulsan, Ekdanai Uawithya, Patimaporn Wongprompitak, Chutikarn Chaimayo, Navin Horthongkham, Nasikarn Angkasekwini, Nanthaya Tisavipat, Naraporn Prayoonwiwat, Natthapon Rattanathamsakul, Kanokwan Boonyapisit, Theerawat Kumutpongpanich, Onpawee Sangsai, Kamonchanok Aueaphatthanawong, Jirawan Budkum, Sasitorn Siritho*
- 
- 80** Effect of a Single-dose Dexmedetomidine on Postoperative Delirium and Intraoperative Hemodynamic Outcomes in Elderly Hip Surgery; A Randomized Controlled Trial Dexmedetomidine for Postoperative Delirium  
*Chidchanok Choovongkomol, Sothida Sinchai, Kongtush Choovongkomol*
- 
- 90** Development of the Purification Process of Gallium-68 Eluted from Germanium-68/ Gallium-68 Generator  
*Tossaporn Sriprapa, Thanete Doungta, Napamon Sritongkul, Malulee Tantawiroon*
- 
- 97** Comparison between Anal Dilatation Protocols Following an Endorectal Pull-through for Hirschsprung Disease  
*Ravit Ruangtrakool, Jirarak Deepor*

## REVIEW ARTICLE

- 106** Beyond Vision: Potential Role of AI-enabled Ocular Scans in the Prediction of Aging and Systemic Disorders  
*Moez Osama Omar, Muhammad Jabran Abad Ali, Soliman Elias Qabillie, Ahmed Ibrahim Haji, Mohammed Bilal Takriti, Ahmed Hesham Atif, Rangraze Imran*



**Executive Editor:** Apichat Asavamongkolkul

**Editorial Director:** Aasis Unnanuntana

**Editor-in-Chief:** Thawatchai Akaraviputh, Mahidol University, Thailand

### Associate Editors

Adisorn Ratanayotha, Mahidol University, Thailand

Chenchit Chayachinda, Mahidol University, Thailand

Pornprom Muangman, Mahidol University, Thailand

Phunchai Charatcharoenwitthaya, Mahidol University, Thailand

Varut Lohsiriwat, Mahidol University, Thailand

### International Editorial Board

Andrew S.C. Rice, Imperial College London, UK

Morris Solomon Odell, Monash University, Australia

Anusak Yiangpruksawan, The Valley Robotic Institute, USA

Moses Rodriguez, Mayo Clinic, USA

Barbara Knowles, The Jackson Laboratory, USA

Nam H. CHO, Ajou University School of Medicine and Hospital, Republic of Korea

Christopher Khor, Singapore General Hospital, Singapore

Nima Rezaei, Tehran University of Medical Sciences, Iran

Ciro Isidoro, University of Novara, Italy

Noritaka Isogai, Kinki University, Japan

David S. Sheps, University of Florida, USA

Paul James Brindley, George Washington University, USA

David Wayne Ussery, University of Arkansas for Medical Sciences, USA

Pauline Mary Rudd, National Institute for Bioprocessing Research and Training

Davor Solter, The Jackson Laboratory, USA

Fosters Avenue Mount Merrion Blackrock Co., Dublin, Ireland

Dennis J. Janisse, Medical College of Wisconsin, USA

Peter Hokland, Aarhus University Hospital, Denmark

Dong-Wan Seo, University of Ulsan College of Medicine, Republic of Korea

Philip A. Brunell, State University of New York At Buffalo, USA

Folker Meyer, Argonne National Laboratory, USA

Philip Board, Australian National University, Australia

Frans Laurens Moll, University Medical Center Utrecht, Netherlands

Richard J. Deckelbaum, Columbia University, USA

G. Allen Finley, Delhousie University, Canada

Richard W. Titball, University of Exeter, USA

George S. Baillie, University of Glasgow, United Kingdom

Robert W. Mann, University of Hawaii, USA

Gregory Bancroft, London School of Hygiene of Tropical Medicine, United Kingdom

Robin CN Williamson, Royal Postgraduate Medical School, United Kingdom

Gustavo Saposnik, St. Michael's Hospital, Canada

Sara Schwanke Khilji, Oregon Health & Science University, USA

Harland Winter, Harvard Medical School, USA

Seigo Kitano, Oita University, Japan

Hidemi Goto, Nagoya University Graduate School of Medicine, Japan

Shomei Ryozaawa, Saitama Medical University, Japan

Ichizo Nishino, National Institute of Neuroscience NCNP, Japan

Shuji Shimizu, Kyushu University Hospital, Japan

Intawat Nookaew, University of Arkansas for Medical Sciences, USA

Stanley James Rogers, University of California, San Francisco, USA

James P. Doland, Oregon Health & Science University, USA

Stephen Dalton, University of Georgia, USA

John Damian Smith, Texas A&M University-San Antonio, USA

Sue Fletcher, Murdoch University, Australia

John Hunter, Oregon Health & Science University, USA

Tai-Soon Yong, Yonsei University, Republic of Korea

Juri Gelovani, Wayne State University, USA

Tomohisa Uchida, Oita University, Japan

Karl Thomas Moritz, Swedish University of Agricultural Sciences, Sweden

Victor Manuel Charoenrook de la Fuente, Centro de Oftalmologia Barraquer, Spain

Kazuo Hara, Aichi Cancer Center Hospital, Japan

Vincent W.S. Chan, University of Toronto, Canada

Keiichi Akita, Tokyo Medical and Dental University Hospital, Japan

Wen-Shiang Chen, National Taiwan University College of Medicine, Taiwan

Kym Francis Faull, David Geffen School of Medicine, USA

Wikrom Karnsakul, Johns Hopkins Children's Center, USA

Kyoichi Takaori, Kyoto University Hospital, Japan

Yasushi Sano, Director of Gastrointestinal Center, Japan

Marcela Hermoso Ramello, University of Chile, Chile

Yik Ying Teo, National University of Singapore, Singapore

Marianne Hokland, University of Aarhus, Denmark

Yoshiki Hirooka, Nagoya University Hospital, Japan

Matthew S. Dunne, Institute of Food, Nutrition, and Health, Switzerland

Yozo Miyake, Aichi Medical University, Japan

Mitsuhiro Kida, Kitasato University & Hospital, Japan

Yuji Murata, Aizenbashi Hospital, Japan

### Editorial Board

Ampaiwan Chuansumrit, Mahidol University, Thailand

Sayomporn Sirinavin, Mahidol University, Thailand

Anuwat Pongkunkorn, Lampang Hospital, Thailand

Suneerat Kongsayreepong, Mahidol University, Thailand

Jarupim Soongswang, Mahidol University, Thailand

Supakorn Rojananin, Mahidol University, Thailand

Nopphol Pausawasdi, Mahidol University, Thailand

Surapol Issaragrasi, Mahidol University, Thailand

Nopporn Sittisombut, Chiang Mai University, Thailand

Suttipong Wacharasindhu, Chulalongkorn University, Thailand

Pa-thai Yenchitsomanus, Mahidol University, Thailand

Vasant Sumethkul, Mahidol University, Thailand

Pornchai O-Charoenrat, Mahidol University, Thailand

Vitoon Chinswangwatanakul, Mahidol University, Thailand

Prapon Wilairat, Mahidol University, Thailand

Watchara Kasinrerak, Chiang Mai University, Thailand

Puttinun Patpituck, Mahidol University, Thailand

Wiroon Laupattrakasem, Khon Kaen University, Thailand

Rungroj Krittayaphong, Mahidol University, Thailand

Yuen Tanniradorn, Chulalongkorn University, Thailand

Saranatra Waikakul, Mahidol University, Thailand

**Journal Manager:** Nuchpraweepon Saleeon, Mahidol University, Thailand

**Medical Illustrator:** Nuchpraweepon Saleeon, Mahidol University, Thailand

**Proofreaders:** Nuchpraweepon Saleeon, Mahidol University, Thailand, Amornrat Sangkaew, Mahidol University, Thailand

# Enhancing Adolescent Self-Esteem: A Pilot Randomized Controlled Trial of the Online Mindfulness-Based Intervention Program (MBSI Online)

Tuksina Krobtrakulchai, M.D.<sup>1</sup>, Tidarat Puranachakere, M.D.<sup>1</sup>, Wanlop Atsariyasing, M.D.<sup>1</sup>, Natee Viravan, M.D.<sup>1</sup>, Kanthip Thongchoi, M.S.<sup>1</sup>, Pennapa Prommin, B.A.<sup>2</sup>

<sup>1</sup>Department of Psychiatry, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, <sup>2</sup>Petchaboon Department of Youth Observation and Protection, Mueang Phetchaboon, Phetchaboon, Thailand.

## ABSTRACT

**Objective:** This study aimed to develop and assess the effectiveness of the MBSI online program in enhancing self-esteem, mindfulness, and resilience among adolescents, while also evaluating its feasibility and participant satisfaction.

**Materials and Methods:** The MBSI online program is an adapted form of mindfulness-based interventions (MBIs), developed by integrating specific aspects of mindfulness that are related to self-esteem and the core processes of Acceptance and Commitment Therapy (ACT), following consultations with mindfulness experts and program trials. The study enrolled 70 adolescents aged 15 to 18 years with low to moderate self-esteem, from diverse Thai regions. Participants were randomly assigned and equally allocated to intervention and waiting-list control groups. The intervention group underwent an 8-week MBSI online program. Self-esteem, mindfulness, and resilience were assessed at baseline, week 4, week 8, 1 month, and 3 months post-intervention.

**Results:** The self-esteem, mindfulness, and resilience scores within the intervention group have significantly increased over time compared to baseline on week 4, week 8, 1 month, and 3 months post-intervention ( $p < 0.001$ ). Furthermore, between-group comparisons revealed statistically significant improvements in self-esteem, mindfulness, and resilience ( $p < 0.05$ ) at corresponding intervals, with medium to large effect sizes. The dropout rate was 25.7%, while participant satisfaction with the MBSI online program was remarkably high, averaging 4.73 out of 5, with 76.9% expressing the highest level of satisfaction.

**Conclusion:** The MBSI online program significantly improved self-esteem, mindfulness, and resilience in adolescents, achieving high participant satisfaction. This program presents a valuable intervention for adolescents with low self-esteem, aiming to prevent psychological issues stemming from diminished self-esteem.

**Keywords:** Adolescent; mindfulness; online group therapy; self-esteem; resilience (Siriraj Med J 2024; 76: 40-51)

## INTRODUCTION

In recent years, there has been increasing attention directed toward the mental health and well-being of adolescents due to the intricate challenges encountered in the contemporary world. Self-esteem, defined as an individual's comprehensive positive self,<sup>1</sup> plays a pivotal role in influencing emotional resilience, academic

achievement, and interpersonal relationships during the transformative phase of adolescence.<sup>2,3</sup>

Low self-esteem is strongly correlated with internalizing symptoms such as depressive mood, somatic complaints, and anxiety. In serious cases, it can lead to various problems, including delinquency, self-inflicted injuries, and even suicide.<sup>4,5</sup> Previous studies have found that one-third to

Corresponding author: Tidarat Puranachakere

E-mail: [tidarat.pua@mahidol.edu](mailto:tidarat.pua@mahidol.edu)

Received 17 November 2023 Revised 9 December 2023 Accepted 28 December 2023

ORCID ID: <http://orcid.org/0000-0001-6768-2200>

<https://doi.org/10.33192/smj.v76i2.266383>



All material is licensed under terms of the Creative Commons Attribution 4.0 International (CC-BY-NC-ND 4.0) license unless otherwise stated.

one-half of adolescents struggle with low self-esteem.<sup>6</sup> During the COVID-19 pandemic, research in Thailand revealed that adolescents experienced a lower level of self-esteem, with 51.9% reporting reduced self-esteem.<sup>7</sup>

Mindfulness-based interventions (MBIs) have demonstrated their effectiveness in enhancing subjective well-being, reducing stress, anxiety,<sup>8</sup> and depression,<sup>9</sup> as well as improving emotion regulation, self-control, and enhancing executive, behavioral, and socio-emotional competences.<sup>10-12</sup> However, in the realm of enhancing self-esteem among adolescents, the outcomes remain inconclusive.<sup>13</sup>

Previous studies on MBIs in adolescents have been limited by factors such as a lack of randomization,<sup>14</sup> absence of a control group,<sup>15</sup> short-term follow-up,<sup>15,16</sup> small-to-medium effect sizes,<sup>17</sup> and feasibility assessments.<sup>18</sup> Some adapted online MBIs did not demonstrate significant changes in improving self-esteem.<sup>19,20</sup> Furthermore, within the context of Thailand, there has been no study on the effectiveness of online MBIs in enhancing self-esteem among adolescents.

The recognition of MBIs has significantly grown with the advent of mindfulness-based stress reduction (MBSR)<sup>21</sup> and mindfulness-based cognitive therapy (MBCT).<sup>22</sup> MBIs have continued to evolve, including approaches like dialectical behavior therapy (DBT)<sup>23</sup> and acceptance and commitment therapy (ACT).<sup>24</sup> Various platforms now offer MBIs, ranging from traditional in-person groups to online programs accessible via computers, laptops, or mobile phones.

However, traditional MBIs often rely on formal therapeutic procedures delivered by psychiatrists or psychologists, heavily emphasizing meditation techniques that may not fully engage adolescents. Explicit references to depression, anxiety, or other mental health conditions can be limited by stigma within participants' cultural contexts. In Thailand, adolescents face challenges accessing MBIs due to barriers such as limited mental health services, time constraints, confidentiality concerns, social stigma, high private healthcare costs, and geographical barriers in rural areas.

To bridge these gaps, we have developed an online mindfulness-based intervention for self-esteem improvement group program (MBSI online). It integrates mindfulness aspects related to self-esteem<sup>25</sup> and core principles of Acceptance and Commitment Therapy (ACT) that pertain to self-esteem.<sup>26</sup> We have employed creative and adolescent-friendly strategies while preserving the essential MBI concepts, informed by consultations with mindfulness experts and program trials.

This program is designed to target primary outcomes

related to self-esteem and secondary outcomes linked to mindfulness and resilience, with a specific emphasis on secondary prevention of psychological illnesses. The choice of the program's name is aimed at promoting a positive direction and addressing social stigma concerns within the participants' context. Additionally, the group intervention for adolescents provides them with the opportunity to share their personal experiences and perspectives, contributing to heightened self-awareness.<sup>27,28</sup> The online platform enhances accessibility, especially in remote areas, saving time and costs,<sup>29</sup> while also addressing concerns related to confidentiality and social stigma.

In the present study, we conducted a pilot randomized controlled trial. The primary objective was to develop and assess the efficacy of the MBSI online program in improving self-esteem in adolescents. The secondary objectives included: 1) investigating the effectiveness of the MBSI online program in enhancing mindfulness and resilience in adolescents, and 2) assessing the feasibility and satisfaction of the MBSI online program.

## **MATERIALS AND METHODS**

The pilot study involved a randomized controlled trial conducted in Thailand, encompassing participant recruitment, interventions, and data collection spanning from June 2022 to March 2023. Approval for the study was granted by the Siriraj Institutional Review Board (SIRB), with the assigned COA number Si 369/2022. The study has been reviewed and approved by the Thai Clinical Trial Registry (TCTR) committee. The TCTR identification number is TCTR20230201004.

### **Participant recruitment**

The research team utilized diverse online platforms, including Line and Facebook, for participant recruitment. Interested adolescents accessed informative documents online, providing study details. Following this, informed consent and contact information were collected, and participants completed an online questionnaire evaluating self-esteem levels using the Revised Rosenberg Self-Esteem Scale (RSES-R).<sup>30</sup>

Eligibility criteria included age (15-18 years), RSES-R score  $\leq 30$ , proficiency in Thai, and internet accessibility. Exclusions encompassed moderate to severe intellectual impairment or severe psychological symptoms. Psychiatric history was assessed via telephone interviews. Withdrawal occurred for those attending fewer than six sessions, with participants informed of their right to withdraw at any time.

Eligible applicants received an online parental consent form, requiring approval. An anonymous list

of participant ID numbers, lacking additional data, was generated using nQuery Advisor Software. Random sequences allocated participants equally to Group A or B. Participants were then informed of their assigned group, given a unique five-digit identification code, and provided links for online questionnaire completion.

**Data collection**

Both groups of participants completed a baseline questionnaire, which included demographic characteristics, RSES-R, Philadelphia Mindfulness Scale in Thai Version (PHLMS\_TH), and Resilience Inventory-9 (RI-9) before the commencement of the intervention program (T0). Following the initiation of the intervention program, both groups were required to complete the RSES-R, PHLMS\_TH, and RI-9 at specific time points: at week 4 (T1), immediately after the intervention in week 8 (T2), at a 1-month follow-up (T3), and at a 3-month follow-up (T4). Data collection of the waitlist group aligned with the intervention group’s timeline. Participants who prematurely discontinued the program or were lost to follow-up before week 4, leading to data insufficiency at the T1 time point, were excluded from the modified intention-to-treat analysis (mITT). Additionally, participants in the intervention group were asked to provide feedback on the program immediately upon its completion. To recognize their participation within the program, each participant received a compensation of 100 baht (approximately 3 USD) for every questionnaire completed.

**MBSI online program**

The MBSI online program is an adapted MBI, that incorporates five specific aspects of mindfulness related to self-esteem: describing, acting with awareness, non-judging of inner experiences, nonreactivity to inner experiences, and being present.<sup>25</sup> Additionally, it includes an element of ACT that involves the exploration of values and committed action to enhance self-esteem,<sup>26</sup> as illustrated in Fig 1.

The MBSI online intervention consisted of eight sessions, with each session lasting 120 minutes. An outline of the eight sessions, detailing the activities and main components of the MBSI, is presented in Fig 2.

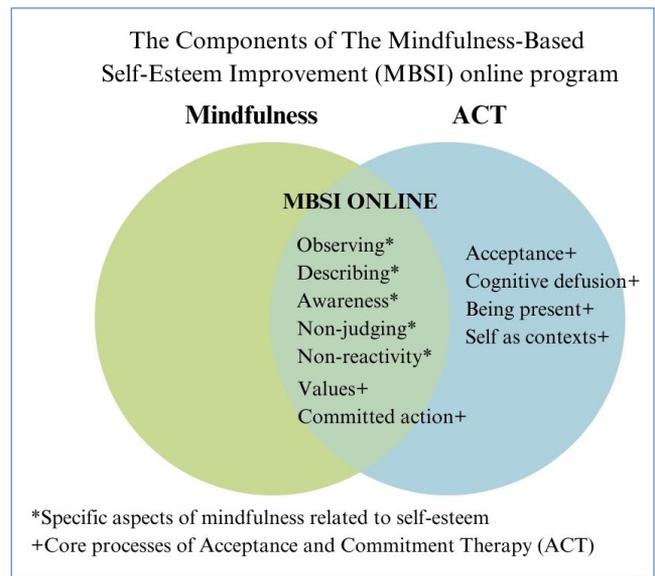


Fig 1. Components of the MBSI Online Program.

8-week MBSI online program								
	Session 1 Say Hi to My Friends	Session 2 Mindful Fitness	Session 3 Emotional Scanner	Session 4 River of Thoughts	Session 5 Mindful Communication	Session 6 Dealing with Negative Emotions	Session 7 Mission to the Moon	Session 8 Extending Your Mindful Skills
<b>Main Activities</b>	- Greeting with friends - Mindfulness insights through movie discussion - Mindfulness fact game - Easy mindfulness practice	- Storytelling awareness game - Listen mindfully to music - Formal sitting meditation and daily life mindfulness	- Guess my emotion game - Body scan - Emotional awareness through your body with 4 music styles - Exploring the nature of emotion	- Watching your river of thoughts - Your inner experiences with thought awareness - Non-judging your judging thoughts - Befriending yourself	- Mindful dialogue - Mindful listening - Mindful sharing of your own experiences - Mindful reflection for your friends	- Presence practice - Mindful breathing and meditation - Body scan relaxation - Self-compassion - Build your own tranquil tempo	- Discovering your inner values - Harnessing character strengths: using and balancing them - Mindful life goal exploration	- SATI reflection hopping: mindfulness concept group sharing - Share your inner change - Build your own plan and commit to it - Gratitude
<b>Main MBSI Components</b>	- Observing - Awareness	- Observing - Awareness - Describing	- Observing - Awareness - Describing - Non-judging - Non-reactivity	- Observing - Awareness - Describing - Non-judging - Non-reactivity	- Awareness - Describing - Non-judging - Values	- Awareness - Describing - Non-judging - Non-reactivity	- Observing - Awareness - Describing - Values - Committed action	- Awareness - Describing - Values - Committed action

Fig 2. An outline of the eight sessions of the MBSI online program.

The MBSI online program was designed with the aim of seamlessly integrating into participants' daily lives. It incorporated group-based activities that encouraged sharing of experiences from diverse perspectives, thereby promoting interpersonal skills. Mindfulness exercises were a key component of each session, conducted in both the whole group and small groups comprising 6-7 participants. During each session, participants were encouraged to practice a 10-minute homework assignment every day (mindfulness in daily activities) and to share their practicing experiences with their group at the beginning of the next session.

In the MBSI online program, facilitators possessed advanced expertise in mindfulness practice, supervised by two advisory professors specializing in mindfulness training. The primary facilitator, boasting four years of mindfulness experience, completed courses in Thailand, including the Human Work Course. This course introduced a meditation technique involving hand movements or walking while attentively observing body movements, thoughts, or emotions with kindness and a nonjudgmental attitude. The facilitator also participated in various workshops such as Tender Heart Meditation, Maitri Meditation, Buddhist Psychotherapy, and training sessions for enhancing participatory learning processes like Semsikkhalai's Training of the Trainer. Notably, the main facilitators did not undergo formal training in any mindfulness-based approaches. Additionally, the MBSI online intervention featured a meticulously organized "MBSI online program manual", ensuring consistency across subgroups.

## Procedure

In the intervention group, participants received an 8-week MBSI online program via the Zoom platform. Conversely, individuals in the control group were assigned to a waiting list and received the intervention only after completing a 3-month follow-up questionnaire. It is noteworthy that blinding was not implemented; both participants and therapists were aware of the interventions.

## Outcomes

### Primary outcome

The Thai Version of the Revised Rosenberg Self-Esteem Assessment (RSES-R)<sup>30</sup> is a 10-item self-report questionnaire designed to assess self-esteem within the Thai cultural context. This version is a translation derived from Morris Rosenberg's original Rosenberg Self-Esteem Scale (RSES).<sup>1</sup> Responses are rated on a scale from 'Strongly Agree' to 'Strongly Disagree,' with positively phrased items scored from 4 to 1 and negatively

phrased items from 1 to 4. Scores on this assessment range from 10 to 40, categorizing self-esteem levels as High (31 - 40 points), Moderate (21 - 30 points), and Low (10 - 20 points). The assessment demonstrates good internal consistency, with a Cronbach's alpha coefficient of 0.84.

### Secondary outcome

The Philadelphia Mindfulness Scale in Thai Version (PHLMS\_TH)<sup>31</sup> is a 20-item self-report tool with 5 response options. It assesses mindfulness, having been translated into Thai from the original PHLMS. The internal consistency, evaluated by Cronbach's alpha coefficients, is 0.87 for awareness and 0.88 for acceptance.

The Resilience Inventory-9 (RI-9),<sup>32</sup> is a 9-item self-report questionnaire with 5 response categories, assessing resilience. It yields scores ranging from 9 to 45, where higher scores signify increased resilience and adaptability. The assessment demonstrates strong internal consistency (Cronbach's alpha = 0.86).

Feasibility evaluation was conducted through the development of a program feedback questionnaire. The assessment encompassed five dimensions of program satisfaction: usefulness of the intervention, user-friendliness, homework satisfaction, facilitator satisfaction, and online platform satisfaction. These dimensions were measured using a 5-point Likert scale, ranging from 1 (very dissatisfied) to 5 (very satisfied).

## Statistical Analysis

The sample size estimation is based on the rules of thumb for pilot trial sample sizes,<sup>33</sup> suggesting a range of 10-75 participants per arm. Anticipating 25 participants per arm and accounting for a 30% dropout rate,<sup>34</sup> we plan to divide the study population into small groups, each comprising 6-7 participants. Therefore, we have estimated a total sample size of 70 participants, with randomization resulting in an equal distribution of 35 participants per arm.

Baseline characteristics were compared between groups using appropriate statistical tests, including Pearson's chi-square test, Fisher's exact test, or Linear-by-Linear Association for categorical variables. The independent t-test was utilized for normally distributed continuous variables, while the Mann-Whitney U test was applied to non-normally distributed continuous variables.

For the within-group analyses, we employed repeated-measures ANOVA to examine differences in mean scores of RSES-R, PHLMS\_TH, and RI-9 across time within each group. Analyses were conducted at five time points (T0-T4) for both group A and B. The Group x Time

interaction for RSES-R, PHLMS\_TH, and RI-9 mean scores was explored between groups A and B during T0–T4.

We conducted a modified intention-to-treat analysis (mITT),<sup>35</sup> excluding participants with insufficient data at the T1 time point. The mITT analysis was performed for both within-group and between-group comparisons of mean scores on the RSES-R, PHLMS\_TH, and RI-9. Between-group analyses were conducted at various time points: T1, T2, T3, and T4 for the intervention and waiting list control groups. To address missing data in follow-up assessments, we applied Last Observation Carried Forward (LOCF)<sup>36</sup> in this study. Statistical significance was set at a p-value threshold of < 0.05. Cohen's d statistic<sup>37</sup> was employed to calculate the effect size, with interpretation categorizing effect sizes as small (d = 0.2), medium (d = 0.5), and large (d = 0.8), respectively.

## RESULTS

A total of 329 individuals from diverse regions of Thailand underwent initial eligibility assessment as depicted in Fig 2.

Among them, 259 were excluded for reasons including failure to meet inclusion criteria due to high RSES-R score (n=238), participation refusal (n=9), and severe psychological illness (n=12). The remaining 70 participants were randomly assigned to either the intervention group (n=35) or the waiting list control group (n=35).

However, six participants from the intervention group and one participant from the control group, who prematurely discontinued the program exhibiting data insufficiency at the T1 time point, were excluded. As a result, a final analyzed cohort comprised 29 participants in the intervention group and 34 participants in the control group for the modified intention-to-treat (mITT) analysis.

A comparative analysis between intervention and waiting list control groups revealed no significant differences in baseline self-esteem, mindfulness, resilience, and characteristics including age, gender, religion, chronic illness, substance use, family household, socioeconomic status, relational support, and prior meditation experience, as depicted in Table 1.

The dropout rate was 25.7% in the intervention group according to prematurely discontinued intervention due to meet withdrawal criteria before 4<sup>th</sup> session (n=4), unable to contact (n=4), Internet problem (n=1), as illustrated in Fig 3.

### Primary outcome

The self-esteem was compared within-group and

between-group using mITT analysis. For the within-group analysis in the intervention group, self-esteem (RSES-R) scores significantly improved over time (p < 0.001). A significant change from baseline was observed from week 4 to the 3-month follow-up. In contrast, the control group demonstrated no statistically significant within-group improvement in self-esteem scores.

The between-group analysis for the intervention group compared to the control group revealed notable changes in self-esteem (RSES-R) scores, indicated by statistically significant differences at week 8 (p < 0.001), the 1-month follow-up (p < 0.001), and the 3-month follow-up (p < 0.001), with large effect sizes for all three time points (d = 1.00, 0.96, 0.96, respectively). There were differences in the outcomes between the two groups at T1, T2, T3, and T4 for the direction and size of outcome differences; see Table 2 and Fig 4.

### Secondary outcomes

In the intervention group, there were significant improvements over time in mindfulness (PHLMS\_TH) and resilience (RI-9) scores (p = 0.001 and p < 0.001, respectively) during the within-group analysis. Notably, a significant change from baseline was evident starting from week 4 and persisted through the 3-month follow-up. Conversely, the control group exhibited no statistically significant within-group enhancements in overall mindfulness and resilience scores.

The analysis comparing the intervention group to the control group unveiled significant alterations in mindfulness (PHLMS\_TH) and resilience (RI-9) scores. These changes were evident at week 8, the 1-month follow-up, and the 3-month follow-up, demonstrating medium to large effect sizes. Noteworthy differences between the two groups were observed at T1, T2, T3, and T4 in terms of the direction and magnitude of outcome variances; see Table 2 and Fig 4.

### Program feedback

The feedback scores for the MBSI online program are summarized in Table 3. Facilitator satisfaction was notably high, with a mean score of 4.92, and a substantial majority (92.3%) of participants expressing complete satisfaction (score 5). Similarly, satisfaction with the online platform and overall program satisfaction garnered mean scores of 4.73, with 76.9% of participants indicating the highest level of satisfaction.

## DISCUSSION

The present study aimed to develop and evaluate the efficacy of the 8-week MBSI online program in enhancing

**TABLE 1.** Baseline demographic and characteristics of participants.

Baseline Characteristics	Intervention Group (n=29) Mean ± SD n (%)	Waiting List Control Group (n=34) Mean ± SD n (%)	p
Age, year	16.48 ± 0.91	16.12 ± 0.73	0.082
Range	15-18	15-18	
Female	19 (65.5)	22 (64.7)	0.946
Religion			0.327
Buddhist	24 (82.8)	32 (90.9)	
Christian	1 (3.4)	0 (0)	
Islamic	2 (6.9)	0 (0)	
Other/None	2 (6.8)	5 (8.1)	
Chronic Illness			0.339
None	16 (59.3)	23 (69.7)	
Physical Illness	6 (22.2)	8 (24.2)	
Mental Illness	5 (18.5)	2 (6.1)	
Substance Use			0.453
Never Used	24 (82.8)	31 (91.2)	
Used Before	5 (17.2)	3 (8.8)	
Family Member			1.000
Both Parents	16 (55.2)	20 (58.8)	
Either Parent	7 (24.1)	8 (23.5)	
Other Relatives	6 (20.7)	6 (17.6)	
Socioeconomic Status			0.534
<550 US/month	7 (24.1)	2 (5.9)	
550-850 US/month	8 (27.6)	18 (52.9)	
>850 US/month	14 (48.3)	14 (41.2)	
Relational support			0.741
No	4 (13.8)	6 (17.6)	
Yes	25 (86.2)	28 (82.4)	
Prior Meditation Experience			0.299
Never	25 (86.2)	25 (75.8)	
Yes	4 (13.8)	8 (24.2)	
Baseline Outcome Scores			
RSES-R	23.7 ± 4.2	25.3 ± 4.1	0.118
PHLMS_TH	55.0 ± 6.7	57.2 ± 5.4	0.136
RI-9	28.3 ± 5.2	28.2 ± 7.2	0.946

**Abbreviations:** RSES-R, Thai Version of the Revised Rosenberg Self-Esteem Assessment; PHLMS\_TH, Philadelphia Mindfulness Scale in Thai Version; RI-9, Resilience Inventory-9.

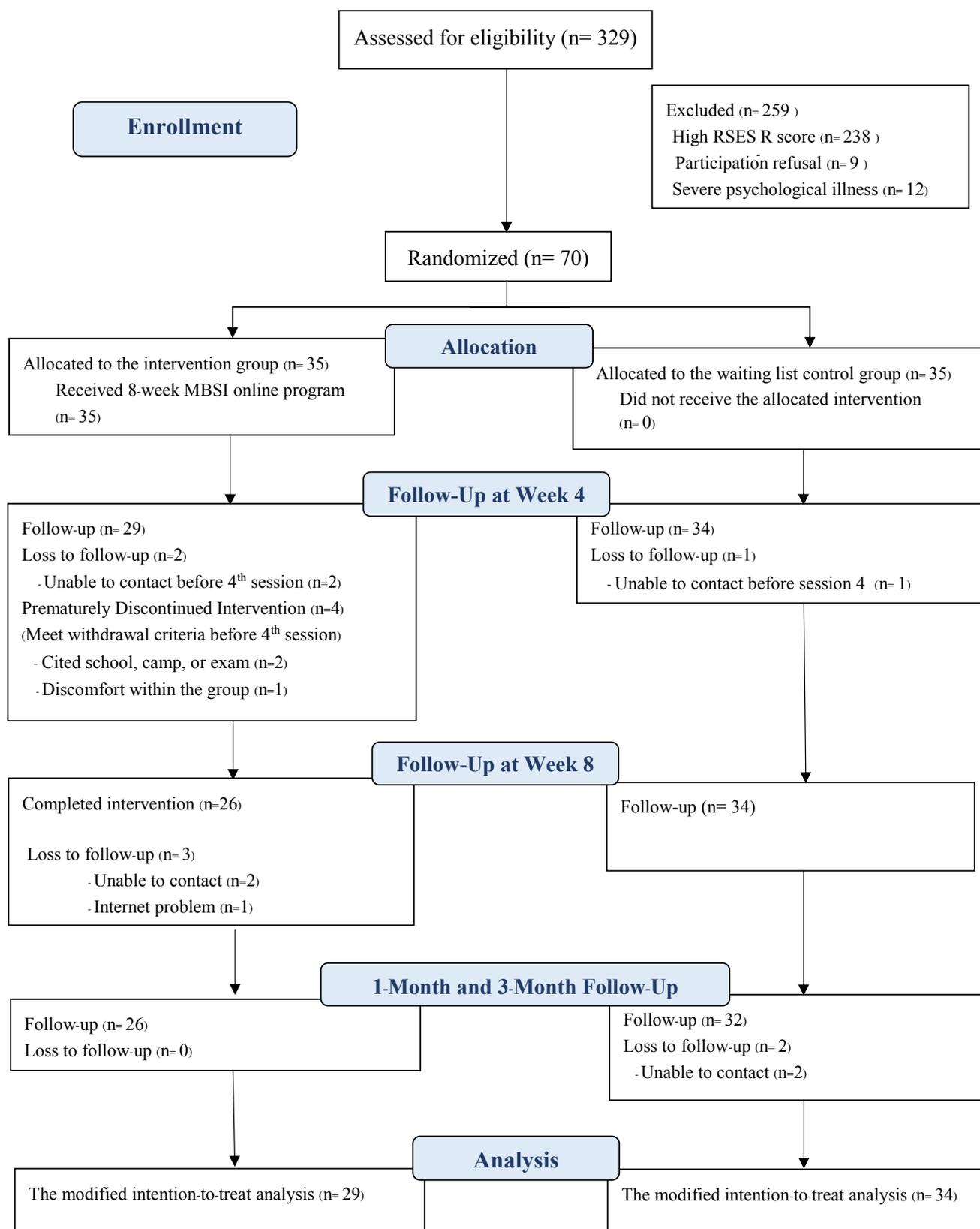
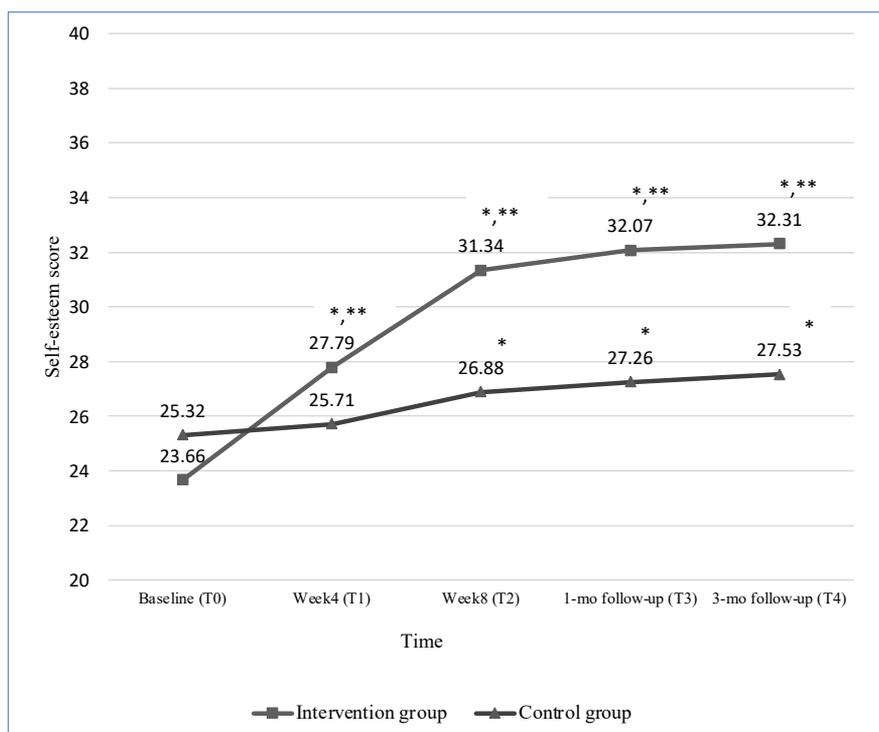


Fig 3. CONSORT flow diagram.

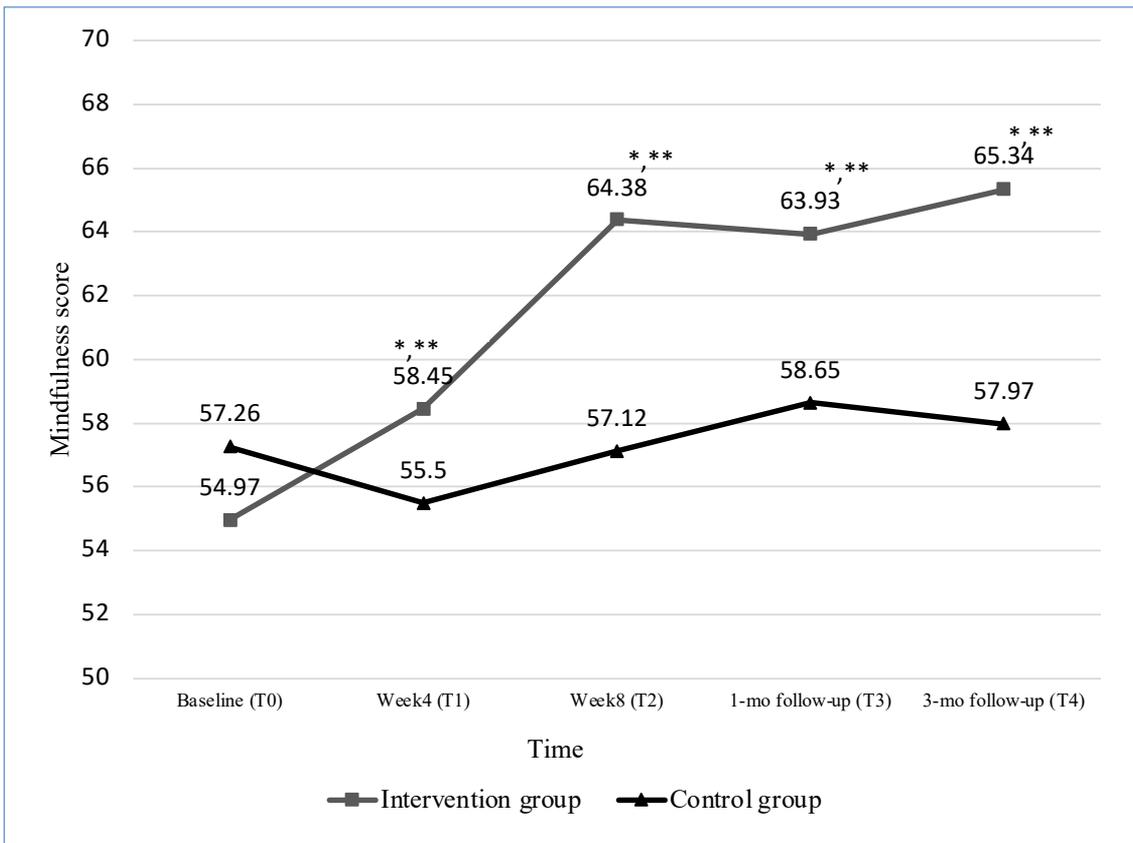
**TABLE 2.** Within-Group and Between-Group Outcomes at Baseline (T0), Week 4 (T1), Week 8 (T2), 1-Month Follow-Up (T3), and 3-Month Follow-Up (T4).

Outcomes	Time point	Intervention group (n=29) Mean ± SD	Waiting list control group (n=34) Mean ± SD	p	d
RSES-R	Time x Group			<0.001	
	Baseline (T0)	23.66 ± 4.24	25.32 ± 4.10	0.118	0.40
	Week 4 (T1)	27.79 ± 4.63 <sup>a</sup>	25.71 ± 4.81	0.086	0.44
	Week 8 (T2)	31.34 ± 4.17 <sup>a</sup>	26.88 ± 4.73	<0.001	1.00
	1-month follow-up (T3)	32.07 ± 4.18 <sup>a</sup>	27.26 ± 5.73	<0.001	0.96
	3-month follow-up (T4)	32.31 ± 4.42 <sup>a</sup>	27.53 ± 5.48	<0.001	0.96
	p (Within-group changes over)	<0.001	0.084		
PHLMS_TH	Time x Group			<0.001	
	Baseline (T0)	54.97 ± 6.71	57.26 ± 5.37	0.136	0.38
	Week 4 (T1)	58.45 ± 8.21 <sup>a</sup>	55.50 ± 7.51	0.142	0.37
	Week 8 (T2)	64.38 ± 10.73 <sup>a</sup>	57.12 ± 7.90	0.003	0.77
	1-month follow-up (T3)	63.93 ± 10.52 <sup>a</sup>	58.65 ± 7.84	0.026	0.57
	3-month follow-up (T4)	65.34 ± 10.31 <sup>a</sup>	57.97 ± 6.87	0.001	0.84
	p (Within-group changes over time)	0.001	0.061		
RI-9	Time x Group			<0.001	
	Baseline (T0)	28.34 ± 5.16	28.24 ± 7.18	0.946	0.02
	Week 4 (T1)	31.97 ± 5.52 <sup>a</sup>	28.85 ± 7.62	0.073	0.47
	Week 8 (T2)	35.21 ± 5.80 <sup>a</sup>	30.06 ± 7.16	0.003	0.79
	1-month follow-up (T3)	36.07 ± 6.63 <sup>a</sup>	29.29 ± 7.80	<0.001	0.94
	3-month follow-up (T4)	36.62 ± 6.04 <sup>a</sup>	29.59 ± 8.63	<0.001	0.94
	p (Within-group changes over time)	<0.001	0.522		

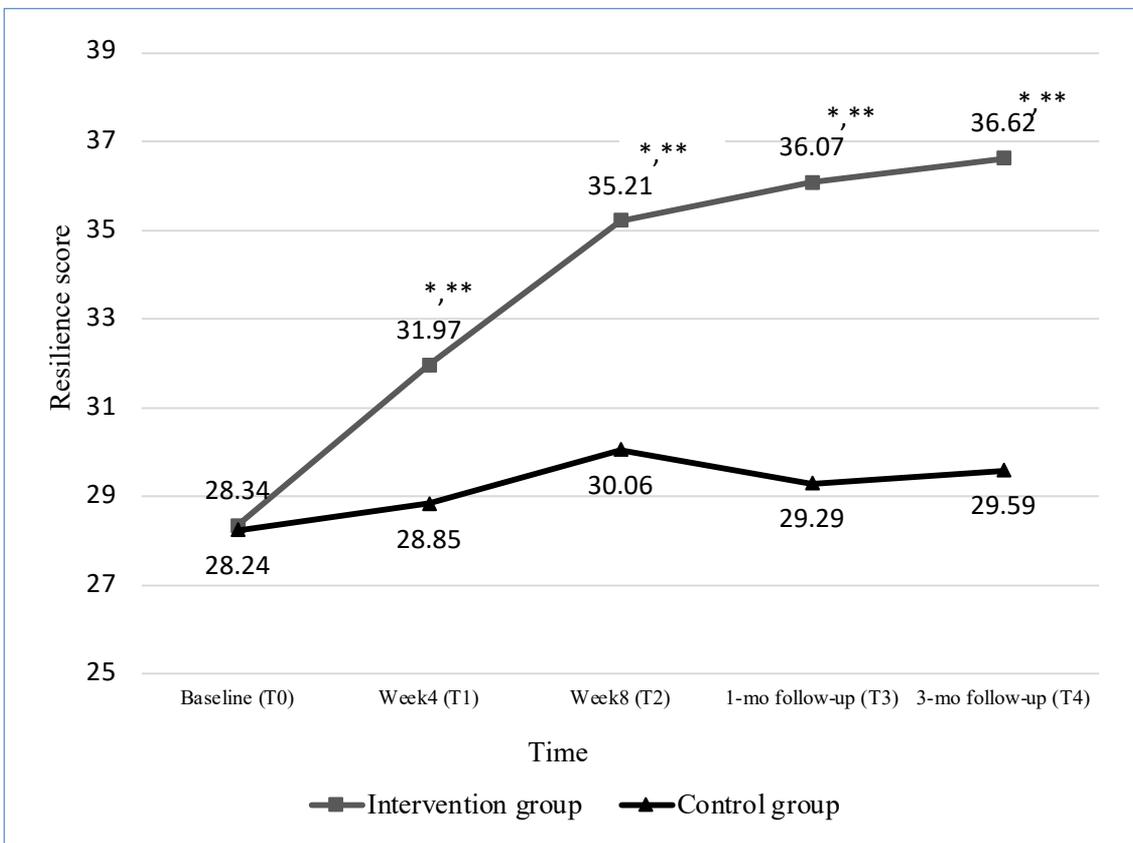
Note. a significant change from baseline P < 0.05



**Fig 4.** Within-group changes over time and between-group outcomes of self-esteem.  
Note. \* P<0.05 (within-group analysis), \*\* P < 0.05 (between group analysis adjusted for baseline characteristic)



**Fig 5.** Within-group changes over time and between-group outcomes of mindfulness.  
 Note. \* P<0.05 (within-group analysis), \*\* P < 0.05 (between group analysis adjusted for baseline characteristic)



**Fig 6.** Within-group changes over time and between-group outcomes of resilience.  
 Note. \* P<0.05 (within-group analysis), \*\* P < 0.05 (between group analysis adjusted for baseline characteristic)

**TABLE 3.** MBSI online program feedback (N=26).

Aspect	Mean score ± SD	Score 5, n (%)	Score 4, n (%)	Score 3, n (%)	Score 2, n (%)	Score 1, n (%)
Usefulness of program	4.58 ± 0.64	17 (65.4)	7 (26.9)	2 (7.7)	0 (0)	0 (0)
User-friendliness of program	4.73 ± 0.53	20 (76.9)	5 (19.2)	1 (3.8)	0 (0)	0 (0)
Homework satisfaction	4.50 ± 0.65	15 (57.7)	9 (34.6)	2 (7.7)	0 (0)	0 (0)
Facilitator satisfaction	4.92 ± 0.27	24 (92.3)	2 (7.7)	0 (0)	0 (0)	0 (0)
Online platform satisfaction	4.73 ± 0.53	20 (76.9)	5 (19.2)	1 (3.8)	0 (0)	0 (0)
Overall program satisfaction	4.73 ± 0.53	20 (76.9)	5 (19.2)	1 (3.8)	0 (0)	0 (0)

**Note:** Scores range from 1 (very dissatisfied) to 5 (very satisfied)

self-esteem, mindfulness, and resilience among low-to-moderate self-esteem adolescents, while also assessing the feasibility of the MBSI online program. Key findings revealed significant improvements in self-esteem, mindfulness, and resilience within the intervention group over time. Between-group analysis demonstrated notable changes at week 8, 1-month follow-up, and 3-month follow-up, with medium to large effect sizes post-intervention and sustained effects at the 3-month follow-up. Differences in outcomes between groups were observed at T1, T2, T3, and T4 regarding the direction and size of outcome differences.

Our findings were congruent with prior research that demonstrated the effectiveness of mindfulness-based interventions in enhancing various psychological aspects including subjective well-being, self-esteem and perceived stress levels among adolescents.<sup>10,38</sup> A previous systematic review<sup>13</sup> of 17 studies showed the majority of studies investigating the impact of MBIs on self-esteem in adolescents reported significant increases in self-esteem.

Multiple strengths of present study were identified adding up to current MBIs research field. First, the study population targeted low to moderate self-esteem adolescents. Since adolescents struggling with diminished self-esteem are at heightened risk for internalizing symptoms like depressive mood, somatic complaints, and anxiety, the MBSI online program was designed to find effective strategies for preventing negative consequences.

Second, the efficacy of the MBSI online program in improving self-esteem demonstrated large effect size with long term effect to the 3-month follow-up. This program enabling practical integration into adolescents' daily lives and the activities were designed specifically for adolescents appeared to resonate well with adolescents'

lifestyles, leading to increased program efficacy. When compared to previous studies,<sup>19,20</sup> it was found that the MBSI online program has better efficacy than other online interventions. In MBSI online, group-based training with the use of consistent subgroups encouraged participants to openly share and maintain group dynamics throughout the training. Co-facilitators within these subgroups ensured precise guidance and consistent practice for each participant. Third, in relation to the secondary outcomes, the intervention demonstrated a statistically significant enhancement in both mindfulness and resilience among the participants. Developing mindfulness and resilience in adolescents is essential for their well-being. Mindfulness helps individuals become more self-aware, attentive and focused on the present, which can enhance their ability to cope with stress and challenges.<sup>39</sup> Resilience enables adolescents to persevere, adapt, and rise to the occasion when faced with difficult circumstances.<sup>40</sup> Our study revealed substantial and lasting improvements in mindfulness and resilience, with medium to high effect sizes immediately post-intervention and persisting at the 3-month follow-up. These findings underscore the efficacy of the MBSI online program in fostering enduring and positive impacts in these vital domains.

Fourth, the MBSI online was an efficient innovative approach facilitated through an online platform which enabled participants from diverse geographical regions to access the intervention and made it a cost-effective approach.

The feasibility of the current study, as assessed by the dropout rate and satisfaction rate, offers insight into the practicality and acceptability of the intervention. The intervention group exhibited a dropout rate of 25.7% aligning with the range reported in a systematic review,<sup>34</sup>

which indicated dropout rates between 16% and 29% for mindfulness-based interventions (MBIs). However, the observed dropout rate raises concerns about potential bias and underscores the need for strategies that promote adherence, especially within online platforms. In terms of program satisfaction, the MBSI online program received consistently high participant satisfaction, as indicated by positive mean scores (ranging from 4.58 to 4.92) and substantial percentages reporting top-level contentment (ranging from 65.4% to 92.3%). These results affirm participants' favorable perceptions, serving as a testament to the program's success in meeting their needs and fostering a constructive learning environment.

The study's limitations necessitate ongoing scrutiny. Firstly, the relatively small sample size of 70 participants may constrain the generalizability of findings to a broader population, affecting the statistical power of analyses. Secondly, the absence of blinding introduces the possibility of biases in reporting and implementation, potentially influencing participant responses. Thirdly, relying on self-report measures for outcomes like self-esteem, mindfulness, and resilience introduces the potential for social desirability bias and measurement inaccuracies. Fourthly, the mITT analysis may be biased due to missing outcome data. Fifthly, there was no analysis conducted on the homework assignment to determine whether it is a factor influencing the program's effectiveness. Lastly, the study's geographical restriction to Thailand may limit the generalizability of findings to other cultural contexts.

However, this research holds significant implications for medical practice, public health, and research implementation, particularly in the realm of addressing low self-esteem among adolescents. Clinicians working with adolescents experiencing low self-esteem can utilize the MBSI online program as an effective tool to support their mental well-being and proactively prevent psychological illnesses. The program's accessibility through online platforms further enhances its reach and impact. By providing evidence of the positive impact of the MBSI program on self-esteem, mindfulness, and resilience, this research serves as a foundation for future studies and the development of similar programs tailored to specific needs.

Future research endeavors should strive to involve larger and more diverse samples, with efforts focused on reducing attrition rates. Exploring double-blinded and active control groups, incorporating objective measures, conducting cross-cultural validation, monitoring and analyzing the effectiveness of homework assignments, and extending follow-up periods would collectively enhance the comprehensiveness of our understanding regarding

the program's efficacy and its potential implications for the well-being of adolescents.

## CONCLUSION

The pilot randomized controlled trial demonstrated the efficacy of the MBSI online program in enhancing self-esteem, mindfulness, and resilience among low self-esteem adolescents, with sustained effects at the 1-month and 3-month follow-ups. The program's innovative combination of MBIs and ACT elements included group-based activities facilitated through the online platform and tailored its design specifically for adolescents. The positive program feedback and high satisfaction ratings underscored the feasibility and acceptability of the MBSI online program. To validate these findings and establish its broader efficacy and applicability, future research should include larger, diverse samples, minimize dropouts, explore various control groups, use objective measures, cross-cultural validation, and extend follow-up periods.

## ACKNOWLEDGEMENT

The authors thank Mr. Suthipol Udompunthurak, Department of Health Research and Development, Faculty of Medicine, Siriraj Hospital, Mahidol University for assistance with the research analysis.

This research was funded by the research department, Faculty of Medicine Siriraj Hospital, under Grant Agreement No R016531069 (Fund 3). This grant provided financial support for survey participant compensation and assistant lecturer remuneration in accordance with expense reimbursement guidelines.

## REFERENCES

1. Rosenberg M. Rosenberg self-esteem scale. *J Relig Health*. 1965.
2. Vaughan-Johnston TI, Lambe L, Craig W, Jacobson JA. Self-esteem importance beliefs: A new perspective on adolescent self-esteem. *Self Identity*. 2020;19(8):967-88.
3. Abdel-Khalek AM. Introduction to the psychology of self-esteem. In: Holloway F, ed. *Self-esteem: perspectives, influences, and improvement strategies*. 1st ed. Nova Science Publisher; 2016.p.1-23.
4. Ngo H, VanderLaan DP, Aitken M. Self-esteem, symptom severity, and treatment response in adolescents with internalizing problems. *J Affect Disord*. 2020;273:183-91.
5. Schoeps K, Tamarit A, Zegarra SP, Montoya-Castilla I. The long-term effects of emotional competencies and self-esteem on adolescents' internalizing symptoms. *Rev de Psicodidactica (Engl Ed)*. 2021;26(2):113-22.
6. Hirsch BJ, DuBois DL. Self-esteem in early adolescence: The identification and prediction of contrasting longitudinal trajectories. *J Youth Adolesc*. 1991;20(1):53-72.
7. Rakpuangchon W, Wisarapun N, Sangsawang N, Sangsawans B, Boondaulyan S, Boonperm P, et al. Factors Influencing

- Self-Esteem Among Adolescents during the COVID-19 Pandemic. *Nursing J CMU*. 2023;50(1):314-28.
8. Lukseng T, Siripornpanich V, Chutabhakdikul N. Long-Term Vipassana Meditation Enhances Executive Function in Adult Meditators. *Siriraj Med J*. 2020;72(4):343-51.
  9. Biegel GM, Brown KW, Shapiro SL, Schubert CM. Mindfulness-based stress reduction for the treatment of adolescent psychiatric outpatients: A randomized clinical trial. *J Consult Clin Psychol*. 2009;77(5):855.
  10. Primasari A, Yuniarti KW. Enjoying Every Moment: Improving Adolescent's Subjective Well-Being Through Adolescent Mindfulness Program. *GamaJPP*. 2021;7(2):115-28.
  11. Zhang A, Zhang Q. How could mindfulness-based intervention reduce aggression in adolescent? Mindfulness, emotion dysregulation and self-control as mediators. *Curr Psychol*. 2023;42(6):4483-97.
  12. Siffredi V, Liverani MC, Hüppi PS, Freitas L, De Albuquerque J, Gimbert F, et al. Mindfulness-based intervention for very preterm young adolescents: An RCT. *MedRxiv*. 2021.
  13. Randal C, Pratt D, Bucci S. Mindfulness and self-esteem: a systematic review. *Mindfulness*. 2015;6:1366-78.
  14. Emavardhana T, Tori CD. Changes in self-concept, ego defense mechanisms, and religiosity following seven-day Vipassana meditation retreats. *J Sci Study Relig*. 1997;36(2):194-206.
  15. Juengsiragulwit D, Thongthammarat Y, Praneetpolgrung P, Choompudsa P, Tantipiwattanasakul P. The efficacy of group mindfulness-based cognitive therapy in prevention of youth depression; a pilot study. *J Ment Health Thai*. 2015;23(3):143-53.
  16. Osborn TL, Wasil AR, Venturo-Conerly KE, Schleider JL, Weisz JR. Group intervention for adolescent anxiety and depression: outcomes of a randomized trial with adolescents in Kenya. *Behav Ther*. 2020;51(4):601-15.
  17. Dunning DL, Griffiths K, Kuyken W, Crane C, Foulkes L, Parker J, et al. Research Review: The effects of mindfulness-based interventions on cognition and mental health in children and adolescents—a meta-analysis of randomized controlled trials. *J Child Psychol Psychiatry*. 2019;60(3):244-58.
  18. White LS. Reducing stress in school-age girls through mindful yoga. *J Pediatr Health Care*. 2012;26(1):45-56.
  19. Chancey JB, Heddy BC, Lippmann M, Abraham E. Using an Online-Based Mindfulness Intervention to Reduce Test Anxiety in Physics Students. *J Cogn Enhanc*. 2023:1-12.
  20. Ierfino DJ. An initial evaluation of an online compassion focused therapy intervention for self-esteem: Canterbury Christ Church University (United Kingdom); 2017.
  21. Kabat-Zinn J. An outpatient program in behavioural medicine for chronic pain patients based on the practice of mindfulness meditation: theoretical considerations and preliminary results. *Gen Hosp Psychiatry*. 1982;4:33-47.
  22. Morgan D. Mindfulness-based cognitive therapy for depression: A new approach to preventing relapse. Taylor & Francis; 2003.
  23. Linehan MM. Cognitive-behavioral treatment of borderline personality disorder: Guilford Publications; 2018.
  24. Hayes S, Strosahl K, Wilson K. Acceptance and commitment therapy: An experiential approach to behaviour change. New York, NY: Guilford Press; 1999.
  25. Pepping CA, O'Donovan A, Davis PJ. The positive effects of mindfulness on self-esteem. *J Posit Psychol*. 2013;8(5):376-86.
  26. Hayes SC, Strosahl KD, Wilson KG. Acceptance and commitment therapy: The process and practice of mindful change: Guilford press; 2011.
  27. Anaby D. Towards a new generation of participation-based interventions for adolescents with disabilities: the impact of the environment and the need for individual-based designs. *Dev Med Child Neurol*. 2018;60(8):735-6.
  28. Visser M, Du Plessis J. An expressive art group intervention for sexually abused adolescent females. *J Child Adolesc Ment Health*. 2015;27(3):199-213.
  29. Spijkerman M, Pots WTM, Bohlmeijer E. Effectiveness of online mindfulness-based interventions in improving mental health: A review and meta-analysis of randomised controlled trials. *Clin Psychol Rev*. 2016;45:102-14.
  30. Tinakon W, Nahathai W. A comparison of reliability and construct validity between the original and revised versions of the Rosenberg Self-Esteem Scale. *Psychiatry Investig*. 2012;9(1):54.
  31. Silpakit C, Silpakit O, Wisajun P. The validity of Philadelphia mindfulness scale Thai version. *J Ment Health Thai*. 2011;19(3):140-7.
  32. Wongpakaran T. Resilient Inventory-9 (RI-9) Psychotherapy Unit and Geriatric Unit 2020 [Available from: <http://www.pakaranhome.com/index.php?lay=show&ac=article&Id=2147602325>].
  33. Whitehead AL, Julious SA, Cooper CL, Campbell MJ. Estimating the sample size for a pilot randomised trial to minimise the overall trial sample size for the external pilot and main trial for a continuous outcome variable. *Stat Methods Med Res*. 2016; 25(3):1057-73.
  34. Kukucska D, Whitehall J, Shorter GW, Howlett N, Wyld K, Chater AM. A systematic review of Positive Psychology Interventions (PPIs) to improve the health behaviours, psychological wellbeing and/or physical health of police staff. *J Police Crim Psychol*. 2023;38:728-42.
  35. Kahan BC, White IR, Edwards M, Harhay MO. Using modified intention-to-treat as a principal stratum estimator for failure to initiate treatment. *Clin Trials*. 2023;20(3):269-75.
  36. Lachin JM. Fallacies of last observation carried forward analyses. *Clin Trials*. 2016;13(2):161-8.
  37. Lakens D. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. *Front Psychol*. 2013;4:863.
  38. Eva AL, Thayer NM. Learning to BREATHE: A pilot study of a mindfulness-based intervention to support marginalized youth. *J Evid Based Complementary Altern Med*. 2017;22(4):580-91.
  39. Hosseinian S, Nooripour R. Effectiveness of mindfulness-based intervention on risky behaviors, resilience, and distress tolerance in adolescents. *Int J High Risk Behav Addict*. 2019;8(4):e39481.
  40. Dwidiyanti M, Wijayanti DY, Munif B, Fahmi Pamungkas AY. Increasing Adolescents' Religiosity and Resilience through Islamic Spiritual Mindfulness. *Gac Med Caracas*. 2022;130:S206.

# Retrospective Analysis of Inpatient Dermatologic Consultations in a Residency Training Program

Pantaree Kobkurkul, M.D., Chanakarn Pisankikitti, B.Sc., Jidapa Rueangkaew, B.Sc., Nattha Angkoolpakdeekul, M.D., Supenya Varothai, M.D., Sumanas Bunyaratavej, M.D., Narumol Silpa-archa, M.D.

Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.

## ABSTRACT

**Objective:** This study assessed the prevalence and clinical characteristics of inpatient dermatologic diseases, examined trends over 3 academic years in a tertiary care hospital in Thailand, and evaluated their relevance to the current dermatology residency curriculum.

**Materials and Methods:** A retrospective review was performed at the Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. Consultation records from July 2018 to June 2021 were assessed. Data extracted included patient age, sex, referring departments, and diagnoses.

**Results:** Of the 1964 consultations, 2002 diagnoses were identified. Consistent with previous findings, the predominant diagnostic categories were drug eruptions (28.02%; 561), eczema (16.18%; 324), and viral infections (9.29%; 186). Internal medicine made the most requests, followed by surgery and orthopedics. While the prevalence of consulted diseases remained constant over the 3 academic years, the total number of consultations increased. Most of the consulted conditions were already covered in the “must-know” section of the dermatology residency curriculum, with a few exceptions. The consultation cases satisfied the inpatient evaluation requirements of Entrustable Professional Activity.

**Conclusion:** The prevalence of inpatient dermatologic diseases was highest for drug eruptions, followed by eczema and viral infections. The consistent trend in the prevalence of these consulted diseases underscores the significance of inpatient dermatology. Incorporating these insights into revisions of the dermatology residency curriculum may enhance the training of dermatologists.

**Keywords:** Dermatologic consultation; Dermatology; Inpatient dermatology; Residency training (Siriraj Med J 2024; 76: 52-60)

## INTRODUCTION

Dermatology predominantly operates within an outpatient context.<sup>1</sup> Common outpatient conditions such as eczema, acne, pigmentary disorders, and alopecia are typically neither urgent nor life-threatening. Conversely, inpatient dermatologic cases are typically more distinct and intricate than their outpatient counterparts.<sup>2</sup> Dermatologic problems may either be the primary cause of a patient’s hospitalization or subsequently emerge during admission for other conditions.<sup>3</sup> Several

inpatient dermatologic conditions demand immediate intervention, for example, severe cutaneous adverse drug reactions, vesiculobullous diseases, and generalized pustular psoriasis. Their impact often extends beyond the skin, with some complex cutaneous consultations substantially elevating morbidity and mortality rates.<sup>3,4</sup> Thus, inpatient dermatologic conditions merit equal, if not greater, emphasis than the dermatoses typically encountered in outpatient settings.

Corresponding author: Narumol Silpa-archa

E-mail: doctornarumol@gmail.com

Received 18 November 2023 Revise 20 December 2023 Accepted 30 December 2023

ORCID ID: <http://orcid.org/0000-0002-1678-5442>

<https://doi.org/10.33192/smj.v76i2.266387>



All material is licensed under terms of the Creative Commons Attribution 4.0 International (CC-BY-NC-ND 4.0) license unless otherwise stated.

Dermatologists are instrumental in diagnosing and managing inpatient cutaneous manifestations. Evidence from prior research indicates that inpatient dermatologic consultations significantly refine diagnostic accuracy and improve both clinical and economic outcomes.<sup>2-6</sup> Without these consultations, incorrect diagnoses and treatments would escalate patient morbidity and mortality.<sup>3,4</sup> Therefore, dermatologists must be well versed in the characteristics of inpatient consultations. While the literature offers insights into inpatient dermatologic consultations in certain countries,<sup>1,3,4,7-12</sup> data for Thailand remain sparse.

This research endeavored to establish the prevalence, clinical features, and trends of inpatient dermatologic consultations within a tertiary care setting in Thailand. These insights will ascertain whether the current dermatology residency curriculum aligns with the afflictions observed among inpatients.

## MATERIALS AND METHODS

We undertook a retrospective study at the Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. The Siriraj Institutional Review Board granted study approval (COA no. Si 559/2022). We included patients aged 18 years and above with dermatologic consultation records between July 2018 and June 2021. We extracted data on age, sex, referring departments, and diagnostic outcomes. Three dermatologists from the dermatology department (N.S., S.V., S.B.) categorized these diagnoses into 22 groups. We subsequently analyzed the prevalence of each diagnostic group and discerned consultation trends.

### Statistical analysis

Categorical data, encompassing sex, consulting departments, and the prevalence of each diagnostic group, were tabulated as numbers and percentages. Continuous data were evaluated using means and standard deviations. Data analyses were executed with PASW Statistics, version 18 (SPSS Inc, Chicago, IL, USA).

## RESULTS

Over the 3 years from July 2018 to June 2021, 1964 consultation cases were documented. The mean age of the patients was  $57.6 \pm 19.1$  years, with males comprising 50.05% of the cohort. The internal medicine department initiated approximately half of the consultations (53.56%; 1052). This was followed by the surgery department (25.46%; 500), orthopedics department (7.74%; 152), obstetrics and gynecology department (3.46%; 68), and ophthalmology department (2.49%; 49; refer to [Table 1](#)).

When accounting for the number of patients admitted

in each department, the physical medicine and rehabilitation department had the highest consultation ratio at 3.78%. Following this were internal medicine (2.01%), radiology (1.30%), orthopedics (1.03%), psychiatry (0.90%), surgery (0.87%), ophthalmology (0.35%), otorhinolaryngology (0.34%) and obstetrics and gynecology (0.11%), as depicted in [Table 2](#).

[Table 3](#) delineates the frequency distribution of each disease category. During the study interval, dermatologists and dermatology residents recorded 2002 diagnoses from the 1964 consultation cases. The top 10 diagnostic categories were drug eruptions (28.02%; 561), eczema (16.18%; 324), viral infections (9.29%; 186), fungal infections (8.44%; 169), vasculitis (5.49%; 110), miscellaneous conditions (4.3%; 86), tumors and malignancies (4.2%; 84), papulosquamous diseases (3.8%; 76), bacterial infections (3.15%; 63), and vesiculobullous diseases (3.05%; 61). Parasitic infection, such as scabies and strongyloidiasis, and mycobacterial disorders were less common. Other infrequent conditions were autoimmune diseases, urticaria, hair and nail diseases, pigmentary diseases, neutrophilic dermatoses, pregnancy dermatoses, skin manifestations in systemic diseases, panniculitis, graft-versus-host diseases, and aging skin signs.

Drug eruptions emerged as the predominant diagnostic category. Most of these eruptions lacked systemic involvement (23.83%; 477). Morbilliform drug eruption dominated this subcategory (17.13%; 343), followed by adverse reactions from chemotherapy (3.35%; 67). Among the drug eruptions with systemic involvement, drug rash with eosinophilia and systemic symptoms was predominant (2.05%; 41). Eczema was second most prominent diagnostic category. Regarding viral infections, the third most prominent category, herpes simplex virus and herpes zoster virus were the principal causative agents (8%; 160). Fungal infections stood fourth, with superficial fungal infections, especially candida and dermatophyte infections, taking precedence (7.26%; 145). In the tumor and malignancy group, benign tumors were the most diagnosed (1.6%; 32), followed by cutaneous lymphoma and leukemia/lymphoma cutis (1.25%; 25), malignant/precancerous tumors (0.95%; 19), and cutaneous metastasis (0.4%; 8). The papulosquamous disease group was predominantly characterized by psoriasis diagnoses (3.65%; 73). Within the bacterial infections, cellulitis was the primary diagnosis prompting consultation (1.00%; 20), followed by folliculitis (0.70%; 14). Last, in the vesiculobullous diseases category, bullous pemphigoid was the most frequent diagnosis (2.10%; 42).

The prevalence of diagnoses requested for consultation by each department is outlined in [Table 4](#). In the internal

**TABLE 1.** Demographic data of dermatologic consultations: July 2018–June 2021.

	<b>Number of patients (%)</b> <b>N= 1,964</b>
Age (year) mean $\pm$ SD	57.6 $\pm$ 19.1
Sex: male	983 (50.05)
Department requesting consultation	
Internal medicine	1,052 (53.56)
Surgery	500 (25.46)
Orthopedics	152 (7.74)
Obstetrics & gynecology	68 (3.46)
Ophthalmology	49 (2.49)
Radiology	40 (2.04)
Physical medicine & rehabilitation	39 (1.99)
Otorhinolaryngology	34 (1.73)
Anesthesiology	17 (0.87)
Psychiatry	13 (0.66)

**TABLE 2.** Ratio of inpatient consultations by department.

	<b>Average number of admitted patients in 3 years</b>	<b>Number of consulted patients in 3 years</b>	<b>Ratio of consulted patient (%)</b>
Physical medicine & rehabilitation	1,032	39	3.78
Internal medicine	52,227	1,052	2.01
Radiology	3,078	40	1.30
Orthopedics	14,769	152	1.03
Psychiatry	1,440	13	0.90
Surgery	57,697	500	0.87
Ophthalmology	13,824	49	0.35
Otorhinolaryngology	9,948	34	0.34
Obstetrics & gynecology	64,029	68	0.11

**TABLE 3.** Dermatologic disease categories in consultations: July 2018–June 2021.

Diagnosis	Number of diagnoses (%) N=2,002
Drug eruptions	561 (28.02)
Drug eruptions without systemic involvement	477 (23.83)
Drug eruptions with systemic involvement	84 (4.20)
Eczema	324 (16.18)
Viral infection	186 (9.29)
Fungal infection	169 (8.44)
Vasculitis	110 (5.49)
Miscellany	86 (4.30)
Tumor/Malignancy	84 (4.20)
Papulosquamous diseases	76 (3.80)
Bacterial infection	63 (3.15)
Vesicubullous diseases	61 (3.05)
Autoimmune diseases	58 (2.90)
Urticaria	58 (2.90)
Hair and Nail diseases	36 (1.80)
Pigmentary diseases	32 (1.60)
Neutrophilic dermatoses	22 (1.10)
Pregnancy dermatoses	18 (0.90)
Skin signs in systemic diseases	17 (0.85)
Mycobacterial infection	14 (0.70)
Panniculitis	9 (0.45)
Parasitic infestation	9 (0.45)
Graft-versus-host disease	6 (0.30)
Aging skin sign	3 (0.15)

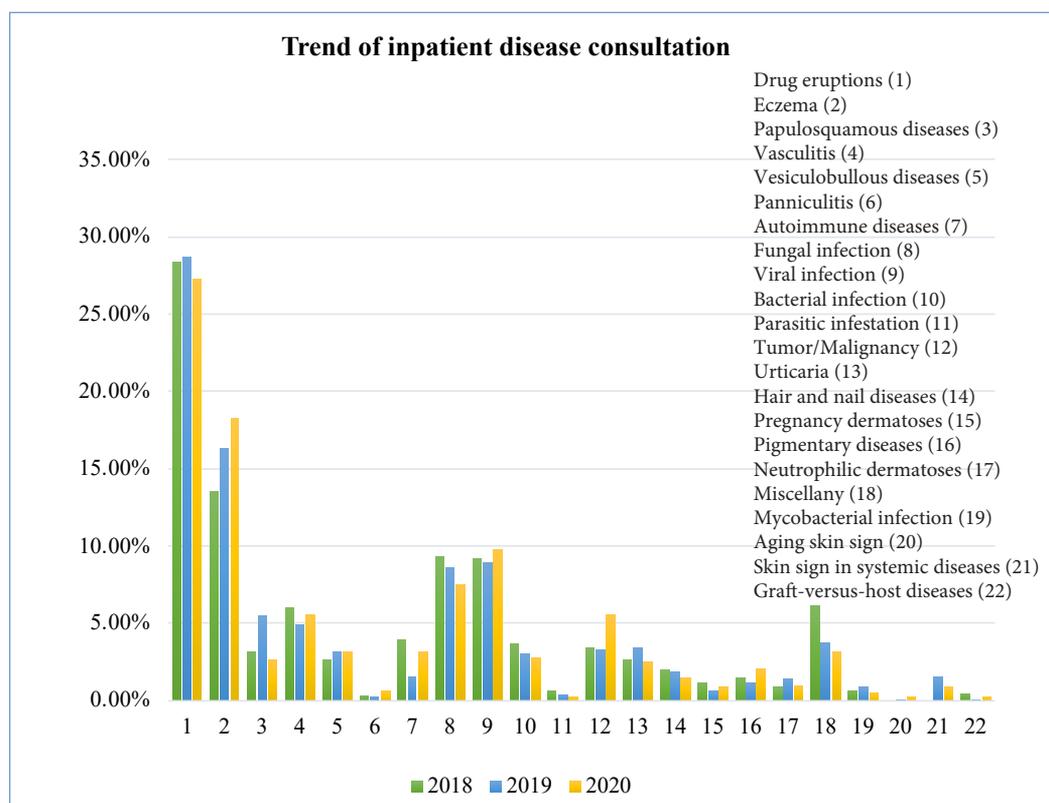
medicine department, the most frequent diagnosis was drug eruptions (30.9%), followed by eczema (10.4%), viral infections (8.2%), vasculitis (7.9%), and other conditions (5.5%). In the surgery department, the most frequent diagnoses were drug eruptions (30.5%), eczema (20.1%), fungal infections (12.7%), viral infections (9.6%), and urticaria (5.1%). In the orthopedics department, eczema was the leading dermatosis requested for consultation (28.4%), followed by fungal infection (17.4%), drug eruptions (14.8%), viral infections (12.9%), and papulosquamous diseases (5.2%). In the obstetrics and gynecology department, pregnancy dermatoses dominated the consultation requests at 25%, followed by eczema

(22.1%), fungal infections (11.8%), drug eruptions (10.3%), and tumors/malignancies (5.9%). For the ophthalmology department, the most common diagnoses were eczema (33.3%), viral infections (19.6%), drug eruptions (17.6%), fungal infections (15.7%), and vasculitis (2%).

**Fig 1** charts the dermatologic consultation trends from July 2018 to June 2021. The 2018 academic year saw 591 consultations, which increased to 669 in 2019 and further to 704 in 2020. Throughout these 3 academic years, the internal medicine department consistently submitted the highest number of dermatologic consultation requests (51.44%, 54.56%, and 54.47%, respectively). Regarding the consultation disease trend, the prevalence

**TABLE 4.** Dermatologic diseases consulted by department: July 2018–June 2021.

Diagnosis	N (%)
<b>Internal medicine (1,070)</b>	
Drug eruptions	331 (30.9)
Eczema	111 (10.4)
Viral infection	88 (8.2)
<b>Surgery (512)</b>	
Drug eruptions	156 (30.5)
Eczema	103 (20.1)
Fungal infection	65 (12.7)
<b>Orthopedics (155)</b>	
Eczema	44 (28.4)
Fungal infection	27 (17.4)
Drug eruptions	23 (14.8)
<b>Obstetrics &amp; Gynecology (68)</b>	
Pregnancy dermatoses	17 (25)
Eczema	15 (22.1)
Fungal infection	8 (11.8)
<b>Ophthalmology (51)</b>	
Eczema	17 (33.3)
Viral infection	10 (19.6)
Drug eruptions	9 (17.6)



**Fig 1.** Disease trend in dermatologic consultations: July 2018–June 2021.

of diagnostic categories remained relatively stable across all 3 years. Drug eruptions consistently appeared as the most frequent diagnosis (28.38%, 28.63%, and 27.23%). Eczema ranked as the second most common diagnosis, with its prevalence notably rising over the 3 years (13.52%, 16.28%, and 18.30%).

Table 5 juxtaposes the diseases in the “must-know” category of the dermatology curriculum with inpatient consultations. Almost all conditions could be categorized into the “must know” diagnostic group of the dermatology residency curriculum. The notable exceptions were adverse reactions from chemotherapy, ulcer/burn conditions, and lymphoma-related diagnoses, including cutaneous lymphoma and leukemia/lymphoma cutis.

## DISCUSSION

Dermatology is traditionally perceived as an outpatient specialty, yet it also plays a vital role in providing inpatient dermatologic consultations. Inpatient dermatologic consultations are integral to in-hospital patient care. The consultations offer not only clinical and educational benefits but also financial advantages to patients, hospitalist teams, and hospitals.<sup>1,3,4</sup> The present investigation focused on the clinical and epidemiological aspects of inpatient dermatologic consultations, evaluating the prevalence of departments soliciting dermatologic consultation, the commonality of consulted inpatient dermatoses, and consultation trends across 3 academic years.

Consistent with prior studies,<sup>1,3,4,7-11</sup> the internal medicine department initiated the largest number of consultations. Research by Vinay et al,<sup>3</sup> Galimberti et al,<sup>4</sup> and Balai et al<sup>10</sup> indicated that almost half of the consultations originated from the internal medicine department. This finding aligns with our data (53.56%). This trend might be due to the substantial number of inpatient admissions in internal medicine, compounded by the intricacy of the conditions treated and the extensive use of medications.<sup>7,10</sup> The high demand for consultations might also be driven by the rigorous and thorough physical examinations performed by internal medicine teams, leading to the detection of more lesions than by other departments. We found that the surgery and orthopedics departments had the second- and third-highest number of consultation requests, respectively, corroborating findings from earlier publications. However, it is imperative to note that our investigation did not encompass patients under 18, meaning that the pediatric department a significant contributor to consultations in some studies<sup>3,4,13</sup> was not considered.

Conversely, when assessing the proportion of dermatologic consultations relative to the total patient

admissions per department, the department with the most pronounced ratio was physical medicine and rehabilitation, followed by internal medicine and then radiology. This finding indicates that even when accounting for the sheer volume of patient admissions, internal medicine remains a prominent requester of consultations. Interestingly, physical medicine and rehabilitation, which primarily admits patients for holistic rehabilitation, emerged as the department with the highest consultation frequency.

The most commonly consulted conditions highlighted in earlier studies encompass infectious dermatoses, eczema, and drug eruptions.<sup>1,8-10,12</sup> However, in our research, drug eruptions accounted for most consultations, followed by eczema and infectious dermatoses (encompassing viral, fungal, and bacterial infections). Drug eruptions skin manifestations more prevalent among inpatients due to the administration of multiple medications during hospitalization constituted the largest category of consulted diseases in inpatient dermatology. Although severe drug eruptions (4.2%; 84) were consulted less frequently than those without systemic involvement (23.83%; 477), the high mortality rate associated with severe drug eruptions underscores the need for emergency care awareness. An updated residency curriculum must incorporate information about novel treatments and the pathogenesis of severe drug eruptions.

The heightened prevalence of infectious lesions might be due to inpatients' significant preexisting comorbidities and immunosuppression during their admission.<sup>3</sup> Our study also identified complicated and atypical infectious dermatologic conditions, such as disseminated herpes infection, nontuberculous mycobacterial infection, and interferon-gamma autoantibody disease. On the other hand, our findings revealed that autoimmune diseases received fewer consultations than cutaneous vasculitis. This is likely because rheumatologists at our tertiary hospital often oversee autoimmune disease consultations. Autoimmune diseases, especially systemic lupus erythematosus, are “must-know” conditions for dermatologists. Therefore, collaboration between dermatologists and rheumatologists remains paramount for both optimal patient care and residency training.

In the internal medicine and surgery departments, the dominant conditions eliciting consultations were drug eruptions, followed by eczema and infections, both fungal and viral a pattern mirroring the general consultation trends. However, this uniformity was absent in other departments. For instance, the obstetrics and gynecology department primarily dealt with pregnancy-related dermatoses. In the orthopedic and ophthalmology departments, where the primary ailments are largely

**TABLE 5.** “Must-Know” disease categories in dermatology residency training curriculum vs. prevalence in inpatient consultations.

Diagnostic group and diseases found in dermatology consultation	Number of diagnoses (%)
Eczema	324 (16.18)
Immunologic diseases	127 (6.34)
Connective tissue disease: Lupus erythematosus	44 (2.20)
Anaphylactic syndrome	
Urticaria	75 (3.74)
Anaphylaxis	2 (0.10)
Graft-versus-host disease	6 (0.30)
Infection	441 (22.03)
Viral infection	186 (9.29)
Fungal infection	169 (8.44)
Bacterial infection	63 (3.15)
Mycobacterial infection	14 (0.70)
Parasitic infestation	9 (0.45)
Photodermatology	-
Papulosquamous eruptions	76 (3.80)
Psoriasis	73 (3.65)
Others	3 (0.15)
Vesiculobullous disease	61 (3.05)
Bullous pemphigoid	42 (2.10)
Pemphigus vulgaris	13 (0.65)
Others	6 (0.30)
Vasculitis	110 (5.49)
Panniculitis	9 (0.45)
Drug eruptions	424 (21.18)
Morbilliform drug eruption	343 (17.13)
DRESS/DIHS	41 (2.05)
SJS	18 (0.90)
TEN	9 (0.45)
Pustular drug eruption	6 (0.30)
Exfoliative dermatitis	5 (0.25)
Generalized bullous fixed drug eruption	2 (0.10)
Non-infectious inflammatory disorder	22 (1.10)
Neutrophilic dermatoses	22 (1.10)
Pigmentary disorder	32 (1.60)
Diseases of hair	5 (0.25)
Diseases of nail	31 (1.55)
Diseases of sebaceous gland	
Diseases of sweat gland	13 (0.65)
Miliaria	13 (0.65)
Diseases of oral mucosa	-
Genodermatoses	-
Disease of nutrition and metabolism	-
Skin neoplasm	59 (2.95)
Benign tumor	32 (1.60)
Malignant/precancerous tumor	19 (0.95)
Cutaneous metastasis	8 (0.40)
Skin signs in systemic disease	17 (0.85)
Occupational and environmental diseases	-
Psychocutaneous disorders	-
Pediatric dermatology	-
Genital diseases	-
Skin diseases in pregnancy	18 (0.90)

organ-specific and do not typically involve extensive drug administration, the leading diagnoses were eczema, fungal and viral infections, and drug eruptions. Such insights could guide dermatologists in tailoring their approach to each department's unique consultation spectrum.

Across the study's 3 academic years, the prevalence trend of consulted diseases remained static and, despite the ongoing COVID-19 pandemic during the study interval, inpatient consultation numbers consistently rose. In contrast, outpatient cases has declined over a similar period of time.<sup>14</sup> This underscores the pivotal role of inpatient dermatologic consultation despite the on-going COVID-19 situation.

Our Dermatology Residency is the largest dermatology residency program in Thailand. As of the 2023 academic year, 23 board-certified dermatologists contribute to the program with 34 total residents. Thai dermatology curriculum spans 4 academic years. First-year residents rotate in the internal medicine department to acquire comprehensive knowledge and skills from the rotations. While second to fourth-year residents primarily practice within the dermatology department. Residents are required to complete 750 hours of outpatient dermatology clinic. For inpatient requirement, each resident participates in an inpatient dermatology rotation for about 8 weeks per year, targeting 50-60 inpatient consultations per month. The curriculum's main assessment in inpatients is Entrustable Professional Activities (EPA). EPA assesses residents on multiple aspects such as patient care, medical knowledge and interpersonal skills. The EPA for inpatients requires residents to pass nine conditions, with three mandatory conditions to meet: severe drug reaction, severe psoriasis, and severe vesiculobullous disease.<sup>15</sup>

We analyzed the dermatology residency training curriculum at the Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand<sup>15</sup> to ascertain its alignment with the diseases identified in our study. The curriculum organizes diseases into 3 tiers: conditions dermatologists must know, diseases they should know, and rare or uncommon conditions. Our review found that the current curriculum encompasses all 10 primary diagnostic groups from our study, positioning these diseases in the "must-know" tier. Almost every prevalent lesion in each of the 10 diagnostic groups was already in the "must-know" category, with the exceptions of adverse drug reactions from chemotherapy, cutaneous lymphoma, leukemia/lymphoma cutis, and ulcers/burns. Notably, cutaneous lymphoma and leukemia/lymphoma cutis, while absent from the "must-know" tier, were frequent within the malignant consultation group in

our study, accounting for 1.25% of all consultations.

When focus on the number of cases of consultations, the study has shown that there were 1964 consultations in 3 years, averaging approximately 54.5 patients per month. This figure closely aligns with the intended number stated in the curriculum. In addition, severe drug eruption, severe psoriasis, and severe vesiculobullous disease are among the top 10 inpatients diagnoses for inpatient EPA assessment.

Given the rising incidence of cancer in Thailand,<sup>16</sup> our study highlighted that adverse reactions to chemotherapy are common within the drug eruptions category. These reactions, such as hand-foot syndrome, papulopustules, paronychia, irregular hair growth, itching, and dryness from epidermal growth factor receptor inhibitors (PRIDE syndrome), are particularly prevalent in the internal medicine department. These conditions have yet to be incorporated into the current curriculum. We advocate for an update to the dermatology residency curriculum to encompass all routinely encountered conditions.

While dermatology is out of reach for some hospitals, telemedicine consultations could be helpful at hospital training institutes that lack sufficient inpatient consultation services. Dermatologists in the tertiary care facility might therefore share cases with the co-training centers and aid in clinical decision making. It is possible for residents to contribute significantly to telemedicine while still fulfilling their educational requirements.<sup>17</sup>

This study is with some limitations. Given its retrospective chart review design, incomplete or missing data were inevitable. Additionally, given Thailand's primarily universal healthcare system, the easy transfer of patients from secondary to tertiary care facilities not only amplifies the workload in tertiary care hospitals but might also skew disease prevalence. Conducted at Siriraj Hospital one of Thailand's largest tertiary care and referral centers our study's disease prevalence may therefore not be representative, limiting the generalizability of our findings to other institutions. Moreover, this research did not explore other facets of inpatient dermatologic consultations, such as comparison of diagnoses from dermatologists and other medical professionals, economic implications or patient treatment impacts, areas deserving future investigations.

In summary, the prevalence of commonly consulted inpatient dermatologic conditions in our study mirrors previous findings. Nevertheless, we identified equally frequent severe and potentially fatal dermatologic conditions. Without an intricate grasp of these intricate inpatient disorders, dermatologists might fail to provide accurate diagnoses or efficacious treatments. The study

also highlighted that distinct diseases were consulted upon by each department. Additionally, the incessant evolution of therapeutic approaches inevitably results in the emergence of novel dermatologic disorders. Integrating this newfound knowledge into residency programs and subsequent curriculum revisions will undoubtedly benefit future dermatologists and, by extension, their patients.

### Funding

None.

### Disclosure of interests

The authors declare that there are no conflicts of interest.

### REFERENCES

- Mancusi S, Festa Neto C. Inpatient dermatological consultations in a university hospital. *Clinics (Sao Paulo)*. 2010;65(9):851-5.
- Noe MH, Rosenbach M. Inpatient Dermatologists-Crucial for the Management of Skin Diseases in Hospitalized Patients. *JAMA Dermatol*. 2018;154(5):524-5.
- Vinay K, Thakur V, Choudhary R, Dev A, Chatterjee D, Handa S. A Retrospective Study to Evaluate the Impact of Inpatient Dermatological Consultations on Diagnostic Accuracy in a Tertiary Care Setting. *Indian Dermatol Online J*. 2021;12(3):417-22.
- Galimberti F, Guren L, Fernandez AP, Sood A. Dermatology consultations significantly contribute quality to care of hospitalized patients: a prospective study of dermatology inpatient consults at a tertiary care center. *Int J Dermatol*. 2016;55(10):e547-51.
- Joseph J, Truong K, Smith A, Fernandez-Penas P. Dermatology inpatient consultations in a tertiary hospital - a retrospective analysis. *Int J Dermatol*. 2022;61(1):48-53.
- Milani-Nejad N, Zhang M, Kaffenberger BH. Association of Dermatology Consultations With Patient Care Outcomes in Hospitalized Patients With Inflammatory Skin Diseases. *JAMA Dermatol*. 2017;153(6):523-8.
- Lorente-Lavirgen AI, Bernabeu-Wittel J, Pulpillo-Ruiz Á, de la Torre-García JM, Conejo-Mir J. Inpatient dermatology consultation in a Spanish tertiary care hospital: a prospective cohort study. *Actas Dermosifiliogr*. 2013;104(2):148-55.
- Fernandes IC, Velho G, Selores M. Dermatology inpatient consultation in a Portuguese university hospital. *Dermatol Online J*. 2012;18(6):16.
- Williams A, Bhatia A, Kanish B, Chaudhary PR, Samuel CJ. Pattern of Inpatient Dermatology Consultations in a Tertiary Care Centre from Northern India. *J Clin Diagn Res*. 2016;10(12):WC07-WC10.
- Balai M, Gupta LK, Khare AK, Mittal A, Mehta S, Bharti G. Pattern of inpatient referrals to dermatology at a tertiary care centre of South Rajasthan. *Indian Dermatol Online J*. 2017;8(1):25-8.
- Peñate Y, Guillermo N, Melwani P, Martel R, Borrego L. Dermatologists in Hospital Wards: An 8-Year Study of Dermatology Consultations. *Dermatology*. 2009;219(3):225-31.
- Dantas LD, Bakos L, Balbinot G, Drechsler CE, Eidt LM. Prevalence of dermatoses in dermatologic evaluation requests from patients admitted to a tertiary hospital for 10 years. *An Bras Dermatol*. 2015;90(5):762-4.
- Fischer M, Bergert H, Marsch WC. Das dermatologische Konsil. *Der Hautarzt*. 2004;55(6):543-8.
- Wamaphutta K, Thasen C, Sereephinan C, Chaweekulrat P, Boonchai W. Impact of the COVID-19 Pandemic on Tertiarycare University Dermatology Outpatient Clinic and Dermatology Procedures. *Siriraj Med J*. 2022;74(12):836-43.
- Residency Training in Dermatology, Department of Dermatology, Faculty of Medicine Siriraj Hospital. Department of Dermatology, Faculty of Medicine Siriraj Hospital 2019.
- Absolute numbers, incidence and mortality, males and females in Thailand [Internet]. World Health Organization 2023 [cited 2023 July 18]. Available from: <https://gco.iarc.fr/overtime/en>.
- Hammond MI, Sharma TR, Cooper KD, Beveridge MG. Conducting inpatient dermatology consultations and maintaining resident education in the COVID-19 telemedicine era. *J Am Acad Dermatol*. 2020;83(4):e317-e8.

# The Effect of Diabetes Self-management Education Provided by Certified Diabetes Educator Compared to Usual Diabetes Education on Glycemic Level and Stage of Behavior Change in Adult with Types 2 Diabetes Mellitus

Kanyarat Wongmuan, CDE, RN<sup>1</sup>, Narinnad Thanaboonsutti, CDE, RN<sup>1</sup>, Wilawan Ketpan, CDE, RN<sup>1</sup>, Sarawoot Uprarat, CDE<sup>2</sup>, Varisara Lapinee, CDE<sup>2</sup>, Lukana Preechasuk, M.D.<sup>2</sup>

<sup>1</sup>Department of Nursing, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, <sup>2</sup>Siriraj Diabetes Center of Excellence, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.

## ABSTRACT

**Objective:** To explore the effect of diabetes self-management education and support (DSMES) provided by Siriraj certified diabetes educators (CDE) compared to usual diabetes education (DE) on glycemic level and stage of behavior change in patients with type 2 diabetes mellitus (T2D).

**Materials and Methods:** Patients with T2D having A1C 8-12% were recruited between 2019-2020 to receive DSMES from CDE. Patients received the usual DE from healthcare professionals in 2016 were randomly selected from their medical records.

**Results:** 76 patients were enrolled in each group. Mean±SD age was 59.4±11.5 years. After receiving DSMES and DE, A1C decreased dramatically at 3 months in both groups without significant difference between the groups (9.4±1.1% to 8.0±1.2% vs. 9.5±1.1% to 8.1±1.5%, respectively). However, the DSMES group can further decrease A1C to 7.8±1.2% while A1C in the usual DE group increased to 8.5±1.6% at 12 months (p=0.028). In the DSMES group, most patients can move to the next stage of behavior change and reported a better QOL (89.4±11.6 vs. 92.6±12.2, p=0.018).

**Conclusion:** The receipt of DSMES from CDE significantly improved the level of A1C, the stage of behavior change, and QOL. Its benefit on the glycemic level can last at least one year.

**Keyword:** Diabetes self-management education; certified diabetes educator; glycemic level; stage of behavior change; quality of life (Siriraj Med J 2024; 76: 61-68)

## INTRODUCTION

Type 2 diabetes mellitus (T2D) is a disorder of carbohydrate metabolism with two main pathophysiologies, including insulin resistance and relative insulin deficiency. The Western Pacific region has the highest number of people living with diabetes in 2021, which is 206 million, and could project to 260 million by 2045.<sup>1</sup> In the same

direction, the prevalence of diabetes in Thailand has increased from 8.9% in 2014 to 9.5% in 2020,<sup>2</sup> and only 33.3% of people with type 2 diabetes can achieve optimal glycemia.<sup>3</sup>

Diabetes Self-Management Education and Support (DSMES) is the process of facilitating the knowledge, skills, and abilities necessary for diabetes self-care. It is

Corresponding author: Lukana Preechasuk

E-mail: Lukana.pre@gmail.com

Received 1 December 2023 Revised 2 January 2024 Accepted 3 January 2024

ORCID ID: <http://orcid.org/0000-0001-8496-6790>

<https://doi.org/10.33192/smj.v76i2.266524>



All material is licensed under terms of the Creative Commons Attribution 4.0 International (CC-BY-NC-ND 4.0) license unless otherwise stated.

an important element in diabetes care that helps people with diabetes make informed decisions, solve problems, develop personal goals and action plans, and cope with emotions and life stresses. It can facilitate behavior change, improve glycemic control, reduce diabetes complications, and improve quality of life.<sup>4</sup> The previous national Thai survey demonstrated the need for competent diabetes educators, adequate time to provide diabetes education, and a clearly defined role for diabetes educators.<sup>5</sup> The Siriraj Diabetes Center of Excellence has established the Certified Diabetes Educator Program, Faculty of Medicine Siriraj Hospital, in 2017, which is the first certified diabetes educator program in Thailand organized by the Faculty of Medicine. After graduating, Siriraj Certified Diabetes Educator (CDE), who works in our hospital, will rotate to work as CDE in DSMES clinic. The objectives of this study were to evaluate the effect of CDE-provided DSMES compared to the usual diabetes education (DE) provided by the health professional on the glycemic level, the behavior change, and the quality of life.

## MATERIALS AND METHODS

### *Design and participants*

This is a prospective cohort with a historical controlled cohort study at Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. The inclusion criteria were 1) adult patients with T2D aged 18-80 years 2) having A1C 8-12% 3) having been diagnosed with T2D for at least 6 months and 4) receiving DSMES from CDE or usual DE from the health care professional. The exclusion criteria were patients with terminal illness or unable to participate in the DSMES program. Patients in the usual DE group were randomly selected from the electronic medical record. They had to receive the usual DE from the healthcare professional in the Outpatient Division or Siriraj Diabetes Center during 2016 before establishing CDE. In the DSMES group, patients were recruited from the DSMES clinic at Siriraj Diabetes Center of Excellence during 2019-2020. They received DSMES by CDE at baseline, 3, 6, and 12 months. Demographic data, glycemic, and lipid levels were collected from both groups. Knowledge of diabetes and quality of life were evaluated at baseline, 6 and 12 months, while the stage of behavior change was evaluated every visit after receiving DSMES.

The protocol was approved by the Siriraj Institutional Review Board (SIRB) of the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, (COA no. Si 715/2019). Written informed consent was obtained from patients in the DSMES group.

### *Diabetes self-management education and support*

DSMES was provided to patients at baseline, 3, 6, and 12 months. It took around 45-60 minutes per session. The DSMES session consisted of four components, including building relationships, assessment, implementation, and evaluation. CDE assessed the knowledge, understanding, attitude, mental, social, needs, and self-care management of patients by determining their problems, using open questions and the motivational interviewing principle. CDE applied deep listening principles and good communication skills using both verbal and nonverbal body language. The topics of DSMES in each session were individualized and depended on the assessment and the problems of the patients. The main topics of DSMES included diabetes pathophysiology, healthy eating and food exchange, acute and chronic diabetic complications, exercise, glucose monitoring, diabetes medication, insulin injection technique and self-care for special conditions. The educational materials used were the Siriraj DM interactive tool, food models, glucose monitoring, and insulin injection devices.

The CDE discussed with patients and their families about their problems and developed suitable solutions together. DSMES was delivered based on the motivation interviewing principle to build patients' confidence in their potential to take care of themselves. For behavior problems related to diabetes, the intention of the patients to change was taken into account by choosing appropriate change processes and counseling techniques that matched each stage, as well as providing psychological and social support. CDE encouraged patients to set SMART goals (specific, measurable, achievable, relevant, and time-based) and evaluated the results at the next visits. During the COVID-19 pandemic, some follow-up visits were made by telephone.

### *Usual diabetes education*

Before the establishment of CDE, DE was provided by a healthcare professional primarily by a nurse or a dietitian. The DE session was mainly content-based teaching, including general knowledge of diabetes, healthy diet, and exercise. It was mainly one-way communication from the healthcare professional to ensure the completeness of the content. At that time, the goal setting and stage of behavior change theory were not applied. Ninety-five percent of the patients received DE only one session.

### *Laboratory measurement*

Plasma glucose, cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C) were measured on a Cobas® 8000 modular analyzer (Roche Diagnostics,

Basel, Switzerland). Plasma levels of low-density lipoprotein cholesterol (LDL-C) were calculated using the Friedewald formula. The A1C level was determined by a turbidimetric inhibition immunoassay (Integra 400 analyzer; Roche Diagnostics).

### ***Diabetes knowledge, stage of change, and quality of life evaluation***

Diabetes knowledge was assessed at baseline, 6 and 12 months using a diabetes knowledge assessment tool that consisted of 24 true-false questions. Patients can also choose unknown as an answer. Sixteen questions were part of an instrument to assess general knowledge of patients with diabetes,<sup>6</sup> and eight questions were from a pretest of our center's T2D camp. The questions covered general knowledge of diabetes, diet, exercise, sickness management, and foot care. The assessment tool was tested in 20 patients with T2D. The reliability of the tool calculated by Kuder-Richardson 20 (KR-20) was 0.754.

Quality of life was assessed at baseline, 6 and 12 months using WHOQOL – BREF – THAI.<sup>7</sup> WHOQOL – BREF, an abbreviated version of WHOQOL – 100, consists of 26 questions in 4 domains, including physical, psychosocial, social, and environment. The score ranges from 26 to 130. Higher scores mean a better quality of life. Quality of life can be classified into 3 groups including 1) a poor quality of life (score 26 – 60), 2) a moderate quality of life (score 61-95) and 3) a good quality of life (score 96-130).

During the DSMES session, CDE and patients discussed unhealthy behaviors that patients would like to change. Stage of behavior change<sup>8</sup> including pre-contemplation, contemplation, preparation, action, maintenance, and relapse were evaluated for each behavior by CDE at baseline and at each visit.

### ***Sample size calculation***

Previous data from our hospital showed that the number of patients having A1C less than 7% at 12 months after receiving usual diabetes education was 25%. We expected that the number of patients having A1C less than 7% at 12 months after receiving DSMES by CDE would increase to 50%. Using these data, an alpha level of 0.05, an allowable error (d) of 0.02, and a 30% increase were required to compensate for the loss of follow-up, a sample size of 76 participants in each group was required.

### ***Statistical analysis***

The baseline characteristics were compared between

the DSMES and the usual DE group. The medical results were compared between baseline and each visit within the group and between the groups. Knowledge of diabetes, stage of behavior change, and quality of life were compared between baseline and 6 to 12 months only in the DSMES group. Paired t-test and unpaired t-test were used for normal distribution data, and the Mann-Whitney test was used for nonnormal distribution data. Chi-square and Fisher's exact test were used for categorical data. Statistical analysis was performed using SPSS version 21 and Python version 3.7.

## **RESULTS**

### ***Baseline characteristics***

Seventy-six patients with T2D were recruited in each group. The mean±SD age was 59.4±11.5 years; the median duration of diabetes (IQR) was 9.2 (3.7, 13.1) years. There were no differences in baseline characteristics between the groups, except for a higher diastolic blood pressure in the control group (Table 1). Around 40% of patients received insulin therapy.

### ***Medical outcomes***

Both groups demonstrated a significant improvement in fasting plasma glucose (Table 2) and A1C after receiving DSMES and the usual DE. However, only DSMES group can maintain glycemic control at 12 months. In the DSMES group, A1C decreased sharply from 9.4±1.1% to 8.0±1.2%,  $p < 0.001$  at 3 months and further decreased to 7.8±1.2% at 12 months,  $p < 0.001$  compare to baseline. In the usual DE group, A1C decreased significantly from 9.5±1.1% to 8.1±1.5%,  $p < 0.001$  at 3 months, but increased slightly to 8.5±1.6%,  $p < 0.001$  at 12 months (Fig 1). A1C was significantly lower in the DSMES group compared to the usual DE group at 12 months (7.8±1.2% vs. 8.5±1.6%,  $p = 0.028$ ).

Triglyceride and LDL-C did not change during the study period. HDL-C significantly increased in DSMES group at 3 months (Table 2).

### ***Diabetes knowledge and quality of life evaluation***

After receiving DSMES from the CDE team, the patient gained more knowledge and had a better quality of life. The diabetes knowledge score increased from 16.9±4.3 at baseline to 20.1±2.5 at 12 months ( $p < 0.001$ ). When comparing between baseline and 6 to 12 months, the QOL score improved statistically from 89.4±11.6 to 92.6±12.2,  $p = 0.018$ , and the number of patients with good quality of life increased from 18 (27.7%) to 27 (41.5%),  $p = 0.064$ .

**TABLE 1.** Baseline characteristics.

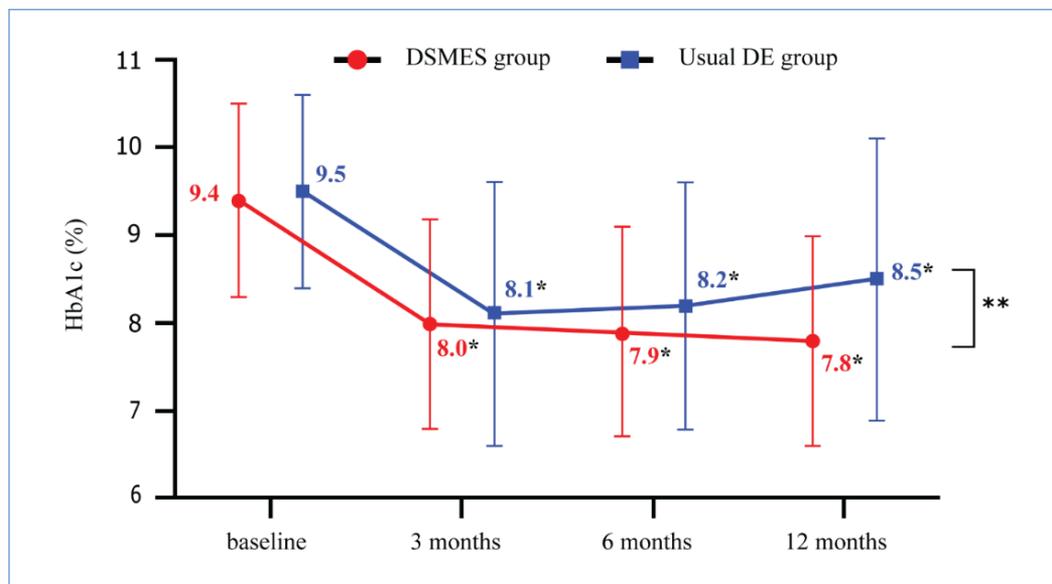
Baseline Characteristics	Total (n=152)	DSMES Group (n=76)	Usual DE Group (n=76)	p-value
Age (Year)	59.4±11.5	58.3±10.6	60.4±12.2	0.265
Gender, Female (%)	95 (62.5)	47 (61.8)	48 (63.2)	0.867
Education Level, n (%)	(n= 88)	(n =75)	(n = 13)	0.855
Elementary school or below	38 (43.2)	33 (44.0)	5 (38.5)	
Secondary school or equivalent	22 (25.0)	19 (25.3)	3 (23.1)	
Bachelor's degree or above	28 (31.8)	23 (30.7)	5 (38.5)	
Type of Insurance				
Civil servant medical benefit	87 (57.2)	42 (56.6)	44 (57.9)	0.628
Universal Health Coverage/ Social Health Insurance	50 (32.9)	27 (35.5)	23 (30.3)	
Self-payment/other	15 (9.9)	6 (7.9)	9 (11.8)	
Duration of diabetes (Year)	9.2 (3.7,13.1)	8.7 (3.2,13.1)	9.3 (5.0,13.5)	0.388
Comorbidity, n (%)				
Hypertension	125 (82.2)	61 (80.3)	64 (84.2)	0.524
Dyslipidemia	114 (75.0)	55 (72.4)	59 (77.6)	0.454
Coronary artery disease	20 (13.2)	7 (9.2)	13 (17.1)	0.150
Cerebrovascular disease	5 (3.3)	2 (2.6)	3 (3.9)	0.649
BMI (kg/m <sup>2</sup> )	27.0±5.8	27.5±6.6	26.4±4.7	0.300
SBP (mmHg)	133.7±15.1	131.8 ± 13	135 ± 16.8	0.106
DBP (mmHg)	73.3±11.5	70.2 ± 12.1	76.5 ± 9.9	0.001*
Oral hypoglycemia agent, n (%)	(n=140)	(n=76)	(n=73)	0.365
1 medications	27 (19.3)	11 (14.9)	16 (24.2)	
2 medications	2 (37.9)	29 (39.2)	24 (36.4)	
≥ 3 medications	60 (42.9)	34 (45.9)	26 (39.4)	
Insulin injection, n (%)	66 (43.4)	28 (36.8)	38 (50.0)	0.102

Data were presented as mean±SD, and median (IQR), \*p < 0.05 comparing between the DSMES and the usual DE group

**TABLE 2.** Comparison of the medical outcome between the DSMES and the usual DE group.

	DSMES group				Usual DE group			
	baseline	3-month	6-month	12-month	baseline	3-month	6-month	12-month
Fasting plasma glucose (mg/dL)	n = 75 182±56	n = 64 152±45*	n = 57 159±53*	n = 60 156±61*	n = 74 195±83	n = 72 151±55*	n = 71 163±61*	n = 76 166±66*
Triglyceride (mg/dL)	n = 47 131 (99,208)	n = 33 119 (92,213.5)	n = 27 119 (96,189)	n = 43 123 (84,159)	n = 48 150 (109,226)	n = 26 147 (98.5,194)	n = 31 146 (103,183)	n = 37 137 (96,195)
HDL-C (mg/dL)	n = 48 45.2±10.9	n = 31 51.9±14.5*	n = 26 50.0±11.2	n = 43 50.9±16.0	n = 44 44.5±13.1	n = 26 46.6±13.8	n = 27 49.4±17.6	n = 34 48.8±14.8
LDL-C (mg/dL)	n = 55 93.9±32.6	n = 34 89.2±39.8	n = 31 91.9±40.4	n = 47 92.8±37.9	n = 48 94.8±36.2	n = 28 98.0±29.7	n = 32 88.9±34.6	n = 39 86.9±36.5

Data were presented as mean±SD, and median (IQR) \* p < 0.05 comparing with baseline value.



**Fig 1.** Hemoglobin A1c level during the study. The error bar represents the standard deviation. \* p < 0.05 comparing with the baseline values. \*\* p < 0.05 comparing between the groups.

### Behavior change

The most common behavioral problems that the patient aimed to change were unhealthy eating (53.7%), followed by inadequate exercise (21.1%), and improper medication use (13.7%). The majority of patients can move to the next stage of behavior change after receiving DSME from CDE. The number of patients in action and maintenance stage increased from 0.6% to 69.9%, p < 0.001 at 12 months (Fig 2).

### The chronic diabetic complications screening rates

The rate of screening for diabetic complications was significantly higher in the DSMES group. When comparing between the DSMES and the usual DE group, the screening rate for diabetic retinopathy was 75 (98.7%) vs. 66 (86.8%), p=0.009; the screening rate for diabetic nephropathy was 70 (92.1%) vs. 55 (72.4%), p=0.002 and the screening rate for diabetic foot problems was 73 (96.1%) vs. 23 (30.3%), p < 0.001.

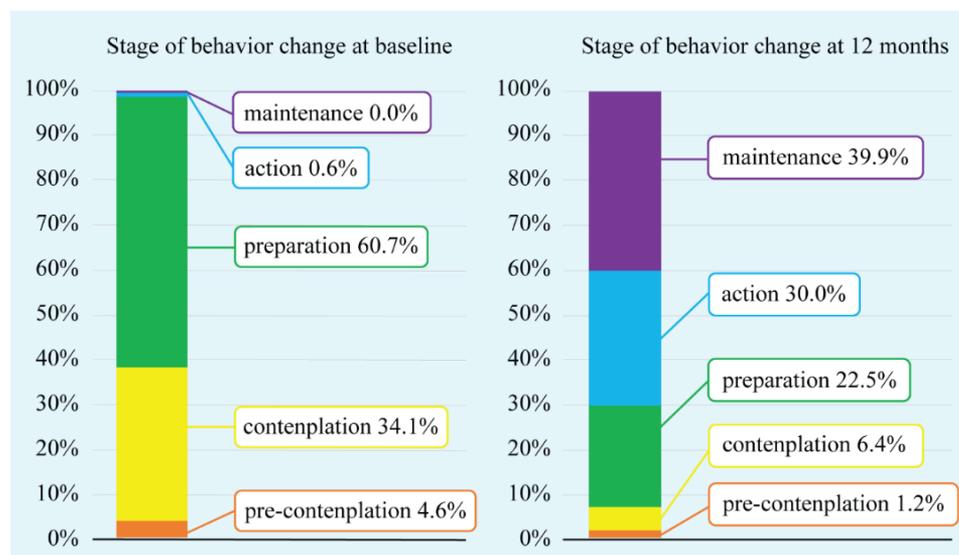


Fig 2. Stage of behavior change at baseline and 12 months in the DSMES group. \*  $p < 0.001$  comparing between baseline and 12 months

## DISCUSSION

The effect of CDE-provided DSMES on the glycemic level in our study was consistent with previous studies in patients with T2D.<sup>9,10</sup> A large meta-analysis in 2016 showed that the overall mean $\pm$ SD reduction in A1C for all patients randomized to DSME was 0.74 $\pm$ 0.63%.<sup>9</sup> A systematic review from the countries of the Middle East showed mean $\pm$ SD reduction of A1C after the DSME program was 1.15 $\pm$ 0.55%.<sup>10</sup> Our study also showed a mean $\pm$ SD of 1.6 $\pm$ 1.5 % of the reduction in A1C after receiving DSME. Although a recent meta-analysis revealed that the DSME contact time > 10 hours exhibited a better rate of A1C reduction than the DSME contact time < 10 hours,<sup>9</sup> our study showed that even a total DSMES contact time of approximately 4 hours could demonstrate an advantage in A1C reduction. The shorter duration of DSMES with favorable medical and psychological outcomes is suitable for public hospitals with high workload in our country.

While there was a slight rebound in A1C level in usual DE group, the effect of DSMES on glycemic level was maintained for 12 months. The authors believe that this sustainability was caused by increasing the knowledge of diabetes, changing unhealthy behavior, and regular follow up throughout 12 months whereas usual DE had only one session at baseline. Although having only diabetes knowledge is not enough to change behavior, it is important to create awareness, which is the first step of behavior change.<sup>11</sup> The most common unhealthy behaviors that our patients would like to change were unhealthy eating, inadequate exercise, and taking medications irregularly. For eating habit, previous meta-analysis showed that delivery of medical nutritional therapy by dietitian reduced A1C by 0.43%

in people with diabetes.<sup>12</sup> Bowen et al. also showed that providing DSMES by CDE using a modified plate method technique improved A1C by 0.83% in people with T2D.<sup>13</sup> Exercise not only improves glycemic control, but also increases cardiovascular fitness, reduces cardiovascular risk factors, contributes to weight loss, and improves well-being.<sup>14</sup> For medication, a study of newly diagnosed patients with diabetes in Singapore found that 35% of patients did not take their medications regularly, and poor adherent patients (proportion of days covered less than 40%) had an increase in A1C by 0.4% during the two years of follow-up.<sup>15</sup> Therefore, changing these unhealthy behaviors should contribute to a better glycemic level.

In the DSMES group, 4.6% of patients was in precontemplation stage, 34% of patients was in contemplation stage, and 60% was in preparation stage at baseline of our study. Interestingly, 70% of patients moved to the action and maintenance stage at 12 months. Using various kinds of techniques by CDE during DSME session might be one factor that result in this significant progression of behavior change. The first important step in our DSMES session was establishing the rapport by appropriate greeting, making an effort to know the patient as a person,<sup>16</sup> and paying attention to the patients. After collecting information and evaluating, CDE designed the content of the session and used the appropriate change process for each patient. If patients are in the pre-contemplation or contemplation stage, CDE will try to raise awareness, increase pros, and overcome cons (decision balance principle). If the patients are in the preparation stage, CDE will encourage the patients to set the SMART goal of behavior change and develop a realistic plan together.<sup>8</sup> Because changing behavior is a continuous process

that can move forward or backward, following up with the patient is a very important step. During follow up sessions, CDE re-evaluated stage of behavior change, worked together with patients to explore barriers and find solutions for behavior change, provided positive feedback and empowered patients to believe in their own ability.

Chronic diabetic complications cause significant comorbidities and disabilities such as coronary artery disease, end stage renal disease, blindness, and amputation.<sup>17-19</sup> Diabetic nephropathy, retinopathy, and foot problems should be screened at least annually for early detection and treatment.<sup>20,21</sup> Our study showed that DSMES can improve the rate of screening for chronic diabetic complications. We hypothesized that the rate of complication screening increased because patients gained more knowledge and awareness about diabetic complications after receiving DSMES. Additionally, CDE are authorized to schedule appointments for complication screenings for patients according to our hospital pathway. Our finding was consistent with the systematic review from the United Kingdom, which indicated that the suggestion of a healthcare provider and their knowledge about the effects of non-attendance on vision were facilitators for retinal screening in patients with diabetes.<sup>22</sup>

Our study had some limitations. First, we used retrospective data from patients who received usual DE from a healthcare professional before the establishment of CDE as a comparator group because DSMES provided by CDE is our standard care in our hospital right now. Therefore, we did not have data on diabetes knowledge, stage of behavior change, and quality of life in the usual DE group. Second, there was a COVID-19 pandemic during our study that resulted in a change in some follow-up visits from face-to-face to telephone consultation.

## CONCLUSION

Our study found that CDE-provided DSMES can decrease A1C 3 months early and maintain its benefit until 12 months. It can also improve diabetes knowledge, stage of behavior change, quality of life, and the rate of chronic diabetic complication screening.

### Practice implications

Proving DSMES by CDE using motivational interviewing, good communication skills and stage of change principle at visits 0 and 3, 6, and 12 months with estimated total contact time of 4 hours can help people with T2D to control their blood glucose and improve quality of life.

## ACKNOWLEDGEMENTS

The authors appreciate all Siriraj Certified Diabetes Educators for contributing to data collection and Siriraj Diabetes Center of Excellence and Department of Nursing, Faculty of Medicine Siriraj Hospital, for facilitating this research.

## Funding disclosure

This study was supported by the Routine to Research Unit (R2R), Faculty of Medicine Siriraj Hospital Mahidol University [grant no. RO16335004].

## Author contributions

K.W., N.T. and L.P. conceived and designed the study plan. K.W., N.T., W.K., S.U., V.L. and L.P. acquired, analyzed, and interpreted the data. K.W., N.T., and L.P. drafted the article. All authors reviewed, edited, and approved the final version of the article.

## REFERENCES

1. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. 2022;183:109119.
2. วิจัย เอกพลภกร. รายงานการสำรวจสุขภาพประชาชนไทยโดยการตรวจร่างกาย ครั้งที่ 6 พ.ศ. 2562 - 2563.
3. Preechasuk L, Tengtrakulcharoen P, Karaketklang K, Rangsin R, Kunavisarut T. Achievement of metabolic goals among different health insurance schemes in Thai patients with type 2 diabetes mellitus: A nationwide study. *Siriraj Med J.* 2020;72(1): 1-9.
4. Powers MA, Bardsley JK, Cypress M, Funnell MM, Harms D, Hess-Fischl A, et al. Diabetes Self-management Education and Support in Adults With Type 2 Diabetes: A Consensus Report of the American Diabetes Association, the Association of Diabetes Care & Education Specialists, the Academy of Nutrition and Dietetics, the American Academy of Family Physicians, the American Academy of PAs, the American Association of Nurse Practitioners, and the American Pharmacists Association. *Diabetes Care.* 2020;43(7):1636-49.
5. Preechasuk L, Sriussadaporn P, Likitmaskul S. The obstacles to diabetes self-management education and support from healthcare professionals' perspectives: a nationwide survey. *Diabetes Metab Syndr Obes.* 2019;12:717-27.
6. Wongwiwatthanukit S, Krittiyanunt S, Wannapinyo A. Development and validation of an instrument to assess the general knowledge of patients with diabetes. *Thai J Pharm Sci.* 2004;28(1-2):17-29.
7. Thai\_WHOQOL-BREF [Available from: <https://www.who.int/tools/whoqol/whoqol-bref/docs/default-source/publishing-policies/whoqol-bref/thai-whoqol-bref>].
8. Prochaska JO, Velicer WF. The transtheoretical model of health behavior change. *Am J Health Promot.* 1997;12(1):38-48.
9. Chivala CA, Sherr D, Lipman RD. Diabetes self-management education for adults with type 2 diabetes mellitus: A systematic

- review of the effect on glycemic control. *Patient Educ Couns*. 2016;99(6):926-43.
10. Mikhael EM, Hassali MA, Hussain SA. Effectiveness of Diabetes Self-Management Educational Programs For Type 2 Diabetes Mellitus Patients In Middle East Countries: A Systematic Review. *Diabetes Metab Syndr Obes*. 2020;13:117-38.
  11. Arlinghaus KR, Johnston CA. Advocating for Behavior Change With Education. *Am J Lifestyle Med*. 2018;12(2):113-6.
  12. Razaz JM, Rahmani J, Varkaneh HK, Thompson J, Clark C, Abdulazeem HM. The health effects of medical nutrition therapy by dietitians in patients with diabetes: A systematic review and meta-analysis: Nutrition therapy and diabetes. *Prim Care Diabetes*. 2019;13(5):399-408.
  13. Bowen ME, Cavanaugh KL, Wolff K, Davis D, Gregory RP, Shintani A, et al. The diabetes nutrition education study randomized controlled trial: A comparative effectiveness study of approaches to nutrition in diabetes self-management education. *Patient Educ Couns*. 2016;99(8):1368-76.
  14. ElSayed NA, Aleppo G, Aroda VR, Bannuru RR, Brown FM, Bruemmer D, et al. 5. Facilitating Positive Health Behaviors and Well-being to Improve Health Outcomes: Standards of Care in Diabetes-2023. *Diabetes Care*. 2023;46(Suppl 1):S68-s96.
  15. Lin LK, Sun Y, Heng BH, Chew DEK, Chong PN. Medication adherence and glycemic control among newly diagnosed diabetes patients. *BMJ Open Diabetes Res Care*. 2017;5(1):e000429.
  16. Ziegelstein RC. Perspectives in Primary Care: Knowing the Patient as a Person in the Precision Medicine Era. *Ann Fam Med*. 2018;16(1):4-5.
  17. Leasher JL, Bourne RR, Flaxman SR, Jonas JB, Keeffe J, Naidoo N, et al. Erratum. Global Estimates on the Number of People Blind or Visually Impaired by Diabetic Retinopathy: A Meta-analysis From 1990-2010. *Diabetes Care* 2016;39:1643-9. *Diabetes Care*. 2016;39(11):2096.
  18. Ezzatvar Y, García-Hermoso A. Global estimates of diabetes-related amputations incidence in 2010–2020: A systematic review and meta-analysis. *Diabetes Res Clin Pract*. 2023;195:110194.
  19. Tangvarasittichai S, Mhaoprasit K. Microalbumin Detection in Diabetes Mellitus Patients. *Siriraj Med J*. 2003;55(5):236-42.
  20. ElSayed NA, Aleppo G, Aroda VR, Bannuru RR, Brown FM, Bruemmer D, et al. 11. Chronic Kidney Disease and Risk Management: Standards of Care in Diabetes-2023. *Diabetes Care*. 2023;46(Suppl 1):S191-S202.
  21. ElSayed NA, Aleppo G, Aroda VR, Bannuru RR, Brown FM, Bruemmer D, et al. 12. Retinopathy, Neuropathy, and Foot Care: Standards of Care in Diabetes-2023. *Diabetes Care*. 2023;46(Suppl 1):S203-S15.
  22. Kashim RM, Newton P, Ojo O. Diabetic Retinopathy Screening: A Systematic Review on Patients' Non-Attendance. *Int J Environ Res Public Health*. 2018;15(1):157.

# Real-world data on the Immunity Response to the COVID-19 Vaccine among Patients with Central Nervous System Immunological Diseases

Punchika Kosiyakul, M.D.<sup>1,2,3</sup>, Jiraporn Jitprapaikulsan, M.D.<sup>1,2</sup>, Ekdanai Uawithya<sup>4</sup>, Patimaporn Wongprompitak, Ph.D.<sup>5</sup>, Chutikarn Chaimayo, M.D., Ph.D.<sup>6</sup>, Navin Horthongkham, Ph.D.<sup>6</sup>, Nasikarn Angkasekwina, M.D.<sup>7</sup>, Nanthaya Tisavipat, M.D.<sup>1,2</sup>, Naraporn Prayoonwiwat, M.D.<sup>1,2</sup>, Natthapon Rattanathamsakul, M.D.<sup>1,2</sup>, Kanokwan Boonyapisit, M.D.<sup>1</sup>, Theerawat Kumutpongpanich, M.D.<sup>1</sup>, Onpawee Sangsai, M.Sc.<sup>2</sup>, Kamonchanok Aueaphatthanawong, B.Sc.<sup>2</sup>, Jirawan Budkum, M.Sc.<sup>2</sup>, Sasitorn Siritho, M.D.<sup>1,2,8</sup>

<sup>1</sup>Division of Neurology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10700, Thailand, <sup>2</sup>Siriraj Neuroimmunology Center, Division of Neurology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10700, Thailand, <sup>3</sup>Department of Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, <sup>4</sup>Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10700, Thailand, <sup>5</sup>Department of Immunology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10700, Thailand, <sup>6</sup>Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10700, Thailand, <sup>7</sup>Division of Infectious disease and tropical medicine, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10700, Thailand, <sup>8</sup>Bumrungrad International Hospital, Bangkok 10110, Thailand.

## ABSTRACT

**Objective:** The effects of immunotherapies on the immune response to various regimens of SARS-CoV-2 vaccines in patients with autoimmune neurological disease have been demonstrated in limited data. Thus, we evaluated the immune responses in each platform of COVID-19 vaccination between patients with autoimmune neurological disease and a healthy population.

**Materials and Methods:** We conducted a prospective observational study. We collected serum from patients with autoimmune neurological diseases to perform serological methods using anti-RBD IgG assay, neutralizing antibodies assay, and interferon SARS-CoV-2 immunoassay. Serological response level was analyzed by platforms of vaccines and types of immune modifying therapy.

**Results:** Fifty-eight patients had tested for an anti-RBD IgG response, and those receiving no immunotherapy/healthy controls had the highest median anti-RBD IgG levels amongst immunotherapy statuses. Rituximab in those who received inactivated or mRNA vaccine regimens had the lowest antibody level compared with other immunotherapies. In vector-based vaccine regimens, significant reductions of anti-RBD IgG response were observed in all other immunotherapy groups except for azathioprine, with the greatest difference seen compared to rituximab. Thirty-five patients with positive anti-RBD responses were further tested for neutralizing antibodies. The mRNA vaccine regimen demonstrated the highest inhibition percentage among the Delta and Omicron variants. Twenty-two patients were tested for T cell responses, with no significant difference in T-cell activity across all groups.

**Conclusion:** We have demonstrated a significant decrease in antibody response against SARS-CoV-2 in patients with autoimmune neurological diseases receiving immunotherapies compared to a healthy population, especially for patients taking rituximab.

**Keywords:** Neuromyelitis optica spectrum disorder; multiple sclerosis; COVID-19 vaccine; immunosuppressant; humoral immune response (Siriraj Med J 2024; 76: 69-79)

Corresponding author: Sasitorn Siritho

E-mail: siritho@yahoo.com

Received 7 December 2023 Revised 26 December 2023 Accepted 26 December 2023

ORCID ID: <http://orcid.org/0000-0002-2562-697X>

<https://doi.org/10.33192/smj.v76i2.266638>



All material is licensed under terms of the Creative Commons Attribution 4.0 International (CC-BY-NC-ND 4.0) license unless otherwise stated.

## INTRODUCTION

The Coronavirus Disease-19 (COVID-19) pandemic has prompted mass vaccination efforts worldwide. Numerous novel vaccines against the severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) have been engineered. The platforms used range from inactivated viruses to protein subunits, viral vectors, and messenger ribonucleic acid (mRNA). Studies in general populations have shown variable vaccine efficacy and adverse events following immunization related to the vaccine with each regimen of vaccine.<sup>1-3</sup> However, patients using immunosuppressive agents as a special very high risk of COVID-19 population were excluded from pivotal phase III vaccine trials.<sup>4</sup>

Immune-mediated neurological diseases, such as multiple sclerosis (MS), neuromyelitis optica spectrum disorder (NMOSD), and other autoimmune neurological diseases (AINDs), are rare diseases.<sup>5,6</sup> The cornerstone of management of immune-mediated neurological diseases is immunomodulatory and immunosuppressive therapies, which are known as immunotherapies (IMTs). Examples include azathioprine (AZA), mycophenolate mofetil (MMF), and rituximab (RTX), which are sometimes used as an alternative to immunomodulators, especially in resource-limited settings.<sup>7,8</sup> AZA and MMF are anti-metabolites that decrease T and B lymphocyte proliferation while RTX specifically suppresses B lymphocytes targeting CD20. While achieving disease control, these agents need to be monitored for their immunosuppressive effects, which could confer greater susceptibility to infection.<sup>9</sup>

Some studies have observed a lower immune response to COVID-19 vaccines in patients taking immunosuppressive agents compared to healthy controls.<sup>10</sup> Low immunoglobulin levels due to B lymphocyte suppression could attenuate vaccine responsiveness, particularly among those taking anti-CD20 RTX.<sup>11</sup> Measurement of specific immunity to SARS-CoV-2, including IgG to a receptor-binding domain (anti-RBD IgG), neutralizing antibody (NAbs), and interferon-gamma release assay (IGRA) could be quantitative surrogate markers for vaccine efficacy. Therefore, we aimed to compare the immune responses after each regimen of COVID-19 vaccination between patients with AIND and a healthy control population.

## MATERIALS AND METHODS

### Participants and samples

This prospective observational study was conducted at Siriraj Hospital, a university hospital referral center in Thailand. Sera from patients with AIND attending the Neurology Clinic at Siriraj Hospital from December 2021 to July 2022 were collected one week before and

one month after the COVID-19 vaccination.

The inclusion criteria were age greater than 18 years and receiving at least two doses of COVID-19 vaccination. Patients lost to follow-up or not able to give post-vaccination sera were excluded. Also, those with a history of COVID-19 infection in the past 6 months or anti-RBD IgG seropositivity at baseline were excluded. Patients with AIND recruited into the study were matched with healthy controls receiving a vaccine against COVID-19 to compare their responsiveness to the vaccine regimens.

Clinical data, including sex, diagnosis, age at disease onset, disease duration, the total number of previous attacks before the first COVID-19 vaccination, current IMT, the number of prior IMTs used, and the most recent follow-up Expanded Disability Status Scale (EDSS) were collected. COVID-19 vaccination status, platforms used, and doses received were documented. We performed matching healthy controls by age, sex, and vaccination regimen.

Participants in the healthy control group were healthy adults aged  $\geq 18$  years in whom sera were collected from two prospective cohort studies of vaccination of either two doses of CoronaVac or two doses of ChAdOx1<sup>12</sup>, and third-dose booster vaccination either BBIBP-CorV, ChAdOx1, 30 $\mu$ g-BNT162b2, or 15 $\mu$ g-BNT162b2.<sup>13</sup> All patients were informed the information about each vaccine and their possible adverse events according to the government brochure.

We defined groups of vaccines for comparison. We defined inactivated vaccine regimen as those having received two doses of an inactivated vaccine. We defined vector-based vaccine regimen as those having received two doses of vector-based vaccines, one dose of each of an inactivated and a vector-based vaccine, and vector-based vaccine as the third dose. Finally, we defined mRNA vaccine regimen as those having received two doses of mRNA vaccine and mRNA vaccine as the third dose.

All participants received their COVID-19 vaccination regimens according to the availability of the vaccines at that time, each patient's preference, and the Thai Ministry of Public Health's recommendations.<sup>14</sup> Most Thai people received a mix-and-match, prime-boost immunization strategy.

The Siriraj Institutional Review Board approved this study (COA no. Si 707/2021). All patients gave written informed consent. The study was performed in accordance with the Declaration of Helsinki of 1975.

### Serum immunologic testing

Serum immunologic tests were anti-RBD IgG assay<sup>15</sup>,

NAbs assay<sup>16</sup>, and interferon SARS-CoV-2 immunoassay.<sup>17,18</sup> We defined an anti-RBD IgG assay titer level of greater or equal to 7.1 BAU/mL as seropositive. The cut off value for inhibition response was set at 30%. The cut-off value for Ag1-Nil and Ag2-Nil was set at 0.2 IU/mL.<sup>18</sup> The details for each test are described in the Appendix.

### Statistical analysis

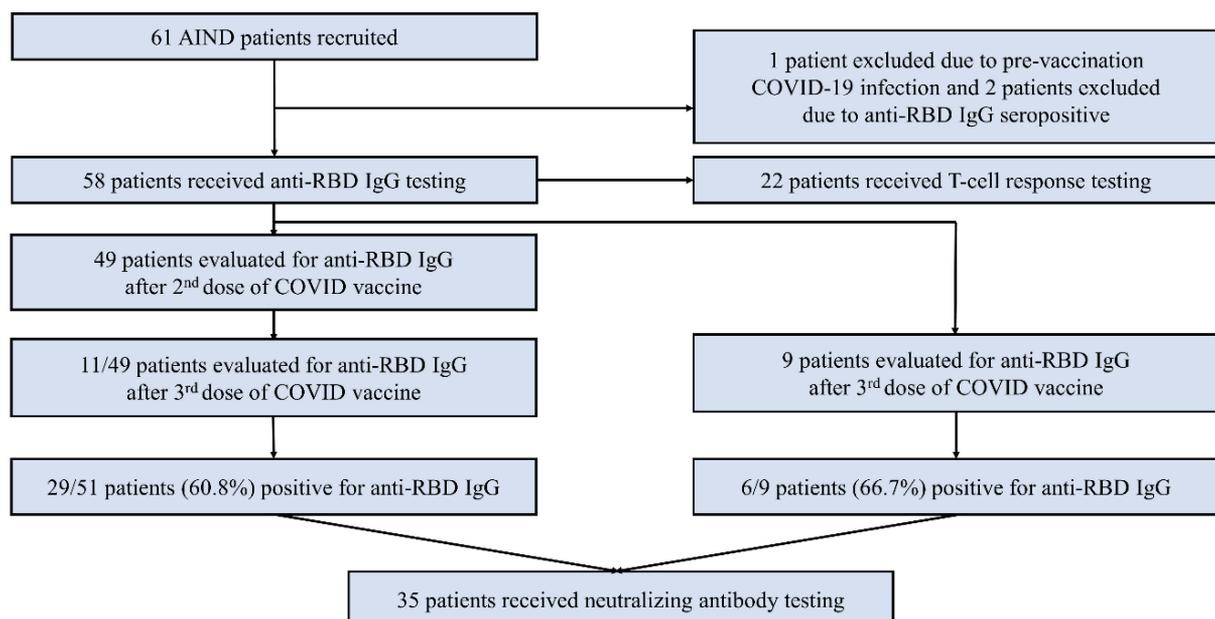
We analyzed the data descriptively. Categorical data are displayed as frequency (%), and continuous data are displayed as median (interquartile range [IQR]). Categorical data was analyzed by the  $\chi^2$  test or Fisher's exact test if less than 75% of cells had expected frequencies of greater than five. Continuous data were compared by nonparametric, rank-based Mann-Whitney U test for pairwise testing or the Kruskal Wallis test if there were more than two groups to compare. Pre- and post-vaccination anti-RBD IgG levels were compared by Wilcoxon signed-rank test for non-parametric, non-independent data. All data were analyzed on PASW Statistics for Windows version 18.0 (SPSS Inc., Chicago, IL) and Graphpad Prism version 9 (GraphPad Software, San Diego, CA).<sup>19</sup> A *p*-value of less than 0.05 was considered significant. No correction for multiplicity was applied.

## RESULTS

Sixty-one patients with AIND were recruited. Three were excluded due to evidence of prior COVID-19 infection (history of COVID-19 infection three months

before eligibility assessment *n* = 1 and anti-RBD-IgG seropositivity *n* = 2). Thus, 58 patients were included in the final analysis (having received inactivated vaccine regimen *n* = 15, having received a vector-based vaccine regimen *n* = 31, and having receive a mRNA vaccine regimen *n* = 22). Of the 58 patients, 46 (78.3%) were female. All 58 patients were evaluated for anti-RBD IgG. The T cell response test was performed in only 22 patients due to limited availability. Thirty-five patients with anti-RBD IgG positivity received further testing for NAbs. Fourteen patients were tested for all the three immunity assays (Fig 1).

Of the 58 patients, the frequencies of immunotherapy status were no immunotherapy (*n* = 6), azathioprine (*n* = 15), MMF (*n* = 13), rituximab (*n* = 18), fingolimod (*n* = 4), glatiramer acetate (*n* = 1), and prednisolone (*n* = 1). Diagnoses included were NMOSD (*n* = 27), MS (*n* = 15), myasthenia gravis (MG) (*n* = 5), clinically isolated syndrome (*n* = 4) [idiopathic single transverse myelitis (*n* = 2), isolated demyelinating brainstem syndrome (*n* = 1), and single optic neuritis (*n* = 1)], autoimmune encephalitis (*n* = 3) [anti-N-methyl D-aspartate encephalitis (*n* = 2) and seronegative autoimmune encephalitis (*n* = 1)], pachymeningitis (*n* = 3) [eosinophilic granulomatosis with polyangiitis with pachymeningitis (*n* = 1), idiopathic pachymeningitis (*n* = 1), and IgG4-related pachymeningitis (*n* = 1)], and myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD) (*n* = 1). All groups of patients had similar age at onset,



**Fig 1.** Flowchart of patients with serum immunologic testings

*Notes:* Data were available as the following: 58 patients had anti-RBD IgG level, 35 patients had neutralizing antibodies, and 22 patients had T cell responses.

**Abbreviations:** AINDs, autoimmune neurological diseases; RBD, receptor-binding domain

EDSS scores, interval from disease onset to the third dose of vaccination, interval from the most recent treatment initiation to the third dose of vaccine administration, and concomitant diseases. Conversely, interval from disease onset to the second dose vaccination and interval from the latest treatment initiation to the second vaccine were significantly different (both  $p < 0.05$ ) (Table 1).

### **Anti-RBD IgG Response to the SARS-CoV2 Virus**

Forty-nine patients had anti-RBD antibodies testing after their second dose of COVID-19 vaccination, of which 15 patients had received two doses of an inactivated vaccine (either CoronaVac or BBIBP-CorV COVID-19 vaccine), 27 patients had received two doses of the vector-based ChAdOx1 nCoV-19 vaccine, 4 patients had received two doses of an mRNA vaccine (either the mRNA-1273 SARS-CoV-2 vaccine or the BNT162b2 nCoV-19 vaccine), and 3 patients had received combinations of CoronaVac and ChAdOx1 nCoV-19. Of the 49 patients, 11 patients had additional anti-RBD antibody testing after the third vaccination. The remaining 9 of the 58 patients had anti-RBD antibody testing performed after the third vaccination. (Fig 1)

#### *Comparison of Anti-RBD IgG Responses by Vaccine Regimen*

Only 13 patients had data on pre-vaccination anti-RBD antibodies levels. Of these, 10 (76.9%) demonstrated a 23-fold rising in antibody titer compared with the baseline value with a median anti-RBD level of 0.21 BAU/mL (IQR 0.16-0.43) pre-vaccination and 4.81 BAU/mL (IQR 0.17- 33.41) post-vaccination ( $p = 0.013$ ). (Supplementary Fig S1)

After the second vaccination, 29 of 49 (59.2%) patients had rising antibody titers above the cut point for seropositivity with a median level of 15.25 BAU/mL (IQR 0.47-152.11).

mRNA vaccine regimens seemed to induce higher anti-RBD levels than the vector-based vaccine regimens, and inactivated vaccine regimen demonstrated the least immunogenicity. For all 68 sera of 58 patients included in the final analysis, the seropositive rates for inactivated vaccine, vector-based vaccine, and mRNA vaccine regimens for anti-RBD were 26.7% (4 of 15), 71% (22 of 31), and 72.7% (16 of 22), respectively. For inactivated vaccine, vector-based vaccine, and mRNA vaccine regimens, the median levels were 0.58 BAU/mL (IQR 0.24-7.50), 52.82 BAU/mL (IQR 5.61-171.79), and 969.77 BAU/mL (IQR 3.41-2382.53) BAU/mL, respectively ( $p < 0.001$ ). (Fig 2A)

The NMOSD group demonstrated the highest anti-RBD IgG level for mRNA vaccine regimens [1062.87 BAU/mL (IQR 93.29-2151.47)], followed by vector-based vaccine regimens [106.22 BAU/mL (IQR 51.39-298.21)], and inactivated vaccine regimens [3.01 BAU/mL (IQR 0.18-91.50)], respectively ( $p = 0.030$ ) (Fig 2B). Conversely, MS patients did not demonstrate any significant difference among mRNA regimens, vector-based regimens, and inactivated vaccine regimens with median levels at 2.78 BAU/mL (IQR 0.39-3803.75), 5.61 BAU/mL (IQR 0.20-14.00), and 0.36 BAU/mL (IQR 0.21-7.50), respectively ( $p = 0.666$ ) (Fig 2C). Similarly, patients in the other AINDs group did not demonstrate any significant differences across all vaccine regimens with median levels at 937.88 BAU/mL (IQR 51.30- 5196.40) for mRNA regimens, 28.41 BAU/mL (IQR 2.51-135.64) for vector-based regimens, and 1.33 BAU/mL (IQR 0.41-34.93) for inactivated vaccine regimens ( $p = 0.069$ ) (Fig 2D).

#### *Comparison of Anti-RBD IgG Responses by Type of Immunotherapy*

Compared to the no IMT/healthy control group, patients treated with RTX having received inactivated vaccine regimen showed a significant reduction of anti-RBD response [112.54 BAU/mL (IQR 53.30-171.32) vs. 0.26 BAU/mL (IQR 0.12-0.36);  $p = 0.010$ ]. (Fig 2E)

For those having received a vector-based vaccine regimen, compared to the no IMT/healthy control group, significant reductions of anti-RBD IgG responses were observed in those treated with RTX, FGM, and MMF [no IMT/healthy controls 235.03 BAU/mL (IQR 96.07-405.77) vs. each of RTX 0.42 BAU/mL (IQR 0.06-61.21);  $p = 0.002$ , FGM 4.81 BAU/mL (IQR 0.20-5.61);  $p < 0.001$ , and MMF 50.91 BAU/mL (IQR 8.18-71.86);  $p = 0.001$ ]. However, no significant reduction was seen comparing no IMT/healthy controls to AZA. (Fig 2F)

For those having received an mRNA vaccine regimen, the no IMT/healthy controls had a remarkably higher anti-RBD-IgG level [4633.13 BAU/mL (IQR 2256.71-7415.94)]. In comparison to the no IMT/healthy controls, RTX showed a significant reduction in anti-RBD level [0.34 BAU/mL (IQR 0.14-49.58);  $p = 0.010$ ] while the FGM group showed some reduction without significance [0.77 BAU/mL (IQR 0.01-1.53);  $p = 0.133$ ]. (Fig 2G)

#### *Neutralizing antibodies for delta and omicron variants*

Amongst the 35 patients seropositive for anti-RBD IgG, 41 serum samples were further evaluated for NAbs against specific COVID-19 variants.

**TABLE 1.** Demographic and characteristics of patients with AINDs

Characteristics	No immunotherapy (n = 6)	Azathioprine (n = 15)	MMF (n = 13)	RTX (n = 18)	Fingolimod (n = 4)	Other: Glatiramer acetate, Prednisolone (n = 2)	P-value
Female gender, n (%)	6 (100)	14 (93.3)	13 (100)	10 (55.6)	2 (50)	1 (50)	0.003
Age at onset, median (range), y	53.5 (29.3, 63.8)	45.0 (35.0, 55.0)	44.0 (26.5-50.5)	35.5 (21.3, 43.8)	38.5 (28.5, 47.0)	40.5	0.447
EDSS <sup>‡</sup> , median (range)	1.25 (1.00, 5.63)	1.0 (1.0, 1.5)	1.25 (1, 4.12)	1 (0, 3.38)	1.0 (0.25, 2.50)	1.0 (1.0, 1.0)	0.799
Duration from disease onset to vaccine administration, median (interquartile range), month							
To 2 <sup>nd</sup> dose	119.34 (NA)	93.8 (29.3, 141.4)	123.98 (50.3, 206.1)	47.9 (21.2, 87.6)	81.9 (36.2, 137.9)	4.73 (NA)	0.027
To 3 <sup>rd</sup> dose	23.1 (20.4, 109.5)	129.94 (NA)	149.8 (59.3, 240.2)	47.2 (32.2, 74.4)	92.76 (NA)	5.82 (NA)	0.166
Duration from the latest treatment initiation to vaccine administration <sup>‡</sup> , median (interquartile range), month							
To 2 <sup>nd</sup> dose	NA	2.72 (1.77, 3.73)	2.72 (0.46, 3.79)	6.80 (3.22, 7.52)	1.91 (1.47, 2.07)	1.97 (NA)	0.002
To 3 <sup>rd</sup> dose	NA	7.92 (NA)	7.56 (5.63, 8.95)	9.96 (8.81, 23.2)	5.90 (NA)	7.00	0.055
Comorbidities <sup>§</sup> , n (%)	4 (66.7)	9 (60)	7 (53.8)	6 (33.3)	4 (100)	1 (50)	0.198
Current disease, n (%)							
MS	1 (16.7)	2 (13.3)	2 (15.4)	5 (27.8)	4 (100)	1 (50)	0.018
NMO	1 (16.7)	9 (60)	8 (61.5)	9 (50)	0	0	0.089
Others AIND*	4 (66.7)	4 (26.7)	3 (23.1)	4 (22.2)	0	1 (50)	0.239
Number of attacks <sup>‡</sup> , median (range)	1.0 (1.0, 2.75)	1.0 (1.0, 5.0)	3.0 (2.0, 10.5)	2.0 (1.0, 3.25)	2.0 (2.0, 3.5)	1.0 (1.0, 1.0)	0.094
Vaccine regimen, n (%)							
Inactivated vaccine regimen	0	4 (26.7)	4 (30.8)	6 (33.3)	1 (25.0)	0	0.194
Vector-based vaccine regimen	2 (20.0)	9 (60.0)	7 (53.8)	8 (44.4)	3 (75.0)	2 (100)	0.035
mRNA vaccine regimen	4 (80.0)	4 (26.7) <sup>††</sup>	6 (46.2) <sup>††</sup>	6 (33.3) <sup>§§</sup>	2 (50.0)	0	0.848
Serum immunologic testing, n (%)							
Tested for RBDs	6 (100)	15 (100)	13 (100)	18 (100)	4 (100)	2 (100)	1
Tested for NAbs**	5 (83.3)	15 (100)	8 (61.5)	6 (33.3)	0 (0)	1 (50.0)	<0.001
Tested for IGRA	3 (50.0)	3 (20.0)	6 (46.2)	7 (38.9)	2 (50.0)	1 (50.0)	0.623

**Abbreviations:** AINDs, autoimmune neurological diseases; EDSS, Expanded Disability Status Scale; IGRA Interferon SARS-CoV-2; IS, immunosuppressive; MS, multiple sclerosis; NAbs, neutralizing antibodies; NMOSD, neuromyelitis optica spectrum disorder; RBDs, anti-RBD IgG level

\*Clinical findings of other patients consisted of myasthenia gravis (n = 5), autoimmune encephalitis (n = 3), MOGAD (n = 1), pachymeningitis (n = 2), and clinically isolated syndromes (n = 5)

<sup>‡</sup>EDSS scores were evaluated in MS and NMOSD patients at the last visit before blood drawing for assay testings<sup>‡</sup>EDSS scores available in 44 patients

<sup>§</sup>For patients receiving immunotherapy

<sup>¶</sup>Of 51 patients, 2 received no immunotherapy

<sup>‡</sup>Of 20 patients, 4 received no immunotherapy

<sup>§</sup>Comorbidities included diabetes mellitus, hypertension, dyslipidemia, vitamin D deficiency, obstructive sleep apnea, obesity, cirrhosis, systemic lupus erythematosus, Hashimoto thyroiditis, coronary artery disease, thymoma, herpes keratoconjunctivitis, and osteoarthritis

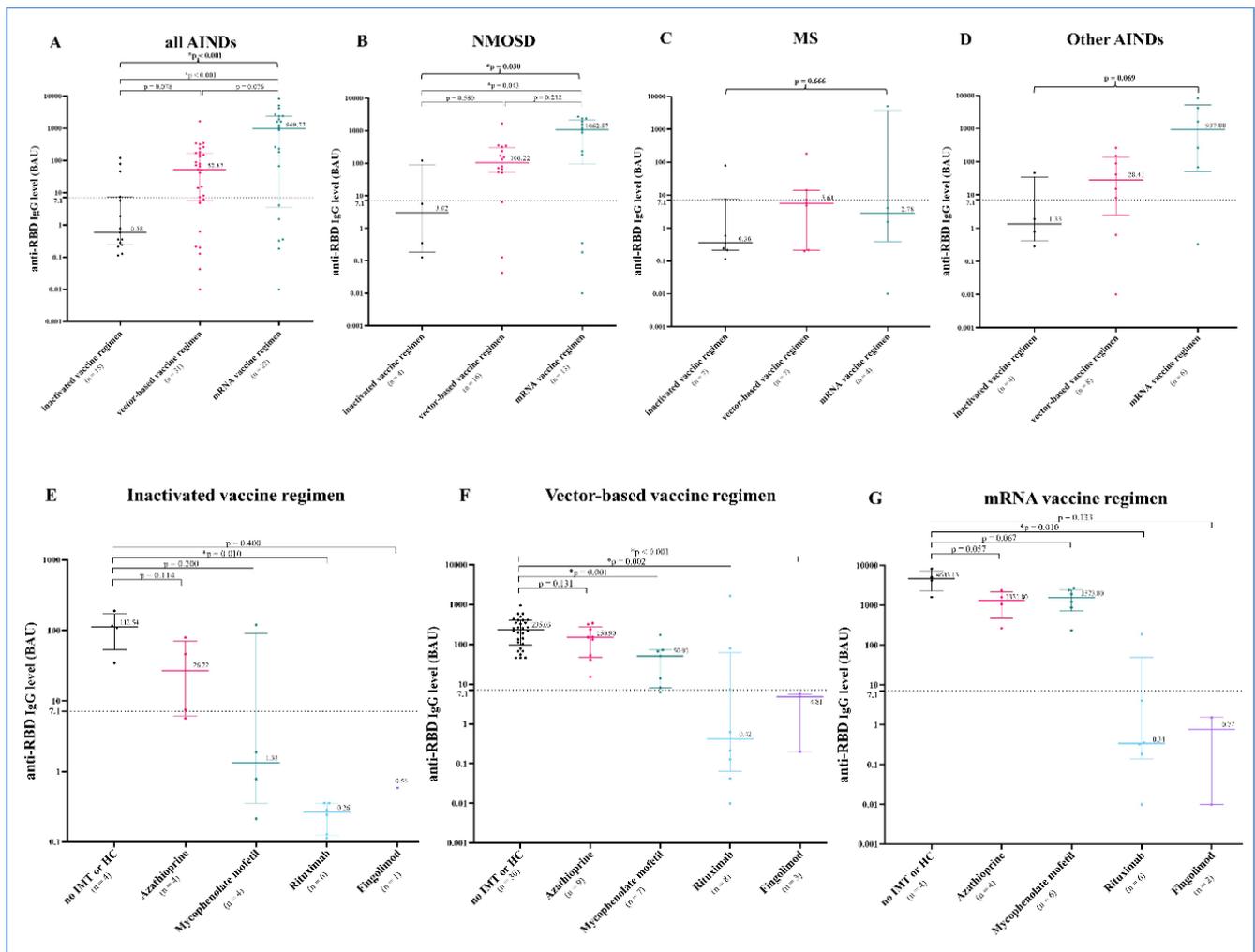
<sup>¶</sup>The number of attacks was evaluated in MS and NMOSD patients

<sup>\*\*</sup>Only patients with positive anti-RBD responses further analysis for neutralizing antibody response

<sup>††</sup>2 patients received vector-based vaccine as the first two doses and mRNA vaccine for third vaccine dose.

<sup>‡‡</sup>4 patients received vector-based vaccine as the first two doses and mRNA vaccine for third vaccine dose.

<sup>§§</sup>1 patient received vector-based vaccine as the first two doses; 1 patient received inactivated vaccine as the first two doses; and both received mRNA vaccine for third vaccine dose.



**Fig 2.** Anti-RBD IgG spike values categorized by types of vaccine regimens and immunotherapies  
**Fig 2A-2D:** Nonparametric distributions are displayed as median (interquartile range) by bar and error bar, respectively. Anti-RBD IgG spike values categorized by type of vaccine regimen in different autoimmune neurological disease groups.  
*Notes for figures A-D:-* (A) All AINDs, (B) NMOSD, (C) MS, and (D) other AINDs.  
**Fig 2E-2G:** Nonparametric distributions are displayed as median (interquartile range) by bar and error bar, respectively Anti-RBD IgG responses in different vaccination categorized by different immunotherapy agents  
*Notes for figures E-G:-* Groups compared were healthy control or no immunotherapy, azathioprine, mycophenolate mofetil, rituximab, and fingolimod(E) two doses of any vaccines, (F) three doses of any vaccines. Statistical significance was calculated by the Mann–Whitney U test. The anti-RBD IgG cut-off value (dash line) was 7.1 BAU/mL. \*denotes a statistically significant result at the  $p < 0.05$  level.  
**Abbreviations:** RBD, receptor-binding domain; AINDs, autoimmune neurological diseases; NMOSD, neuromyelitis optica spectrum disorder; MS, multiple sclerosis; HC, healthy control; IMT, immunotherapy

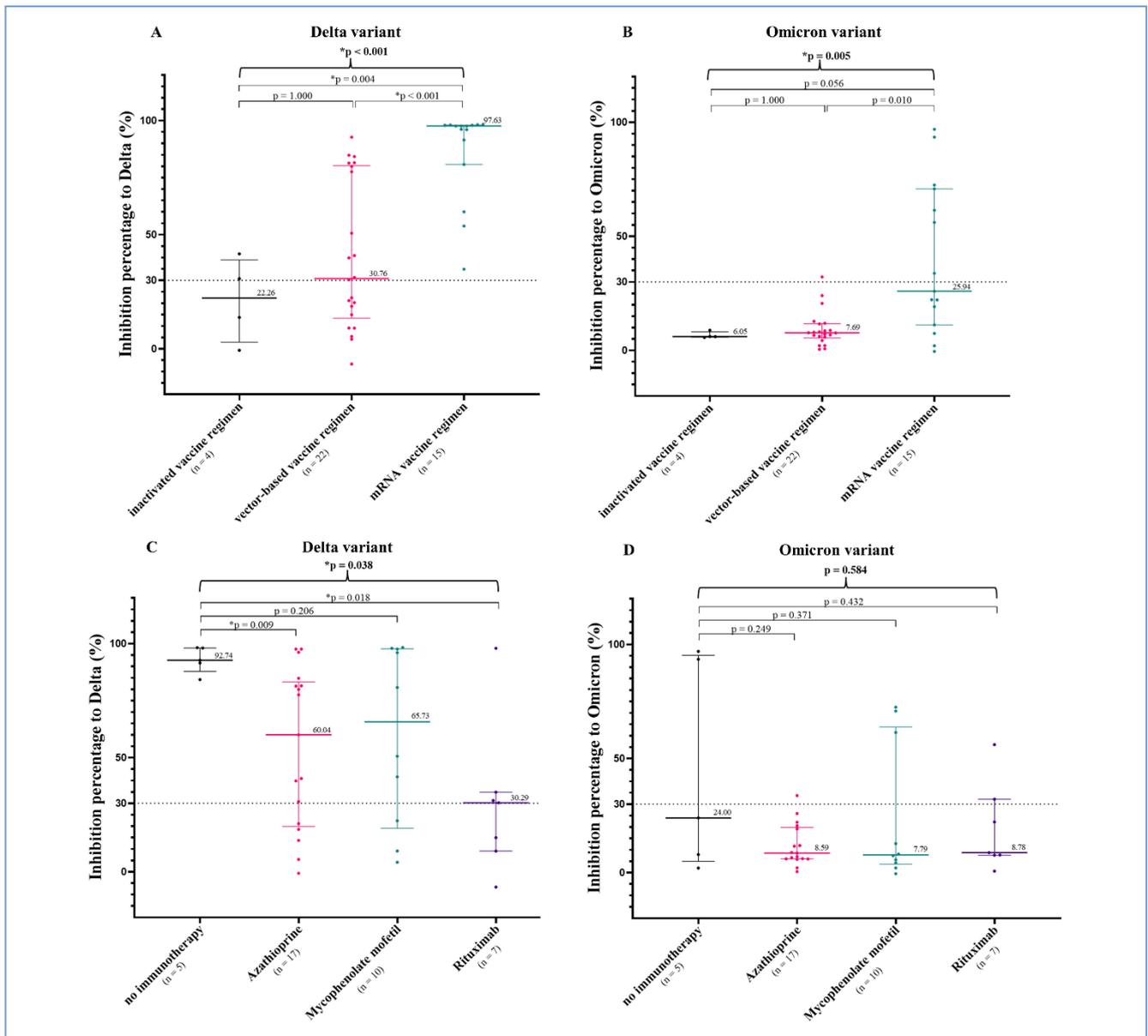
*Inhibition percentage categorized by vaccine regimen*

The overall median inhibition percentages were higher in the Delta variant than the Omicron one regardless of vaccine regimen [53.84% (IQR 20.69-94.40) vs. 8.59% (IQR 6.05-23.09);  $p < 0.001$ ].

The median NAb inhibition percentage for the Delta variant was the highest in mRNA vaccine regimens [97.63% (80.77, 98.00)], followed by vector-based vaccine regimens [30.76% (IQR 13.48-80.32)], and inactivated vaccine regimens [22.3% (IQR 2.94-38.92)], respectively ( $p < 0.001$ ). There were significant pairwise differences between mRNA and inactivated vaccine regimens ( $p = 0.004$ ) along with between mRNA and vector-based

vaccine regimens ( $p < 0.001$ ). (Fig 3A)

The same pattern was mostly seen in the Omicron variant. mRNA vaccine regimens had the highest median inhibition percentage [25.94% (IQR 11.15-70.84)], followed by vector-based regimens [7.69% (IQR 5.44-11.67)] and inactivated vaccine regimens [6.05 (IQR 5.77-8.13)] ( $p = 0.005$ ). Also, there was a significant pairwise difference between mRNA vaccines and vector-based vaccines ( $p = 0.010$ ) and a trend to significance between mRNA vaccine regimens and inactivated vaccine regimens ( $p = 0.056$ ). None of the regimens showed an inhibition percentage greater than 30% for the Omicron variant. (Fig 3B)



**Fig 3.** Neutralizing antibody inhibition percentage categorized by each vaccine regimen and each type of immunotherapy  
**Fig 3A & 3B:** Inhibition percentage of neutralizing antibody categorized by each vaccine regimen in different COVID-19 variants for (A) Delta variant, (B) Omicron variant.

*Notes for figures A-B:-* Nonparametric distributions are displayed as median (interquartile range) by bar and error bar, respectively. The cut off value (dashed line) for inhibition response was set at 30%. Statistical significance was calculated by the Kruskal-Wallis test. \*denotes a statistically significant result at the  $p < 0.05$  level.

**Fig 3C & 3D:** Inhibition percentage of neutralizing antibody categorized by immunotherapy in different COVID-19 variants (C) Delta variant, (D) Omicron variant

*Notes for figures C and D:-* Nonparametric distributions are displayed as median (interquartile range) by bar and error bar, respectively. Statistical significance was calculated by the Mann-Whitney U test. \*denotes a statistically significant result at the  $p < 0.05$  level. The cut-off value (dashed line) for inhibition response was set at 30%.

#### *Inhibition percentage categorized by type of immunotherapy*

Patients tested for the Delta variant showed a significantly greater median inhibition percentage than the Omicron variant across all IMTs ( $p < 0.001$ ). Compared to the no IMT group [92.74% (IQR 87.88-98.15)], AZA and RTX groups showed significant attenuation of inhibition

percentages against the Delta variant [AZA 60.04% (IQR 19.87-83.22);  $p = 0.009$ ] and RTX 30.29% (IQR 9.06-34.93);  $p = 0.018$ ] (Fig 3C). For the Omicron variant, the no IMT group showed no significant difference in inhibition percentages compared with either IMT use groups. (Fig 3D)

### T cell Responses to the SARS-CoV2 Virus

Twenty-two patients with 23 serum samples were tested for T cell responses with seven samples tested after the second vaccination, and 16 samples tested after the third vaccination. One patient was tested after the second and third dose of vaccination.

The T cell response did not significantly differ between the second and third vaccination; Ag1- Nil 0.21 IU/mL (IQR -0.01-1.88) vs. 0.23 IU/mL (IQR -0.03-3.59),  $p = 0.343$  and Ag2- Nil 0.22 IU/ml (IQR 0.00-2.41) vs. 0.57 IU/mL (IQR -0.05-8.67),  $p = 0.131$ , respectively.

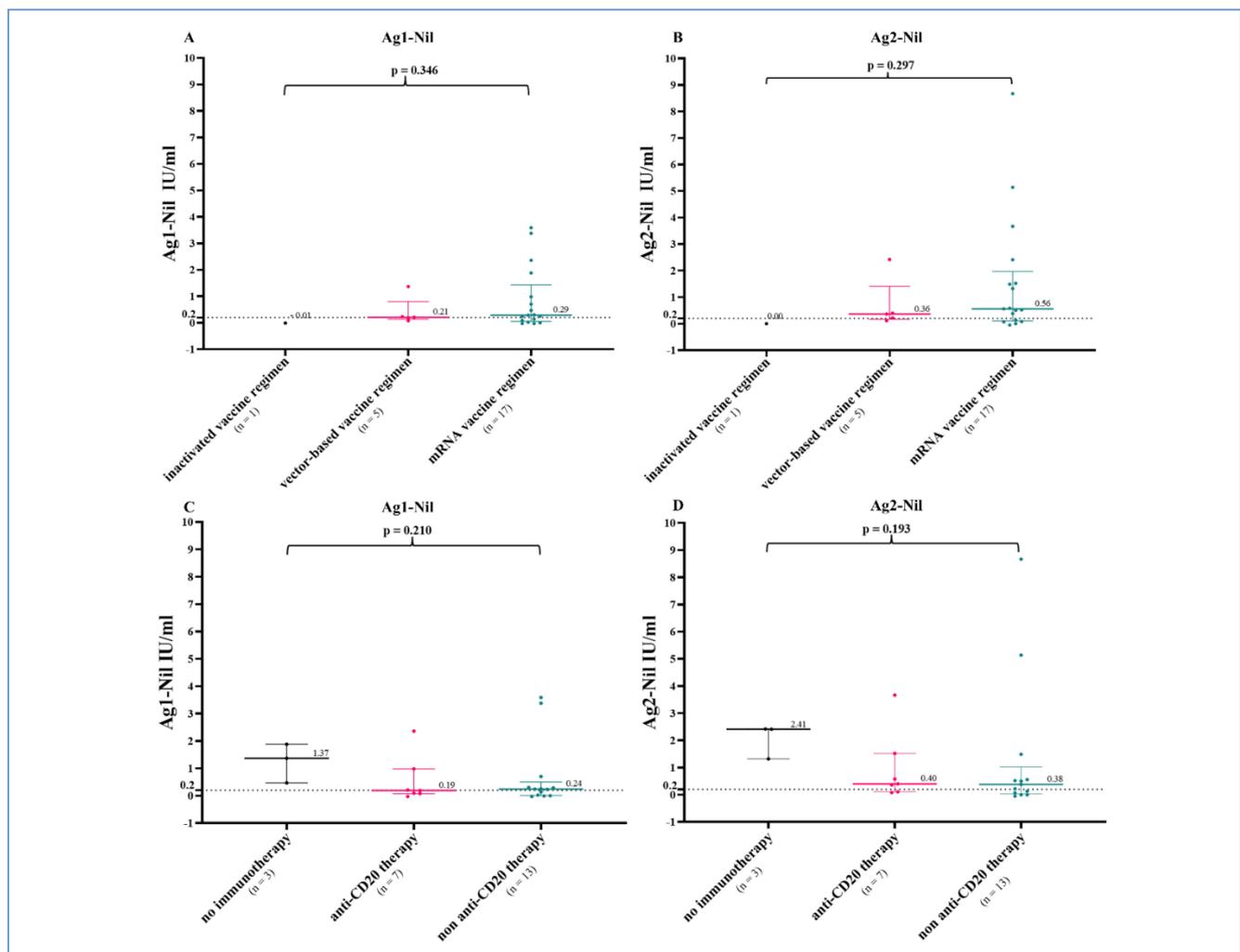
#### T cell Responses Categorized by Vaccine Regimen

T cell response measured by Ag1- Nil and Ag2- Nil were no significantly different across all vaccine regimens ( $p = 0.346$  for Ag1- Nil and  $p = 0.297$  for Ag2- Nil). The mRNA vaccine regimens had the highest positive rates for Ag1- Nil response at 64.71% (11 of 17) compared

with 60% (3 of 5) in vector-based regimens and 0% (0 of 1) in inactivated vaccine regimens. However, the positive rate for Ag2- Nil response was the highest in vector-based vaccine regimens at 80% (4 of 5), followed by 12 of 17 (70.59%) in mRNA regimens, and 0% (0/1) in inactivated vaccine regimen. (Fig 4A-4B)

#### Anti-CD20 Therapy Demonstrates Similar T cell Responses to Non-anti-CD20 Treatment

Among the group treated with no IMTs ( $n = 3$ ), anti-CD20 RTX ( $n = 7$ ), and non-anti-CD20 therapy ( $n = 13$ ), both Ag1- Nil and Ag2- Nil revealed no significant differences (1.37 IU/mL [IQR 0.47-1.88], 0.19 IU/mL [IQR 0.08-0.98], and 0.24 IU/mL [IQR 0.01-0.50];  $p = 0.210$  for Ag-1 Nil, and 2.41 IU/mL [IQR 1.32-2.42], 0.40 IU/mL [IQR 0.11-1.52], and 0.38 IU/mL [IQR 0.04-1.03];  $p = 0.193$  for Ag2- Nil, respectively). (Fig 4C-4D)



**Fig 4.** T cell responses for each vaccine regimen and each type of immunotherapy

**Fig 4A & 4B:** T cell responses for each vaccine regimen (A) Ag1- Nil, (B) Ag2- Nil

**Fig 4C & 4D:** T cell responses categorized by type of immunotherapy (C) Ag1- Nil, (D) Ag2- Nil

Notes for figures 4A-4D:- Nonparametric distributions are displayed as median (interquartile range) by bar and error bar, respectively. The cut-off value (dashed line) for Ag1- Nil and Ag2- Nil was set at 0.2 IU/mL. Statistical significance was calculated by the Kruskal-Wallis test. \*denotes a statistically significant result at the  $p < 0.05$  level.

## Immune Response Categorized by Disease; MS, NMOSD, and Other AINDs

All anti-RBD IgG assays, neutralizing antibody assays, and interferon SARS-CoV-2 immunoassays were categorized by disease. The details for each immune response are described in the Results section and [Supplementary Fig S2 in the Supplementary Appendix](#).

## DISCUSSION

Our study shows COVID-19 vaccination augments the immune system in both T cell functions and B cell functions, demonstrated by anti-RBD IgG, surrogate NAbs, and interferon SARS-CoV-2 immunoassay. The response differed depending on the vaccine regimen, the IMT type, and the variant of COVID-19.

Similar to previous studies, vaccination platforms played a role in the responsiveness of antibodies.<sup>20</sup> Our study also showed inactivated vaccine regimens induced the lowest response as in [Fig 2 and 3](#). mRNA vaccine regimens showed a significantly higher antibody response compared with inactivated and vector-based vaccine regimens among patients with central nervous system immunological diseases measured by the anti-RBD IgG antibody.

Compared to those with no IMT or healthy controls, there was a significant reduction of anti-RBD IgG levels in patients treated with RTX in all vaccine regimens.

The present study also showed neutralizing activity against SARS-CoV-2 measured by NAbs depended on the variant. The overall median inhibition percentages were higher for the Delta variant than for the Omicron variant regardless of vaccine regimen group and type of IMT used. However, for the Omicron variant, those having received an inactivated vaccine, a vector-based vaccine, or an mRNA vaccine regimen all showed a median inhibition percentage of no more than 30%. This supports a previous study showing the estimated effectiveness of two doses of a COVID-19 vaccine was high against symptomatic Delta infection while it was lower against symptomatic Omicron infection.<sup>21</sup>

Although mRNA vaccines seem to stimulate the highest response for the Delta variant, RTX and AZA showed a significant attenuation of the inhibition percentages to the Delta variant compared to the no IMT group.

The T cell response showed no significant difference among vaccine platforms after the second or third dose vaccination or among the groups treated with RTX, non-anti-CD20 therapy, and no IMTs.

These findings support the evidence that anti-CD20 therapy, such as RTX, attenuates vaccine responsiveness due to B lymphocyte suppression, concordant with previous

reports.<sup>10,11</sup> A cohort study<sup>10</sup> showed patients with chronic inflammatory disease receiving B cell depletion therapy would have lower humoral responsiveness measured by spike IgG, NAb, and circulating S-specific plasmablast after COVID vaccination. Some studies<sup>22,23</sup> measured antibody response for the mRNA vaccine regimens in neurological patients, showing similar results. FGM and anti-CD20 therapy, such as RTX, showed no detectable immune response to the COVID-19 vaccines. Based on the drug mechanism, RTX can mediate B cell depletion by binding to the CD20 receptor on the B cell surface, resulting in eliciting fewer B cells immune responses.<sup>24</sup> FGM might play a role in inhibiting germinal center formation, resulting in fewer immune responses and less recall of antigens.<sup>25</sup> Theoretically, anti-CD-20 depleting agents, such as ocrelizumab and RTX, might cause a lower protective response to COVID-19 vaccines.<sup>26</sup> Nevertheless, several studies showed anti-CD20 treatments decreased the humoral response, but did not interfere with the cellular response.<sup>27,28</sup> B cell immunity and T cell response are essential for fighting COVID-19 infection, and the T cell level is accepted to be one of the predictors of COVID-19 severity.<sup>29</sup> We only evaluated one modality of B-cell function, which was the antibody response as levels of anti-RBD and NAbs.<sup>30,31</sup> The NAb assay in the present study is a surrogate neutralizing antibody assay (sVNT), which is a blocking ELISA detection kit that detects the presence of neutralizing antibodies against SARS-CoV-2 RBD.<sup>32,33</sup> However, NAbs do not necessarily correlate with anti-RBD activity. It should also be noted that immunoglobulin classes other than IgG could be important, especially in patients who received anti-CD20 therapy.

This prospective observational study had several limitations. The sample sizes in the compared groups were small, so comparisons may have been underpowered to detect true differences. Since our study is a real-life practice amongst consecutive cases, we could not test all the patients on all three tests, immunoglobulin classes, and adverse events of each patient due to time and resource limitations and the lack of correlation with clinical outcomes.

In conclusion, we have investigated the immunity response to COVID-19 vaccination in Thai patients with autoimmune neurological disease. In general, all DMDs/ISs may slightly decrease antibody level response, but they may not decrease cellular immunity. This cellular immunity may be sufficient to reduce the severity of COVID-19 infection. However, in MS patients on RTX or FGM therapy, choosing an mRNA vaccine regimen might be desirable (if possible) because of its superior

immunogenicity evidenced by a higher B cell response. To date, there has been no consensus on suggestions or recommendations for specific COVID vaccine platforms for patients with autoimmune neurological disease. Therefore, generalizing the results of the present study to other diseases and geographic areas requires caution.

## ACKNOWLEDGMENTS

We would like to thank Khemjira Karaketklang for data analysis.

## Declarations of interest

None.

## Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

## Funding

This research project was supported by Siriraj Research Development Fund, Grant number (IO) R016533016, Faculty of Medicine Siriraj Hospital, Mahidol University.

## REFERENCES

- Andrews N, Stowe J, Kirsebom F, Toffa S, Rickeard T, Gallagher E, et al. Covid-19 Vaccine Effectiveness against the Omicron (B.1.1.529) Variant. *N Engl J Med*. 2022;386(16):1532-46.
- Jara A, Undurraga EA, Gonzalez C, Paredes F, Fontecilla T, Jara G, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. *N Engl J Med*. 2021;385(10):875-84.
- Lopez Bernal J, Andrews N, Gower C, Robertson C, Stowe J, Tessier E, et al. Effectiveness of the Pfizer-BioNTech and Oxford-AstraZeneca vaccines on covid-19 related symptoms, hospital admissions, and mortality in older adults in England: test negative case-control study. *BMJ*. 2021;373:n1088.
- Frenck RW, Jr., Klein NP, Kitchin N, Gurtman A, Absalon J, Lockhart S, et al. Safety, Immunogenicity, and Efficacy of the BNT162b2 Covid-19 Vaccine in Adolescents. *N Engl J Med*. 2021;385(3):239-50.
- Tisavipat N, Jitpratoom P, Siritho S, Prayoonwivat N, Apiwattanakul M, Boonyasiri A, et al. The epidemiology and burden of neuromyelitis optica spectrum disorder, multiple sclerosis, and MOG antibody-associated disease in a province in Thailand: A population-based study. *Mult Scler Relat Disord*. 2023;70:104511.
- Hor JY, Asgari N, Nakashima I, Broadley SA, Leite MI, Kissani N, et al. Epidemiology of Neuromyelitis Optica Spectrum Disorder and Its Prevalence and Incidence Worldwide. *Front Neurol*. 2020;11:501.
- Holroyd K, Vogel A, Lynch K, Gazdag B, Voghel M, Alakel N, et al. Neuromyelitis optica testing and treatment: Availability and affordability in 60 countries. *Mult Scler Relat Disord*. 2019; 33:44-50.
- Mathew T, John SK, Kamath V, Murgod U, Thomas K, Baptist AA, et al. Efficacy and safety of rituximab in multiple sclerosis: Experience from a developing country. *Mult Scler Relat Disord*. 2020;43:102210.
- Zheng C, Kar I, Chen CK, Sau C, Woodson S, Serra A, et al. Multiple Sclerosis Disease-Modifying Therapy and the COVID-19 Pandemic: Implications on the Risk of Infection and Future Vaccination. *CNS Drugs*. 2020;34(9):879-96.
- Deepak P, Kim W, Paley MA, Yang M, Carvidi AB, Demissie EG, et al. Effect of Immunosuppression on the Immunogenicity of mRNA Vaccines to SARS-CoV-2: A Prospective Cohort Study. *Ann Intern Med*. 2021;174(11):1572-85.
- Louapre C, Ibrahim M, Maillart E, Abdi B, Papeix C, Stankoff B, et al. Anti-CD20 therapies decrease humoral immune response to SARS-CoV-2 in patients with multiple sclerosis or neuromyelitis optica spectrum disorders. *J Neurol Neurosurg Psychiatry*. 2022;93(1):24-31.
- Angkasekwinai N, Sewatanon J, Niyomnaitham S, Phumiamorn S, Sukapirom K, Sapsutthipas S, et al. Comparison of safety and immunogenicity of CoronaVac and ChAdOx1 against the SARS-CoV-2 circulating variants of concern (Alpha, Delta, Beta) in Thai healthcare workers. *Vaccine X*. 2022;10:100153.
- Angkasekwinai N, Niyomnaitham S, Sewatanon J, Phumiamorn S, Sukapirom K, Senawong S, et al. The immunogenicity and reactogenicity of four COVID-19 booster vaccinations against SARS-CoV-2 variants of concerns (Delta, Beta, and Omicron) following CoronaVac or ChAdOx1 nCoV-19 primary series. 2022.
- Department of Disease Control MoPH, Thailand. Guidelines for vaccination against COVID-19 in Thailand - 2021 epidemic situation 2022 [Available from: <https://ddc.moph.go.th/vaccine-covid19/getFiles/11/1628849610213.pdf>].
- Hassold N, Brichler S, Ouedraogo E, Leclerc D, Carroue S, Gater Y, et al. Impaired antibody response to COVID-19 vaccination in advanced HIV infection. *AIDS*. 2022;36(4): F1-F5.
- Company NGB. Instruction for use: cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit 2022 [cited 2022 Aug 26]. Available from: [https://www.genscript.com/gfiles/techfiles/GS-SOP-CPTS001G-05\\_L00847-C.pdf?1109908894](https://www.genscript.com/gfiles/techfiles/GS-SOP-CPTS001G-05_L00847-C.pdf?1109908894).
- QIAGEN Company. Instructions for use: QuantiFERON SARS-CoV-2 ELISA Kit [Internet]. 2022 [cited 2022 Aug 26]. Available from: <https://www.qiagen.com/at/resources/download.aspx?id=3d27842e-c811-442c-bcad-a9d42945e59c&lang=en>.
- Johnson SA, Phillips E, Adele S, Longet S, Malone T, Mason C, et al. Evaluation of QuantiFERON SARS-CoV-2 interferon-g release assay following SARS-CoV-2 infection and vaccination. *Clin Exp Immunol*. 2023.
- GraphPad Software Company. Prism: a statistical analysis software [Internet]. 2022 [cited 2022 Aug 26]. Available from: <https://www.graphpad.com/scientific-software/prism>.
- Coyle PK, Gocke A, Vignos M, Newsome SD. Vaccine Considerations for Multiple Sclerosis in the COVID-19 Era. *Adv Ther*. 2021;38(7):3550-88.
- Buchan SA, Chung H, Brown KA, Austin PC, Fell DB, Gubbay JB, et al. Estimated Effectiveness of COVID-19 Vaccines Against Omicron or Delta Symptomatic Infection and Severe Outcomes. *JAMA Netw Open*. 2022;5(9):e2232760.
- Mariottini A, Bertozzi A, Marchi L, Di Cristinzi M, Mechi C, Barilaro A, et al. Effect of disease-modifying treatments on antibody-mediated response to anti-COVID19 vaccination

- in people with multiple sclerosis. *J Neurol*. 2022;269(6):2840-7.
23. Sormani MP, Inglese M, Schiavetti I, Carmisciano L, Laroni A, Lapucci C, et al. Effect of SARS-CoV-2 mRNA vaccination in MS patients treated with disease modifying therapies. *EBioMedicine*. 2021;72:103581.
  24. Luo W, Yin Q. B Cell Response to Vaccination. *Immunol Invest*. 2021;50(7):780-801.
  25. Han S, Zhang X, Wang G, Guan H, Garcia G, Li P, et al. FTY720 suppresses humoral immunity by inhibiting germinal center reaction. *Blood*. 2004;104(13):4129-33.
  26. Mateen FJ, Rezaei S, Alakel N, Gazdag B, Kumar AR, Vogel A. Impact of COVID-19 on U.S. and Canadian neurologists' therapeutic approach to multiple sclerosis: a survey of knowledge, attitudes, and practices. *J Neurol*. 2020;267(12):3467-75.
  27. Apostolidis SA, Kakara M, Painter MM, Goel RR, Mathew D, Lenzi K, et al. Cellular and humoral immune responses following SARS-CoV-2 mRNA vaccination in patients with multiple sclerosis on anti-CD20 therapy. *Nat Med*. 2021;27(11):1990-2001.
  28. Iannetta M, Landi D, Cola G, Malagnino V, Teti E, Fraboni D, et al. T-cell responses to SARS-CoV-2 in multiple sclerosis patients treated with ocrelizumab healed from COVID-19 with absent or low anti-spike antibody titers. *Mult Scler Relat Disord*. 2021;55:103157.
  29. Peng Y, Mentzer AJ, Liu G, Yao X, Yin Z, Dong D, et al. Broad and strong memory CD4(+) and CD8(+) T cells induced by SARS-CoV-2 in UK convalescent individuals following COVID-19. *Nat Immunol*. 2020;21(11):1336-45.
  30. Tauzin A, Gendron-Lepage G, Nayrac M, Anand SP, Bourassa C, Medjahed H, et al. Evolution of Anti-RBD IgG Avidity following SARS-CoV-2 Infection. *Viruses*. 2022;14(3).
  31. Lo Sasso B, Agnello L, Giglio RV, Gambino CM, Ciaccio AM, Vidali M, et al. Longitudinal analysis of anti-SARS-CoV-2 S-RBD IgG antibodies before and after the third dose of the BNT162b2 vaccine. *Sci Rep*. 2022;12(1):8679.
  32. Pang NY, Pang AS, Chow VT, Wang DY. Understanding neutralising antibodies against SARS-CoV-2 and their implications in clinical practice. *Mil Med Res*. 2021;8(1):47.
  33. Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med*. 2021;27(7):1205-11.

# Effect of a Single-dose Dexmedetomidine on Postoperative Delirium and Intraoperative Hemodynamic Outcomes in Elderly Hip Surgery; A Randomized Controlled Trial

## Dexmedetomidine for Postoperative Delirium

Chidchanok Choovongkomol, M.D.<sup>1</sup>, Sothida Sinchai, M.D.<sup>1</sup>, Kongtush Choovongkomol, M.D.<sup>2</sup>

<sup>1</sup>Department of Anesthesiology, Maharat Nakhon Ratchasima Hospital, Nakhon Ratchasima, Thailand, <sup>2</sup>Department of Orthopedics, Maharat Nakhon Ratchasima Hospital, Nakhon Ratchasima, Thailand.

### ABSTRACT

**Objective:** Postoperative delirium (POD) is common in elderly patients. The growing evidences suggesting the potential benefits of dexmedetomidine (DEX) infusion in reducing POD. However, the administration of a single-dose DEX remains controversial. This study aims to investigate the effect of a single-dose DEX on POD in elderly patients undergoing hip surgery.

**Materials and Methods:** This prospective, randomized, double-blinded trial enrolled patients aged over 65 years who underwent hip surgery under spinal anesthesia. Patients were assigned to either the DEX (received intravenous DEX 0.3-0.5 µg/kg after incision), or the normal saline solution (NSS). Delirium assessments were conducted at the post anesthetic care unit (PACU) and at 24, 48 and 72 hours postoperatively using the Confusion Assessment Method (CAM).

**Results:** A total of 200 patients were randomized, with 100 in the DEX and 100 in the NSS. The incidence of POD was significantly lower in the DEX compared to the NSS ( $P < 0.001$ , RR 0.45, 95%CI: 0.28, 0.73). This difference remained significant at each postoperative assessment time point. There was no significant difference in sedation score and perioperative hemodynamics, except for a slightly lower intraoperative heart rate ( $P=0.015$ ) and systolic blood pressure ( $P=0.029$ ) observed at the PACU in the DEX, but these differences were not clinically significant. Additionally, the length of stay after surgery in the DEX was significantly shorter compare to the NSS ( $P=0.006$ ).

**Conclusion:** A single-dose dexmedetomidine can reduce the incidence of POD within 72 hours postoperatively in elderly patients undergoing hip surgery without compromising intraoperative hemodynamic stability.

**Keywords:** Elderly; Dexmedetomidine; Delirium; Postoperative complications; Hip fractures; Hemodynamics (Siriraj Med J 2024; 76: 80-89)

### INTRODUCTION

Hip fractures are a prevalent issue among the elderly population. Surgical intervention has emerged as the definitive and efficacious method for reducing morbidities and mortality.<sup>1,2</sup> However, due to the aging population, patients frequently present with multiple

medical comorbidities, which may increase the likelihood of complications after surgery.<sup>1,2</sup>

Postoperative delirium (POD) represents a significant and common complication, particularly among elderly population with reported incidence rates ranging from 10 to 70%<sup>3-6</sup>, depending on the surgical procedure and studied

Corresponding author: Chidchanok Choovongkomol

E-mail: [thisismemoen@gmail.com](mailto:thisismemoen@gmail.com)

Received 8 December 2023 Revised 30 December 2023 Accepted 31 December 2023

ORCID ID: <http://orcid.org/0000-0002-6589-678X>

<https://doi.org/10.33192/smj.v76i2.266653>



All material is licensed under terms of the Creative Commons Attribution 4.0 International (CC-BY-NC-ND 4.0) license unless otherwise stated.

population. Notably, vascular, cardiac and orthopedic surgeries especially urgent or semi-urgent repair of hip fractures exhibit a higher occurrence of POD.<sup>6-8</sup> The etiology of POD is multifactorial, stemming from patient-related factors such as advanced age or coexisting medical conditions, operative factors such as prolonged operative time or severe blood loss, and environmental factors such as staying in the ICU, where patients may have difficulty recognizing the time or being in an unfamiliar place. Some of these factors can be preventable, but others cannot. POD contributes to unfavorable outcomes, including prolonged hospitalization, increased costs of care, higher rates of morbidities and mortality, as well as, compromised functional and cognitive recovery.<sup>4,6,8-10</sup> Patients may develop post-operative cognitive dysfunction<sup>11</sup>, leading to permanent brain damage if POD is not detected early.

Dexmedetomidine (DEX) is a highly selective  $\alpha$ -2 adrenergic receptor agonist that has a range of potentially beneficial effects in the postoperative period. It possesses anxiolytic, sedative, analgesic and neuroprotective properties while causing minimal respiratory depression.<sup>12,13</sup> In recent years, the role of DEX in the perioperative period has garnered attention. Several studies have demonstrated that the perioperative use of DEX can substantially decrease the incidence of POD in the elderly patients.<sup>13-18</sup> However, the prescribed dose of DEX, along with its infusion technique - 1 mg/kg over 10 minutes than 0.3-0.8  $\mu$ g/kg/hr until the end of the operation - is a considerable dosage that can pose challenges of clinicians during perioperative management. Studies suggest that administering DEX via intravenous infusion may result in unstable hemodynamics and heightened levels of sedation in elderly patients.<sup>15,17,20</sup>

Accordingly, this study was designed to investigate the effect of a single-dose of DEX on the incidence of POD in elderly patients undergoing hip surgery. Moreover, we hypothesized that a smaller single-dose of DEX, compared to the prescribed dose, would be adequate to reduce POD with minimal impact on hemodynamics and sedation status. This consideration takes into account the pharmacokinetics and pharmacodynamics of DEX in the geriatric population, which advise reducing the dose by half. By employing this approach, we anticipate that anesthesiologists will be empowered to effectively prevent POD in elderly patients undergoing hip surgery.

## **MATERIALS AND METHODS**

This prospective, randomized, double-blinded, placebo-controlled trial, based on the CONSORT 2010 guidelines<sup>21</sup>, was conducted after obtaining approval from the author's affiliated institution (Institutional

Review Board number 100/2022, approval date: August 18<sup>th</sup>, 2022) and registered prior to patient enrollment at the Thai Clinical Trials Registry (TCTR20221004001, approval date: October 4<sup>th</sup>, 2022). Written informed consent was obtained from all elderly patients with hip fractures before their enrollment. The study was conducted at a tertiary referral center in Thailand from October 2022 to June 2023.

The inclusion criteria were patients aged 65 years or older who were scheduled for hip surgery under spinal anesthesia with an appropriate peripheral nerve block during that time. The exclusion criteria were patients who had: 1) a known history of anaphylaxis to DEX, 2) comorbidities with delirium or dementia before the operation, 3) a history of stroke and residual neurological deficit, epilepsy, or Parkinson's disease 4) a psychological disease or the use of psychological drugs 5) conversion from spinal anesthesia to general anesthesia due to failure, 6) a history of sick sinus syndrome, severe sinus bradycardia (HR < 50 bpm) or second- or third- degree atrioventricular block without a pacemaker, 7) severe hepatic dysfunction (Child-Pugh grade C or higher), or renal failure requiring renal replacement therapy, or 8) unstable hemodynamics (HR < 60 bpm or > 150 bpm, BP < 90/60 mmHg or > 180/100 mmHg).

All investigators responsible for preoperative screening, data collection and assessment were trained and qualified by psychiatrists prior to the study. Cognitive function and delirium status were evaluated before the operation using the Thai Mini-Mental State Examination (MMSE)<sup>22,23</sup> which allows a maximum score of 30. A score of 14, 17 or 22 or higher (depending on the preoperative patient status) is considered normal. Additionally, the Confusion Assessment Method (CAM)<sup>24</sup> was employed before the operation.

Upon entering the operating theater, all patients were monitored for SBP, DBP, SpO<sub>2</sub> and ECG. A peripheral intravenous line was established in the upper limb, and a crystalloid solution (normal saline solution, lactated Ringer's or acetated Ringer's) was administered at a rate of 5-10 ml per kilogram before the initiation of anesthesia. All hip surgery was performed under spinal anesthesia with an ultrasound-guided peripheral nerve block for postoperative analgesia. An anesthesiologist used 0.5% isobaric bupivacaine (Marcaine®) at a dose of 10-15 mg for spinal anesthesia to achieve sensory level at less thoracic level 10, and 0.33% bupivacaine at a dose of 60-100 mg for an ultrasound guided-iliaca fascial space block which was a proper peripheral nerve block for postoperative analgesia.

All patients were randomized after confirming their

normal test results on the MMSE, CAM and ensuring that spinal anesthesia and an iliaca fascial space block were sufficient for the surgery. Randomization was performed using a block size of four, with each block contained a computer-generated random sequence of two groups in equal numbers. The first group received dexmedetomidine (Precedex® 200 µg/2 ml) at a dose of 0.3-0.5 µg/kg, administered intravenously through a slow injection within 10 minutes after the start of the operation. The second group received an equivalent volume of normal saline solution (NSS). An anesthesiologist, who was not otherwise involved in the trial, would open opaque envelopes for each group and prepared the trial syringe, ensuring that the solutions in identical-looking 10-ml syringes, matched the assigned sequential randomization numbers. Both investigators and patients were therefore completely blinded to treatment allocation. However, in case of emergencies or unexpected deterioration in a patient's clinical status, the attending anesthesiologist could adjust or discontinue drug administration if they deemed clinically necessary. Unmasking was allowed only when clearly needed for clinical purposes.

Throughout the operation, all patients would receive standard anesthesia care. No additional anesthetic, sedative agents or pain relief medication were allowed during the operation, except in necessary situations with the agreement of attending anesthesiologists. At the end of the operation, patients would be transferred to the post-anesthetic care unit (PACU) for close monitoring for one hour.

The primary end point of this study is postoperative delirium, while the sedative score as a secondary endpoint. Two investigators would independently assess delirium and sedation score, using the CAM for non-ventilated patients or the CAM for the Intensive Care Unit (CAM-ICU) for ventilated patients<sup>24,25</sup> and the Richmond agitation sedation scale<sup>26</sup>, respectively, at the PACU, and at 24, 48 and 72 hours postoperatively. In cases of discrepant results, the investigators would re-assess the patients together to determine the reliable responses. Delirium is a complex condition that naturally fluctuates over time, so we decided to evaluate it consistently each morning.

All patients who were identified as having POD by the CAM or the CAM-ICU were referred to a consulting psychiatrist for the diagnosis and treatment of delirium. In terms of postoperative pain control, all patients would receive multimodal analgesia, which included a peripheral nerve block, non-opioid analgesic drugs such as acetaminophen or nonsteroidal anti-inflammation drugs (NSAIDs), unless contraindicated. The use of opioid drugs was minimized to the necessary extent.

For other secondary endpoints, hemodynamic values were documented and reported every 5 minutes throughout the perioperative and recovery periods as part of standard anesthetic care. We selected the greatest change in hemodynamic values from the preoperative baseline to represent the worse outcome associated with DEX. Similarly, for safety outcomes, we compared the changes in hemodynamic status to the preoperative baseline during each period. The definition of bradycardia is heart rate (HR) less than 60 bpm, or a decrease of more than 30% from baseline; tachycardia is HR more than 100 bpm; hypotension is Systolic blood (SBP) less than 90 mmHg, or a decrease of more than 30% of the baseline; hypertension is SBP more than 180 mmHg, or an increase of more than 30% from baseline; hypoxia is pulse oxygen saturation less than 90%. The intervention included adjustment of study drug dose and/or administration of medication, fluid, oxygen or physical therapy. Additionally, we recorded and reported all intraoperative and in-hospital complications, the total length of stay during this visit and the length of stay after the operation.

## Statistics

The sample size was calculated based on the primary endpoint from the study conducted by Xie S and Xie M.<sup>25</sup> In their study, the incidence of delirium in elderly patients undergoing hip surgery was reported as 4% in the DEX group and 17% in the NSS group. The calculated sample size of 86 patients per treatment group would be required to achieve a statistical power of 0.80 with a two-tailed significance level of 0.05 in order to detect a difference. Therefore, we decided to increase the sample size to 100 patients per treatment in order to account for potential dropouts after randomization.

All analyses were performed on an intention-to-treat basis, including all patients in the groups to which they were randomized. The normal distribution of data was assessed using the Shapiro-Wilk test. Primary outcomes, which consisted of categorical data, were reported as frequencies and percentages for each group. Other outcomes, including continuous data, were presented as means ± standard deviations (SDs) or median (Q1, Q3), depending on the normality of the data. Categorical data were also reported as frequencies and percentages. The analyses were conducted using STATA version 16.0 (StataCorp, College Station, TX, USA) with two-sided significance tests at a p-value < 0.05. Fisher's exact test was used to analyse categorical data, while the Student t-test or Mann-Whitney U test, as appropriate, were employed for continuous data. Regression analysis was employed to assess differences in hypothesis testing.

**RESULTS**

A total of 249 patients were eligible for this study from October 2022 to June 2023. Among them, 49 patients were excluded as they met the exclusion criteria. Eight patients declined to participate and 24 patients had a history of delirium or dementia before the operation. Additionally, three patients had a history of head injury and still had neurological deficits, nine patients refused spinal anesthesia, and five patients had failed spinal anesthesia and were converted to general anesthesia before the operation. No delirium assessments were terminated due to deep sedation (Richmond agitation sedation scale less than -2) or coma and no emergency unblinding was necessary. The final intention-to-treat analysis included 100 patients in each group: the DEX and the NSS (Fig 1).

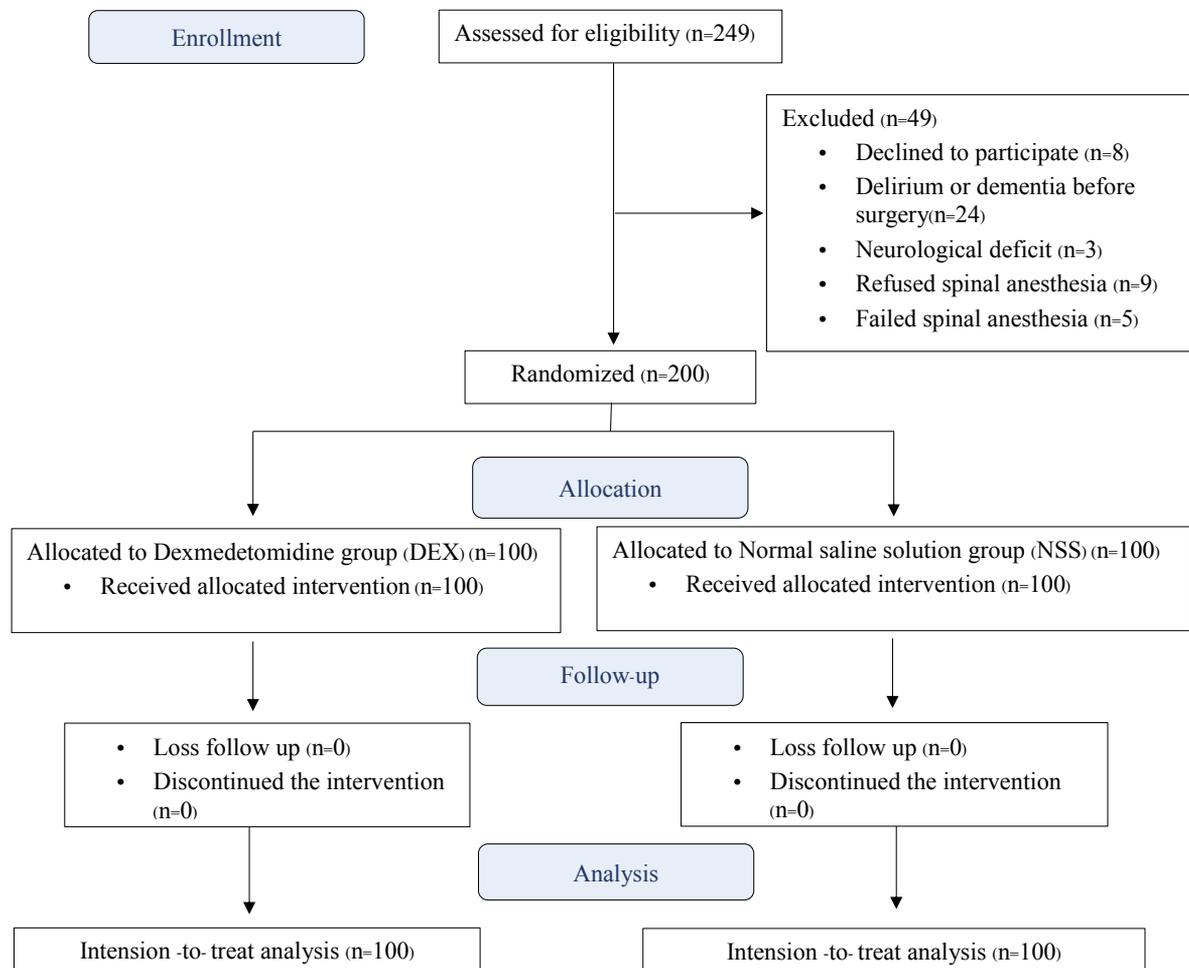
There were no significant differences between the two groups in terms of demographic data. The mean estimated dose of DEX was  $0.43 \pm 0.1$   $\mu\text{g}/\text{kg}$  in the DEX group (Table 1).

The preoperative MMSE indicated normal cognitive function within our population, and the preoperative

screening using the CAM did not detect any signs of delirium. The overall incidence of POD, defined as the occurrence of any delirium assessed at any time using the CAM, was significantly lower in the DEX compared to the NSS, with a p-value of less than 0.001 and a relative risk of 0.45 (95% CI 0.28, 0.73), and this difference remained significant at each postoperative assessment time point (Table 2).

All patients underwent the CAM screening for POD, except for three patients in the DEX and two patients in the NSS, who were intubated at 24-48 hours postoperatively, and they were assessed using the CAM-ICU for indicated POD.

There was no statistical difference between the treatment groups in terms of sedation score (Table 2), which used a cut-off point of less than -2 to indicate deep sedation requiring intervention, as well as perioperative hemodynamics values (Table 3). However, there were some notable differences in certain parameters were observed. The intraoperative heart rate (HR) of patients in the DEX was 75.9 (70, 85) bpm, whereas it was 80.8 (70, 90) bpm in the NSS. Additionally, the systolic blood



**Fig. 1** CONSORT-Study Flow Diagram

**TABLE 1.** Baseline patients characteristic.

Variables	DEX group	NSS group	P value
Age (yr.)	78.8±8.8	80.4±7.8	0.053
Gender (M/F)	29/71	18/82	0.095
BMI (kg/m <sup>2</sup> )	21.0±3.7	20.9±4.2	0.792
ASA physical status II/III/IV	5/95/0	1/95/4	0.051
Diagnosis			
Intertrochanteric fracture	66	63	0.177
Neck of femur fracture	29	36	
Subtrochanteric fracture	5	1	
Operations			
Cephalomedullary nail	69	65	0.543
Dynamic hip screw	0	1	
Multiple screws	2	0	
Hemiarthroplasty	22	27	
Total hip arthroplasty	7	7	
Co-morbidities			
HT	59	66	0.381
CAD, Valvular heart	7	10	0.613
Asthma/COPD/interstitial lung	6	6	1.000
Stroke with full recovery	8	8	1.000
DM	39	28	0.134
Thyroid disease	4	2	0.683
Chronic kidney disease (stage III-IV)	4	6	0.748
Liver cirrhosis (Child–Pugh grade A-B)	4	1	0.369
Medication			
Antihypertensives	60	65	0.557
Antihistamines	22	30	0.259
Diuretics	15	24	0.153
Opioids	18	23	0.484
NSAIDs	35	29	0.449
Benzodiazepines	30	22	0.259
Pre-operative lab values			
Hb (g/dl)	10.2 (9.1, 11.6)	10.2 (9, 11.3)	0.501
Sodium < 135 or > 145 mM/L	19	19	1.000
Potassium < 3.5 or > 5.5 mM/L	4	3	1.000
Perioperative Data			
Operative time (min)	115.5 (90, 135)	108.5 (90, 120)	0.073
Estimate intraoperative blood loss (ml)	131.7 (50, 1150)	118.4 (50, 150)	0.966
Intraoperative infusion (ml)	1081.7 (850, 1300)	961.6 (750, 1125)	0.051
Allogenic blood transfusion(ml)	95.7 (0, 235)	72.7 (0, 198)	0.255

**TABLE 2.** Incidence of post-operative delirium & sedation score.

Timing	DEX group n (%)	NSS group n (%)	RR (95% CI)	P value
Overall-Delirium	18 (18)	40 (40)	0.45 (0.28, 0.73)	<b>&lt; 0.001*</b>
PACU	1 (1)	11 (11)	0.09 (0.01, 0.69)	<b>0.020*</b>
24 h	13 (13)	33 (33)	0.39 (0.22, 0.70)	<b>0.002*</b>
48 h	11 (11)	34 (34)	0.32 (0.17, 0.60)	<b>&lt; 0.001*</b>
72 h	15 (15)	29 (29)	0.52 (0.30, 0.90)	<b>0.021*</b>
Over all-Sedation score	6 (6)	9 (9)	0.67 (0.25, 1.8)	0.425
PACU	0	0	-	-
24 h	2 (2)	7 (7)	0.29 (0.06, 1.32)	0.112
48 h	2 (2)	1 (1)	0.50 (0.05, 5.43)	0.569
72 h	0	3 (3)	-	-

\*A p value < 0.05 indicates statistical significance

**TABLE 3.** Perioperative hemodynamic values.

Parameters	DEX group n (Q1, Q3)	NSS group n (Q1, Q3)	P value
HR (/min)			
Preoperative	87.9 (80, 96)	88.9 (79, 99)	0.579
Intraoperative	75.9 (70, 85)	80.8 (70, 90)	<b>0.015*</b>
PACU	80.0 (70, 87.5)	82.1 (70, 90)	0.190
SBP (mmHg)			
Preoperative	146.9 (131, 160)	142 (130.5, 154.5)	0.260
Intraoperative	105.9 (100, 120)	107.3 (100, 120)	0.570
PACU	115.8 (105, 122.5)	120.6 (110, 130)	<b>0.029*</b>
DBP (mmHg)			
Preoperative	79.9 (71, 90)	77.9 (68, 86.5)	0.082
Intraoperative	62.1 (60, 70)	63.5 (60, 70)	0.084
PACU	68.5 (60, 70)	68.9 (60, 75)	0.604
SpO <sub>2</sub> (%)			
Preoperative	96.1 (95, 98)	96.4 (95, 98)	0.350
Intraoperative	97.9 (98, 100)	98.1 (97, 100)	0.215
PACU	98.8 (98, 100)	98.5 (98, 100)	0.311

\*A p value < 0.05 indicates statistical significance

pressure (SBP) observed at the PACU in the DEX was 115.8 (105, 122.5) mmHg, compared to 120.6 (110, 130) mmHg in the NSS. Both of these hemodynamic changes were significantly lower in the DEX compared to the NSS, with p-values of 0.015 and 0.029 for intraoperative HR and SBP observed at the PACU, respectively.

The opioid consumption within the initial 72 hours postoperatively was evaluated, with a protocol in place to minimize opioid use by specifically choosing intravenous morphine. Consequently, no significant differences were observed in the dose of morphine between the two groups during this period.

For the safety outcome, there was no statistical difference between the treatment groups. (Table 4) None of the patients received additional anesthetic, sedative agents or pain relief medication during the operation. For postoperative pain control, patients received acetaminophen, or NSAIDs, with opioids such as morphine (0.1-0.2 mg/kg) administered as needed only during the first 24 hours postoperatively. None of the patients received benzodiazepines or other sedative drugs postoperatively, except for the patients diagnosed with delirium, for whom they were prescribed by a psychiatrist.

There was also no statistical difference between the treatment groups for postoperative outcomes, including the total length of stay and in-hospital complications. However, the length of stay after the operation in the DEX was significantly shorter compare to the NSS (4.9 [3, 6] vs. 6[4, 6.5] days) with a p-value of 0.006 (Table 5).

## DISCUSSION

This study has provided evidence that dexmedetomidine effectively reduces the incidence of postoperative delirium within 72 hours after surgery, consistent with numerous prior studies.<sup>14,16,18,19,25-28</sup> According to our findings, both groups demonstrated a higher incidence of delirium compared to previous studies<sup>17,18</sup>, including the study conducted by Xie S and Xie M, which was employed for samples size calculation.<sup>25</sup> The higher incidence can be attributed to the enrollment of older patients, with ages of 78.8±8.8 and 80.4±7.8 years in the two treatment groups. besides, these patients experienced pain from hip fractures and may have been in a dehydrated status due to the restrictions on food and drink before the operation.

To ensure outcome accuracy, we made efforts to adjust controllable confounding factors, that have been identified in previous studies<sup>29,30</sup> as contributing to postoperative delirium in elderly patients with hip fracture. These factors include screening and preventing infection, maintaining adequate volume status during admission, screening and treating abnormal electrolyte levels, avoiding multiple drug used, ensuring adequate pain control and selecting neuraxial techniques with peripheral nerve blocks.

This trial stands out as the only one that utilized a different technique for administering DEX, specifically a small single-dose infusion. We opted for this technique considering the pharmacokinetic and pharmacodynamic

**TABLE 4.** Safety outcomes.

Parameters	Intraoperative periods				PACU			
	DEX group n (%)	NSS group n (%)	RR (95% CI)	P value	DEX group n (%)	NSS group n (%)	RR (95%CI)	P value
Bradycardia	18 (18)	13 (13)	1.38 (0.72, 2.67)	0.332	10 (10)	10 (10)	1.00 (0.44, 2.30)	1.000
with intervention	8 (8)	7 (7)	1.14 (0.43, 3.03)	0.789	2 (2)	1 (1)	2(0.18, 21.70)	0.563
Tachycardia	3 (3)	3 (3)	1.00 (0.21, 4.84)	1.000	0	1 (1)	-	-
with intervention	0	0	-	-	0	0	-	-
Hypotension	48 (48)	35 (35)	1.37 (0.98, 1.92)	0.065	16 (16)	11 (11)	1.45 (0.71, 2.98)	0.305
with intervention	28 (28)	25 (25)	1.12 (0.71, 1.78)	0.631	6 (6)	3 (3)	2.00 (0.51, 7.78)	0.317
Hypertension	0	0	-	-	0	1 (1)	-	-
with intervention	0	0	-	-	0	0	-	-
Hypoxia	2 (2)	1 (1)	2.43 (0.18, 21.70)	0.569	0	0	-	-
with intervention	0	0	-	-	0	0	-	-

**TABLE 5.** Postoperative outcomes.

Parameters	DEX group n (Q1, Q3)	NSS group n (Q1, Q3)	P value
Total length of stay (day)	12.5 (8.5, 14)	13.9 (9, 15.5)	0.341
Length of stay after operation (day)	4.9 (3, 6)	6.0 (4, 6.5)	<b>0.006*</b>
Intraoperative complications			
vasopressors	40	35	0.468
convert to general anesthesia	3	0	0.082
shivering	1	2	0.563
arrhythmia	1	0	0.319
In-hospital complications			
death	1	0	0.319
pulmonary embolism	1	0	0.319
myocardial infarction	2	1	0.563
respiratory failure	1	0	0.319
sepsis	1	1	1.000
pneumonia	2	1	0.563
acute kidney injury	1	0	0.319
urinary tract infection	3	7	0.196
volume overload	1	1	1.000
lung atelectasis	1	0	0.319
Total complications	8	13	0.251

\*A p value < 0.05 indicates statistical significance

of DEX in elderly patients. It is important to note that the distribution phase of DEX is rapid, with a distribution half-life of approximately six minutes and an elimination half-life of two hours in a healthy population[10], which may be longer in the elderly population. Moreover; the operative time of hip surgery at our institute was relatively short, with a mean duration of 115.5 (90, 135) and 108.4 (90, 120) minutes in the two treatment groups. Therefore, a single dose of DEX would be sufficient for elderly patients undergoing hip surgery.

Furthermore, considering the main concern, the infusion technique also involved potential adverse effects, such as hypotension and bradycardia, as shown in previous studies.<sup>14,18,19,28</sup> We hypothesized that a single small dose of DEX in this study would result in a lower incidence or no statistically significant difference between the treatments group for hypotension and bradycardia. Our results confirmed this hypothesis when comparing to previous studies<sup>28,31</sup> However, in our study, the DEX

also exhibited statistically lower intraoperative heart rate and lower systolic blood pressure observed at the PACU. Nevertheless, these values did not show clinical significance nor required any intervention.

Another side effect of DEX is sedation, which was assessed using the Richmond agitation sedation scale. The desired sedation level ranged from 0 to -2, indicating patients' status were claim to light sedation. In our study, there was no significant difference in sedation between the two groups. Furthermore, no patients in the DEX, experienced deep sedation or coma at the PACU, which is believed to be within the elimination half-life of DEX. During the intraoperative periods, two patients in the DEX and one patient in the normal saline solution group experienced hypoxia with SpO<sub>2</sub> level below 90%. However, no intervention was required as these episodes of hypoxia were of short duration (less than ten minutes) and the patients' SpO<sub>2</sub> levels manually increased to above 90%.

Additionally, there was a significant difference

in the length of stay after the operation, excluding the waiting time for surgery, between the two groups. Specifically, the DEX had a slightly shorter duration of stay compared to the NSS. It is possible that patients in the DEX experienced less POD than those in the NSS, which might have allowed patients to return home more quickly. However, several factors affect the length of stay after the operation, so we cannot conclusively attribute the reduction in length of stay solely to DEX.

All perioperative complications showed no significant difference between the two groups, consistent with previous studies<sup>14,19,28</sup> Considering the most common complication, which is the need of vasopressors, it is expected that the DEX, which had more patients experiencing hypotension, would also have more patients requiring vasopressors. Three patients in the DEX requested a conversion to general anesthesia after realizing that spinal anesthesia alone was not sufficient for their comfort during the lengthy operation (lasting over four hours). An 85-year-old patient in the DEX developed hospital acquired pneumonia (HAP) with respiratory failure and sepsis from a Covid infection five days after surgery. Unfortunately, the patient's condition deteriorated and he passed away after twenty days of admission. Additionally, one patient in the DEX developed atrial fibrillation (AF) with a rapid ventricular response of 170 bpm within 24 hours after surgery. After receiving appropriate treatment and stabilization, the patient was diagnosed with a myocardial infarction by cardiologist. Finally, they were successfully treated and discharge from the hospital after a 15-day admission.

The strength of this study lies in its design as a randomized, double-blinded, placebo-controlled trial, which demonstrated the significant efficacy of DEX in reducing POD. Moreover, this trial introduced a difference administration technique of DEX in prevention of POD. We believe that this approach could greatly facilitate perioperative management for anesthesiologists and potentially encourage the wider utilization of this drug among the elderly population.

There were several limitations to this study. Firstly, it was conducted at a single-center, which could introduce selection and treatment biases. The uniformity of care provided by the same team and the influence of local practices and personal experience may limit the generalizability of the findings. In addition, the selection criteria limited the inclusion of a border range of elderly patients with hip fractures, thus reducing the applicability of the results. Secondly, the follow-up period was relatively short, spanning only 72 hours postoperatively. While we believed that most cases of

POD would occur within this mentioned timeframe by the study of Whitlock et al.[6], there is a possibility that some patients may have experienced POD beyond this period. Thirdly, this study failed to include brain imaging, quantitative EEG information, sophisticated neurophysiologic testing and blood markers to determine and classify delirium. Fourthly, we were unable to defined all preoperative predictors for POD, such as preoperative cognitive function classified by other screening tools or preoperative functional impairment, due to missing data that cannot be corrected from medical records or patient information. Finally, delirium is a multifactorial condition, and despite our efforts to control confounding factors, there may still uncontrollable variables influencing its occurrence.

## CONCLUSION

The administration of a single-dose dexmedetomidine demonstrated efficacy in reducing the incidence of postoperative delirium within 72 hours postoperatively in elderly patients undergoing hip surgery without affecting intraoperative hemodynamic stability. Further investigation into the long-term outcomes of dexmedetomidine in the elderly is warranted.

## REFERENCES

1. Simunovic N, Devereaux PJ, Sprague S, Guyatt GH, Schemitsch E, Debeer J, et al. Effect of early surgery after hip fracture on mortality and complications: systematic review and meta-analysis. *CMAJ*. 2010;182(15):1609-16.
2. Tay E. Hip fractures in the elderly: operative versus nonoperative management. *Singapore Med J*. 2016;57(4):178-81.
3. Saczynski JS, Inouye SK, Kosar CM, Tommet D, Marcantonio ER, Fong T, et al. Cognitive and brain reserve and the risk of postoperative delirium in older patients: analysis of data from a prospective observational study. *The Lancet Psychiatry*. 2014;1(6):437-43.
4. Schenning KJ, Deiner SG. Postoperative Delirium in the Geriatric Patient. *Anesthesiol Clin*. 2015;33(3):505-16.
5. Van Rompaey B, Schuurmans MJ, Shortridge-Baggett LM, Truijzen S, Elseviers M, Bossaert L. A comparison of the CAM-ICU and the NEECHAM Confusion Scale in intensive care delirium assessment: an observational study in non-intubated patients. *Crit Care*. 2008;12(1):R16.
6. Whitlock EL, Vannucci A, Avidan MS. POSTOPERATIVE DELIRIUM. *Minerva Anestesiol*. 2011;77(4):448-56.
7. Maldonado JR. Neuropathogenesis of delirium: review of current etiologic theories and common pathways. *Am J Geriatr Psychiatry*. 2013;21(12):1190-222.
8. Bruce AJ, Ritchie CW, Blizard R, Lai R, Raven P. The incidence of delirium associated with orthopedic surgery: a meta-analytic review. *Int Psychogeriatr*. 2007;19(2):197-214.
9. Malik A, Quatman C, Phieffer L, Ly T, Khan S. Incidence, risk factors and clinical impact of postoperative delirium following open reduction and internal fixation (ORIF) for hip fractures: an

- analysis of 7859 patients from the ACS-NSQIP hip fracture procedure targeted database. *Eur J Orthop Surg Traumatol.* 2019;29(2): 435-46.
10. Kaur M, Singh PM. Current role of dexmedetomidine in clinical anesthesia and intensive care. *Anesth Essays Res.* 2011;5(2): 128-33.
  11. Suenghataiphorn T, Songwisit S, Tornsatitkul S, Somnuke P. An Overview on Postoperative Cognitive Dysfunction; Pathophysiology, Risk Factors, Prevention and Treatment. *Siriraj Med J.* 2022; 74(10):705-13.
  12. Mo Y, Zimmermann AE. Role of dexmedetomidine for the prevention and treatment of delirium in intensive care unit patients. *Ann Pharmacother.* 2013;47(6):869-76.
  13. Li J, Xiong M, Nadavaluru PR, Zuo W, Ye JH, Eloy JD, et al. Dexmedetomidine Attenuates Neurotoxicity Induced by Prenatal Propofol Exposure. *J Neurosurg Anesthesiol.* 2016;28(1):51-64.
  14. Hu J, Zhu M, Gao Z, Zhao S, Feng X, Chen J, et al. Dexmedetomidine for prevention of postoperative delirium in older adults undergoing oesophagectomy with total intravenous anaesthesia: A double-blind, randomised clinical trial. *Eur J Anaesthesiol.* 2021;38 (Suppl 1):S9-S17.
  15. Lin C, Tu H, Jie Z, Zhou X, Li C. Effect of Dexmedetomidine on Delirium in Elderly Surgical Patients: A Meta-analysis of Randomized Controlled Trials. *Ann Pharmacother.* 2021;55(5):624-36.
  16. Liu Y, Ma L, Gao M, Guo W, Ma Y. Dexmedetomidine reduces postoperative delirium after joint replacement in elderly patients with mild cognitive impairment. *Aging Clin Exp Res.* 2016;28(4): 729-36.
  17. Yan C, Ti-jun D. Effects of Intraoperative Dexmedetomidine Infusion on Postoperative Delirium in Elderly Patients Undergoing Total Hip Arthroplasty. *International Surgery.* 2021;105(1-3): 328-35.
  18. Hong H, Zhang DZ, Li M, Wang G, Zhu SN, Zhang Y, et al. Impact of dexmedetomidine supplemented analgesia on delirium in patients recovering from orthopedic surgery: A randomized controlled trial. *BMC Anesthesiol.* 2021;21(1):223.
  19. Li CJ, Wang BJ, Mu DL, Hu J, Guo C, Li XY, et al. Randomized clinical trial of intraoperative dexmedetomidine to prevent delirium in the elderly undergoing major non-cardiac surgery. *Br J Surg.* 2020;107(2):e123-32.
  20. Zeng H, Li Z, He J, Fu W. Dexmedetomidine for the prevention of postoperative delirium in elderly patients undergoing noncardiac surgery: A meta-analysis of randomized controlled trials. *PLoS One.* 2019;14(8):e0218088.
  21. Schulz KF, Altman DG, Moher D, the CONSORT Group. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *BMC Med.* 2010;8(1):18.
  22. Kurlowicz L, Wallace M. The Mini-Mental State Examination (MMSE). *J Gerontol Nurs.* 1999;25(5):8-9.
  23. Tanglakmankhong K, Hampstead BM, Ploutz-Snyder RJ, Potempa K. Cognitive screening assessment in Thai older adults: a prospective study of the reliability and validity of the Abbreviated Mental Test. *J Health Res.* 2022;36(1):99-109.
  24. Marcantonio ER, Ngo LH, O'Connor M, Jones RN, Crane PK, Metzger ED, et al. 3D-CAM: Derivation and Validation of a 3-Minute Diagnostic Interview for CAM-defined Delirium. *Ann Intern Med.* 2014;161(8):554-61.
  25. Miranda F, Arevalo-Rodriguez I, Díaz G, Gonzalez F, Plana MN, Zamora J, et al. Confusion Assessment Method for the intensive care unit (CAM-ICU) for the diagnosis of delirium in adults in critical care settings. *Cochrane Database Syst Rev.* 2018; 2018(9):CD013126.
  26. MDCalc [Internet]. [cited 2023 Jun 11]. Richmond Agitation-Sedation Scale (RASS). Available from: <https://www.mdcalc.com/calc/1872/richmond-agitation-sedation-scale-rass>
  27. Xie S, Xie M. Effect of dexmedetomidine on postoperative delirium in elderly patients undergoing hip fracture surgery. *Pak J Pharm Sci.* 2018;31(5(Special)):2277-81.
  28. Zhang W, Wang T, Wang G, Yang M, Zhou Y, Yuan Y. Effects of Dexmedetomidine on Postoperative Delirium and Expression of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  in Elderly Patients After Hip Fracture Operation. *Front Pharmacol.* 2020;11:678.
  29. Ming S, Zhang X, Gong Z, Xie Y, Xie Y. Perioperative dexmedetomidine and postoperative delirium in non-cardiac surgery: a meta-analysis. *Ann Palliat Med.* 2020;9(2):264-71.
  30. Duan X, Coburn M, Rossaint R, Sanders RD, Waesberghe JV, Kowark A. Efficacy of perioperative dexmedetomidine on postoperative delirium: systematic review and meta-analysis with trial sequential analysis of randomised controlled trials. *Br J Anaesth.* 2018;121(2):384-97.
  31. Suwanpasu S, Grinslade S, Wu YWB, Porock D. Risk factors of delirium in elderly patients with hip fracture. *Asian Biomedicine.* 2014;8(2):157-65.
  32. Yang Y, Zhao X, Dong T, Yang Z, Zhang Q, Zhang Y. Risk factors for postoperative delirium following hip fracture repair in elderly patients: a systematic review and meta-analysis. *Aging Clin Exp Res.* 2017;29(2):115-26.
  33. Deiner S, Luo X, Lin HM, Sessler DI, Saager L, Sieber FE, et al. Intraoperative Infusion of Dexmedetomidine for Prevention of Postoperative Delirium and Cognitive Dysfunction in Elderly Patients Undergoing Major Elective Noncardiac Surgery: A Randomized Clinical Trial. *JAMA Surg.* 2017;152(8):e171505.

# Development of the Purification Process of Gallium-68 Eluted from Germanium-68/Gallium-68 Generator

Tossaporn Sriprapa<sup>1</sup>, Thanete Doungta<sup>2</sup>, Napamon Sritongkul<sup>1</sup>, Malulee Tantawiroon<sup>1</sup>

<sup>1</sup>Department of Radiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, <sup>2</sup>Thailand Institute of Nuclear Technology, Bangkok, Thailand.

## ABSTRACT

**Objective:**  $^{68}\text{Ga}$  has a half-life of 68 minutes, with 89% of its decay is through positron emission. It is available from generator systems and possesses suitable property for labeling radioligands. These aspects make  $^{68}\text{Ga}$  a promising tracer for positron emission tomography (PET) imaging. This study aims to develop the purification process of the  $^{68}\text{Ga}$  eluates from  $^{68}\text{Ge}/^{68}\text{Ga}$  generator after its recommended shelf-life and ensuring the quality through the radiolabeling process.

**Materials and Methods:** In this study, we explored the development of a purification method for  $^{68}\text{Ga}$  eluted from a  $^{68}\text{Ge}/^{68}\text{Ga}$  generator before radiolabeling was investigated. Cation and anion exchange chromatography techniques were combined to remove trace amounts of competing metal ion impurities. Post-purification, the eluate's metal contents were analyzed using inductively coupled plasma atomic emission spectroscopy (ICP-AES). Breakthrough of  $^{68}\text{Ge}$  was measured using a multi-channel analyzer (MCA) spectrometer with high-purity germanium (HPGe) radiation detectors. Additionally, the radiochemical purity of  $^{68}\text{Ga}$ -NOTA-RGD was analyzed by high-performance liquid chromatography (HPLC).

**Results:** Metal impurities including Fe(II), Zn(II) and Al(III) were reduced by 61%, 38% and 44% respectively. The  $^{68}\text{Ge}$  breakthrough was approximately  $\sim 10^{-3}\%$ . The labeling efficiency with NOTA-RGD, a tracer for angiogenesis imaging, resulted in an average yield of  $^{68}\text{Ga}$ -NOTA-RGD (not corrected for decay) of around 50%, with a radiochemical purity by HPLC of approximately 98%–99%.

**Conclusion:** Cation exchange in combination with anion exchange chromatography was thus proven to be an efficient method for purification of the  $^{68}\text{Ga}$  eluate from a  $^{68}\text{Ge}/^{68}\text{Ga}$  generator prior to labeling the  $^{68}\text{Ga}$  PET radiotracer.

**Keywords:**  $^{68}\text{Ge}/^{68}\text{Ga}$  generator; purification; radiolabeling; ion exchange purification; PET (Siriraj Med J 2024; 76: 90-96)

## INTRODUCTION

The use of positron emission tomography (PET) has widely expanded in recent years. With its excellent resolution, high sensitivity, and potential for precise quantitative analysis, PET imaging is one of the most effective diagnostic tools in nuclear medicine today. The majority of radiopharmaceuticals used in PET are short-lived positron-emitting compounds. The four-

basic cyclotron-produced radionuclides most widely employed are:  $^{18}\text{F}$ ,  $^{11}\text{C}$ ,  $^{15}\text{O}$ , and  $^{13}\text{N}$ . It is also possible to obtain radionuclides that emit positrons from generator systems, with some examples being  $^{82}\text{Rb}$  from  $^{82}\text{Sr}$  and  $^{68}\text{Ga}$  from  $^{68}\text{Ge}$ . While standard PET radionuclides and non-standard PET radionuclides are usually just made up of four basic radionuclides, such as  $^{68}\text{Ga}$ , the range

Corresponding author: Tossaporn Sriprapa

E-mail: Tossaporn.sip@mahidol.ac.th

Received 1 November 2023 Revised 18 December 2023 Accepted 20 December 2023

ORCID ID: <http://orcid.org/0009-0001-1626-0056>

<https://doi.org/10.33192/smj.v76i2.266113>



All material is licensed under terms of the Creative Commons Attribution 4.0 International (CC-BY-NC-ND 4.0) license unless otherwise stated.

has expanded in recent years in both preclinical and clinical studies.<sup>1</sup>

As <sup>68</sup>Ga decays, 89% of it is decayed through positron emission. In addition, the <sup>68</sup>Ge parent material decays with a half-life of 270.8 days through electron capture. Currently, <sup>68</sup>Ga is usually available from an in-house <sup>68</sup>Ge/<sup>68</sup>Ga generator that most users will have on site but independent of an on-site cyclotron. <sup>68</sup>Ge with a long physical half-life is ideal for clinical settings with its suitable lifespan. However, if used for more than a year, the eluting of <sup>68</sup>Ga usually requires a hydrochloric acid solution containing <sup>68</sup>GaCl<sub>3</sub> as its chemical form.<sup>2,3</sup>

The use of radiolabeled peptides in nuclear oncology is increasing. In particular, positron-emitting peptides have now been developed. <sup>68</sup>Ga-labeled compounds are becoming increasingly popular in clinical PET.<sup>4</sup> <sup>68</sup>Ga-labeled DOTA-peptides are the most generally used radiotracers for PET imaging, especially <sup>68</sup>Ga-DOTA-TOC and <sup>68</sup>Ga-DOTA-TATE in the diagnosis of neuroendocrine tumors.<sup>5,6</sup> More recently, NOTA a commonly used bifunctional chelator, has been shown to possess a superior <sup>68</sup>Ga-binding ability, and so <sup>68</sup>Ga-NOTA-RGD was developed as a radiotracer for the visualization of angiogenesis.<sup>7</sup>

During the past few years, gallium has seen a change in its role in infection imaging. While <sup>67</sup>Ga-citrate has been extensively used for the past four decades but with limitations, now <sup>68</sup>Ga citrate and <sup>68</sup>GaCl<sub>3</sub> are usually used for infection imaging.<sup>8</sup>

In several <sup>68</sup>Ge/<sup>68</sup>Ga generator systems, <sup>68</sup>Ge is adsorbed on a wide range of solid supports, including metal oxides, such as SnO<sub>2</sub>, TiO<sub>2</sub>, and Al<sub>2</sub>O<sub>3</sub>; organic materials, e.g., pyrogallol-formaldehyde resins; and inorganic materials, e.g., silica.<sup>9-11</sup> The main drawback of these systems is that the <sup>68</sup>Ga eluate is usually contaminated with long-life <sup>68</sup>Ge and trace metallic impurities, which could potentially compete with the <sup>68</sup>Ga ion when labeled with nanomole levels of conjugated peptides/biomolecules or other carrier ligands. In addition, the eluate from <sup>68</sup>Ge/<sup>68</sup>Ga generators usually has a relatively large volume and high HCl concentration from 0.1–1N, which causes problems in the labeling process. Therefore, dedicated procedures to purify and concentrate <sup>68</sup>Ga before labeling are needed.<sup>12</sup>

There has been a recent report describing a method of purifying <sup>68</sup>Ga for performing <sup>68</sup>Ga-labeled radiopharmaceuticals from <sup>68</sup>Ge/<sup>68</sup>Ga generator eluates eluted with HCl/acetone mixtures on a micro-cation exchange column.<sup>13-15</sup> In other studies, several approaches for purification and concentration have been reported. An effective post-processing technique for <sup>68</sup>Ge/<sup>68</sup>Ga generators

using cation and anion exchange chromatography was developed to provide high <sup>68</sup>Ga recovery, <sup>68</sup>Ge removal, the removal of metallic impurities, lower acidity, and minimized volumes, which would all be useful for direct radiolabeling reactions with a high labeling efficiency of <sup>68</sup>Ga-NOTA-RGD.

The aim of this study was to develop a purification method that makes use of both cation exchange and anion exchange processes to purify <sup>68</sup>Ga eluates with a high radiochemical purity (RCP) and short purification times. The system should also provide a <sup>68</sup>Ga eluate that can be directly used for labeling with a high radiolabeling yield and the highest radionuclidic purity.

## MATERIALS AND METHODS

### Purification process of the <sup>68</sup>Ga eluate

The <sup>68</sup>Ge/<sup>68</sup>Ga generator was purchased from iThemba LABS (Somerset West, South Africa) and had been previously checked for metallic impurities. The elution was done using sterilized 0.6N HCl<sup>12</sup> prepared from ultra-purified hydrochloric acid and tri-distilled water. In order to elute the generator, 6 mL HCl 0.6N was used, and the eluate was divided into five equal portions of 1.2 mL each. The first portion was the non-purified eluate. The second through fifth portions were used for eluate purification by loading the eluate on the top of the cation exchange column. The column was pre-conditioned with 0.5 mL of 98% acetone/0.05 N HCl. The eluate in each column was eluted using 2 mL of 97.6% acetone with different concentrations of HCl (0.05N, 0.10N, 0.15N, and 0.2N). Each eluate from 50 mg of the cation exchange resin, which by now was in the form of [<sup>68</sup>GaCl<sub>4</sub>]<sup>-</sup>, was passed down to 50 mg of the anion exchange resin. The trapped [<sup>68</sup>GaCl<sub>4</sub>]<sup>-</sup> in the anion exchange resin was eluted with 1 mL ultra-purified water. After the purification process, the purity of the eluate in aqueous solution was investigated by inductively coupled plasma atomic emission spectrometry (ICP-AES).

### Study of the metal impurities by ICP-AES

Metal impurities in the eluate were analyzed using a Spectro Arcos 165 ICP-AES system equipped with a Cetac ASX-520 autosampler. The measurements were performed using an ICP-AES spectrometer to investigate the metal ions, whereby the most prominent atomic and ionic analytical lines were chosen, including Fe at 238.104 nm, Zn at 206.2 nm, Ge at 265.118 nm, and Al at 396.153 nm. The concentrations of the following metal ions were determined by comparing them to 50, 100, 200, and 400 ppb solutions prepared from the 1000 ppb standard solution.

### Germanium-68 breakthrough

Germanium-68 breakthrough was calculated by comparing the daughter radionuclide ( $^{68}\text{Ga}$ ) to the parent radionuclide ( $^{68}\text{Ge}$ ). Germanium-68 breakthrough was measured after complete  $^{68}\text{Ga}$  decay (<48 h) by a multi-channel analyzer (MCA) equipped with a high-purity germanium (HPGe) detector.

### Radiolabeling of $^{68}\text{Ga}$ -NOTA-RGD

Purified  $^{68}\text{Ga}$  (111 MBq in 1 mL of tri-distilled sterile water) was added to a 20 µg lyophilized NOTA-RGD kit (supplied by Jae Min Jeong Seoul National University, Jongro-gu, Seoul, Korea)<sup>16</sup>, and the pH was adjusted to 5.0 with 0.1M ammonium acetate buffer. The pH of the mixture was checked with a pH indicator strip and then the mixture was heated in a water bath at 100 °C for 15 min. After cooling, the labeled product  $^{68}\text{Ga}$ -NOTA-RGD was sterilized by 0.22 µm Millipore filtration.

### Quality control of $^{68}\text{Ga}$ -NOTA-RGD

The labeling efficiencies and radiochemical purities of the purified  $^{68}\text{Ga}$ -NOTA-RGD were determined by high-performance liquid chromatography (HPLC) (Agilent Tech., Series 1200) with a Phenomenex Jupiter column C-18, 5 µm, 4.6 × 250 mm. The solvents were 0.1% (m/v) trifluoroacetic acid (TFA) in deionized water (A) and 100% acetonitrile (B). Elution was carried out at a flow

rate of 1 mL/min, under UV-visible illumination (200, 280 nm) with a gamma-ray detector (Raytest Gabi Star), using the elution program in Table 1.

As a result, the peak for free  $^{68}\text{Ga}$  appeared at 3.5 to 4 min, whereas peaks for small particles of the radiochemical impurities appeared at 3.0 to 3.5 min, while  $^{68}\text{Ga}$ -NOTA-RGD showed an earlier retention time of 9.0 to 9.5 min.

### RESULTS

The small amounts of Fe(III), Zn(II), Ge(IV), and Al(III) were found in the eluate. The concentration of Ge(IV) was less than 1 ppb while all the other metals were less than 1 ppm. After the purification process, Fe(II), Zn(II), and Al(III) were reduced by 61%, 38%, and 44%, respectively, compared to the initial non-purified evaluation (Table 2).

The  $^{68}\text{Ga}$  eluted from the  $^{68}\text{Ge}/^{68}\text{Ga}$  generator usually contains small amounts of metallic impurities that represent metals that can compete with  $^{68}\text{Ga}$ (III) in the radiopharmaceutical labeling process, thereby adversely affecting both the  $^{68}\text{Ga}$  labeling yields and the specific activity of the labeled compound. These metal impurities, especially Fe(III), and the  $^{68}\text{Ge}$  breakthrough need to be effectively removed. Here, the concentrations of metallic ions before and after purification are shown in Table 2.

**TABLE 1.** HPLC gradient for the elution of  $^{68}\text{Ga}$ -NOTA-RGD

Time (min)	0.1% Trifluoroacetic acid (TFA) in deionized water (A)	Acetonitrile (ACN) (B)
0–4	90%	10%
4–10	30%	70%
10–13	10%	90%

**TABLE 2.** Metal impurities concentrations in  $^{68}\text{Ga}$  eluates eluted with different acetone / hydrochloric acid mixtures.

Metal	Non-purified	98% Acetone/ 0.05N HCl	98% Acetone/ 0.1N HCl	98% Acetone/ 0.15N HCl	98% Acetone/ 0.2N HCl
Al(III)	1.46E-04	5.16E-05	2.88E-04	6.31E-05	8.17E-05
Fe(III)	6.36E-04	6.52E-04	5.18E-04	4.50E-04	2.48E-04
Zn(II)	1.73E-04	7.59E-05	1.14E-04	2.78E-04	1.06E-04
Ge(IV)	7.97E-06	2.04E-06	1.11E-06	1.23E-06	4.08E-07

**<sup>68</sup>Ge breakthrough**

The <sup>68</sup>Ge breakthrough in 12 samples using a SnO<sub>2</sub>-based <sup>68</sup>Ge/<sup>68</sup>Ga generator. After the complete decay of <sup>68</sup>Ga (> 48 h), the quantitative measurement of <sup>68</sup>Ge breakthrough was performed using a calibrated gamma spectrometer equipped with a coaxial (HPGe) detector. The <sup>68</sup>Ge breakthrough from the generator was found to be approximately 10<sup>-3</sup>% of the eluted <sup>68</sup>Ga activity after purification. This is in accord with many publications that have reported breakthrough of the <sup>68</sup>Ge parent radionuclide as usually less than 0.001%

**Elution efficiency after purification and the labeling of RGD peptide with <sup>68</sup>Ga**

The entire purification process, including two purification steps, cation exchange and anion exchange chromatography, was completed within 30 min. The mean activity at the first elution was 2.89 ± 0.11 mCi. The radioactivity levels of <sup>68</sup>Ga at the first elution and after passing through the cation and anion exchange columns and labeling with NOTA-RGD peptide are shown in Table 3. Without correction for the decay, the mean activities after the two purification steps and labeling were 2.00 ± 0.10, 1.74 ± 0.10, and 1.44 ± 0.10

mCi, respectively. In this study, the elution efficiencies after the purification steps were found to range between 50% to 70%. The elution efficiency decreased to about 69.02 ± 2.88% after the first purification step, 60.03 ± 2.54% after the second step, and 49.89 ± 2.49% after the labeling, see Table 3. In this study, it was approximately 70% over a processing time of not more than 30 minutes. However, the labeling efficiency was 99% and only a small amount of free <sup>68</sup>Ga was detected when labeling NOTA-RGD peptide with the purified <sup>68</sup>Ga, see Table 4.

The complexation yield of Ga-NOTA-RGD was validated by HPLC studies. Fig 1 shows the typical HPLC pattern of <sup>68</sup>Ga-NOTA-RGD. The <sup>68</sup>Ga-NOTA-RGD peak was collected at a retention time of 9.0–9.5 min, while free <sup>68</sup>Ga peak appeared at 3.5–4 min, and small traces of radiochemical impurities at 3.0–3.5 min.

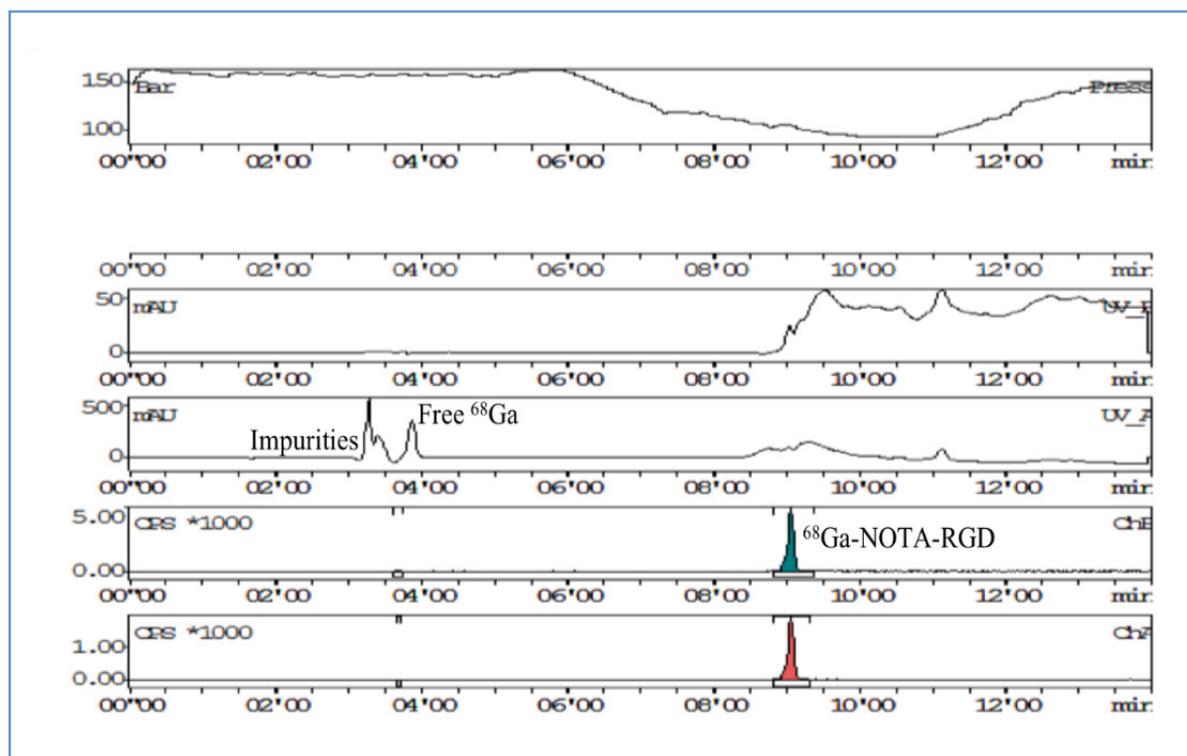
The efficacy of <sup>68</sup>Ga for the preparation of radiopharmaceuticals for PET imaging was confirmed by radiolabeling NOTA-RGD with a very high complexation yield. The radiochemical purity (RCP) of <sup>68</sup>Ga-NOTA-RGD was higher than 99%. The process of labeling was completed within 30 minutes. The mean labeling efficiency was 99.31 ± 0.32%, while the unlabeled <sup>68</sup>Ga was 0.69 ± 0.32%, as shown in Table 4.

**TABLE 3.** Radioactivity in mCi of <sup>68</sup>Ga at the first elution, and after the two purification steps and labeling (The percentage reduction of radioactivity is in parenthesis).

Test no	Radioactivity of <sup>68</sup> Ga in mCi and the percentage reduction (%)					
	First elution	After purification by cation exchange		After purification by anion exchange		After labeling
1	2.93	2.05	(69.97)	1.75	(59.73)	1.48 (50.51)
2	2.82	1.95	(69.15)	1.69	(59.939)	1.43 (50.71)
3	2.94	1.99	(67.69)	1.73	(58.84)	1.44 (48.98)
4	3.02	1.95	(64.57)	1.72	(56.95)	1.41 (46.69)
5	2.95	2.11	(71.53)	1.84	(62.37)	1.53 (51.86)
6	2.77	1.83	(66.06)	1.63	(58.843)	1.39 (50.18)
7	2.62	1.94	(74.05)	1.65	(62.98)	1.40 (53.44)
8	2.90	2.02	(69.66)	1.72	(59.31)	1.46 (50.34)
9	2.98	2.12	(71.14)	1.92	(64.43)	1.61 (54.03)
10	3.01	2.14	(71.10)	1.89	(62.79)	1.44 (47.84)
11	2.91	1.88	(64.60)	1.67	(57.393)	1.34 (46.05)
12	2.85	1.96	(68.77)	1.62	(56.84)	1.37 (48.07)
<b>Mean</b>	2.89	2.33	(69.02)	2.24	(60.03)	2.06 (49.89)
<b>S.D.</b>	0.11	0.11	(2.88)	0.13	(2.54)	0.10 (2.49)

**TABLE 4.** Analysis of  $^{68}\text{Ga}$ -NOTA-RGD and unlabeled  $^{68}\text{Ga}$  by HPLC.

Test no	Area count (cps)			% Area	
	Region 1	Region 2	Total count	Region 1	Region 2
1	80.11	18020.06	18706.17	0.43	99.57
2	100.45	17896.08	17996.53	0.56	99.44
3	77.45	18223.69	18301.14	0.42	99.58
4	256.22	17745.32	18001.54	1.42	98.58
5	59.91	19304.39	19364.30	0.31	99.69
6	147.25	17593.25	17740.50	0.83	99.17
7	201.43	17620.13	17821.56	1.13	98.87
8	81.11	18375.34	18465.45	0.44	99.56
9	154.6	20362.14	20516.74	0.75	99.25
10	145.64	18122.65	18268.29	0.80	99.20
11	93.01	16934.67	17027.68	0.55	99.45
12	105.56	17291.59	17397.15	0.61	99.39
<b>Mean <math>\pm</math> SD</b>				<b>0.69 <math>\pm</math> 0.32</b>	<b>99.3 <math>\pm</math> 0.32</b>

**Fig 1.** HPLC chromatogram patterns of  $^{68}\text{Ga}$ -NOTA-RGD.

## CONCLUSION AND DISCUSSION

The  $^{68}\text{Ge}/^{68}\text{Ga}$  generator used in this study was a  $\text{SnO}_2$ -based generator. By measuring the metallic impurities by ICP-AES, it was verified that metallic impurities were present that could interfere with the formation of Ga(III) complexes. Therefore, before the radiolabeling of peptides, the  $^{68}\text{Ga}$  eluate would need to be purified using a cationic exchange column, an anionic exchange column, or both. In this study,  $^{68}\text{Ga}$  solutions were purified on both cation and anion exchange columns. We eluted the  $^{68}\text{Ga}$  eluate using different concentrations of acetone/hydrochloric acid mixtures after each transfer. Schultz et al.<sup>17</sup> used different sets of chromatographic columns, 50W X8 cation, and UTEVA resin, and eluted with 0.1M HCl. Metal analysis by ICP-AES demonstrated that the stable metals were reduced to less than 0.2 ppm, but Fe(III) could not be removed, while the breakthrough of  $^{68}\text{Ge}$  was less than 0.02% of the  $^{68}\text{Ga}$  activity.

Germanium-68 is strongly absorbed by metal oxides or organic materials, making  $^{68}\text{Ge}$  breakthrough highly unlikely. However, the metal impurities from  $^{68}\text{Ge}$  breakthrough in the eluate are lesser problems compared to patient exposure to radiation when used for the radiolabeling of peptides or other biomolecules. As a minimum, the radionuclidic purity of  $^{68}\text{Ga}$  chloride solution should be limited to 99.9% of the total radioactivity, whereas  $^{68}\text{Ge}$  should not exceed 0.001% (EU Pharm). Some have even reported values less than  $10^{-4}\%$  to  $10^{-5}\%$ . Konstantin et al.<sup>18</sup> reported that the initial amount of  $^{68}\text{Ge}(\text{IV})$  was decreased by a factor of  $10^4$  when using a  $\text{TiO}_2$ -based generator. Roesch<sup>20</sup> reported that  $^{68}\text{Ge}$  breakthrough levels ranged from 0.01% to 0.001% for fresh generators, but they increase with extended use. Since the  $^{68}\text{Ge}$  breakthrough has been shown to increase over the lifetime of the generator and our generator had been used for more than 18 months, our presented result of  $10^{-3}\%$  of the eluted  $^{68}\text{Ga}$  activity is considered an acceptable result. This means our generator also fulfills the requirement of the European Pharmacopeia (EU) concerning the radionuclide purity for  $^{68}\text{Ga}$  of 99.9%. Concerning the radiation absorbed dose, a recent publication reported that  $^{68}\text{Ge}$  is rapidly excreted in the urine, which greatly diminishes the potential radiation absorbed dose. Lin M et al.<sup>21</sup> demonstrated that the elution of  $^{68}\text{Ge}$  from a commercial titanium-dioxide-based  $^{68}\text{Ge}/^{68}\text{Ga}$  generator resulted in markedly low  $^{68}\text{Ge}$  breakthrough, in the order of 14 to 25 nCi. When labeled with DOTATOC, spectroscopic analysis of the synthesis components demonstrated that the  $^{68}\text{Ge}$  breakthrough in the final products was quantitatively removed. Sudbrock et al.<sup>22</sup> reported that the content of long-lived  $^{68}\text{Ge}$  breakthrough increased

to more than 100 ppm over the entire period of use of the generator, while the chelator DOTA eliminated  $^{68}\text{Ge}$  efficiently during labeling. The maximum  $^{68}\text{Ge}$  activity found in the labeled product (below 10 Bq) and the effective doses received by the patient from  $^{68}\text{Ge}$  in the  $^{68}\text{Ga}$ -DOTATATE final product were lower than  $0.1 \mu\text{Sv}$ , meaning practically insignificant for patients.

After the elution of  $^{68}\text{Ga}$ , the activity of  $^{68}\text{Ga}$  was measured immediately using a calibrated ionization chamber to determine the elution efficiency. The  $^{68}\text{Ga}$  elution yields dropped with increasing its usage frequency or shelf-life. In this study, the elution yield of our generator was found to be lower than 50% because it has been used for almost 18 months. Roesch<sup>23</sup> reported that the  $^{68}\text{Ga}$ -eluted yields range from about 70% to 80% for fresh generators, but these decrease over time. The initial yield of the generator has been reported to range from 75% to 100% and the long-term yield from 60% to more than 80%. Patrascu et al.<sup>24</sup> reported an elution efficiency of 80% and Konstantin et al.<sup>18</sup> reported an initial activity of more than 97% from a  $\text{TiO}_2$ -based generator.

The time spent processing the generator eluate, synthesizing the labeled product, and purifying it reduced the production yield. In this study without correction for the decay, the final yield of  $^{68}\text{Ga}$ -NOTA-RGD peptide was less than 50% ( $49.89 \pm 2.49\%$ ). A final yield of  $46 \pm 5\%$  for the  $^{68}\text{Ga}$ -labeled DOTA-conjugated octreotide was reported by Roesch and Filosofov.<sup>25</sup> Further, the decay-corrected yields of  $^{68}\text{Ga}$  radiopharmaceuticals did not exceed 60% to 70%.

For the clinical application of  $^{68}\text{Ga}$  produced by  $^{68}\text{Ge}/^{68}\text{Ga}$  generators, it is important to obtain  $^{68}\text{Ga}$  in a purified chemical form, maximize the elution yield of  $^{68}\text{Ga}$ , and reduce the elution volume while maintaining a permissible level of  $^{68}\text{Ge}$  impurity in the eluate. There is a possibility of regularly eluting  $^{68}\text{Ge}/^{68}\text{Ga}$  from the generator in a way that provides an acceptable radioactive concentration, yield, and purity. It was reported by Asti et al.<sup>19</sup> that the concentration of  $^{68}\text{Ge}$  breakthrough increased with time, with approximately a 15% increase per month, ranging from  $1.1 \times 10^{-2}\%$  to  $2.6 \times 10^{-2}\%$  of the  $^{68}\text{Ga}$  activity within their 7 months of evaluation. Moreover, the elution yields of  $^{68}\text{Ga}$  from these generators decreased from 82% to 69% when elution was repeated, i.e., 100 times, over the period of 7 months.<sup>19</sup>

The RCP of the  $^{68}\text{Ga}$ -labeled product should be greater than 99%, which would result in a high efficiency of radiolabeling with a small volume and low acidity. In the present study, the efficacy of  $^{68}\text{Ga}$  for the preparation of radiopharmaceuticals for PET imaging was confirmed by radiolabeling NOTA-RGD with a very high yield.

At present, an automatic synthesis module to produce  $^{68}\text{Ga}$ -NOTA-RGD may be used. The module was connected to a line elution  $^{68}\text{Ge}/^{68}\text{Ga}$  generator with a purification part and controlled by a personal computer program for easy production and to make it suitable for routine work. However, a variety of post-processing methods, such as anionic exchange and cationic exchange purification, are required to purify the eluate. It is also of particular importance for the labeled products to have high specific activities.

## ACKNOWLEDGEMENTS

The dissertation was financially supported under Research Fellowship, provided by Siriraj Graduate Scholarship. We are grateful for Radioisotope Center, Thailand Institute of Nuclear Technology for the permission to include copyrighted photographs as part of my thesis. I would like to thank Mr. Jatupol Sangsuriyan and Miss Nipavan Poramatikul for their valuable and constructive suggestions during the planning of this research work.

## REFERENCES

- Banerjee SR, Pomper MG. Clinical applications of gallium-68. *Appl Radiat Isot.* 2013;76:2-13.
- Nelson BJB, Andersson JD, Wuest F, Spreckelmeyer S. Good practices for  $^{68}\text{Ga}$  radiopharmaceutical production. *EJNMMI Radiopharm Chem.* 2022;7(1):27.
- Sriprapa T, Doungta T, Sakulsamart N, Taweewatthanasopon N, Madputeh L, Ragchana P, et al. Evaluation of the efficacy and safety of the ITM  $^{68}\text{Ge}/^{68}\text{Ga}$  generator after its recommended shelf-life. *Siriraj Med J.* 2023;75(10):752-8.
- Dash A, Chakravarty R. Radionuclide generators: the prospect of availing PET radiotracers to meet current clinical needs and future research demands. *Am J Nucl Med Mol Imaging.* 2023; 9(1):30-66.
- Hennrich U, Benešová M. [ $^{68}\text{Ga}$ ]-DOTA-TOC: The first FDA-approved  $^{68}\text{Ga}$ -radiopharmaceutical for PET imaging. *Pharmaceuticals (Basel).* 2020;13(3):38.
- Özgülven S, Filizoğlu N, Kesim S, Öksüzöğlü K, Şen F, Öneş T, et al. Physiological biodistribution of  $^{68}\text{Ga}$ -DOTA-TATE in normal subjects. *Mol Imaging Radionucl Ther.* 2021;30(1): 39-46.
- Chen C-J, Chan C-H, Lin K-L, Chen J-H, Tseng C-H, Wang P-Y, et al.  $^{68}\text{Ga}$ -labelled NOTA-RGD-GE11 peptide for dual integrin and EGFR-targeted tumour imaging. *Nucl Med Biol.* 2019;68:22-30.
- Aghanejad A, Jalilian AR, Ardaneh K, Bolourinovin F, Yousefina H, Samani AB. Preparation and quality control of  $^{68}\text{Ga}$ -citrate for PET applications. *Asia Ocean J Nucl Med Biol.* 2015;3(2): 99-106.
- <https://rusatom-energy.com/media/rosatom-news/rosatom-facility-gains-record-revenues-from-stable-isotopes-sales/>
- Romero E, Martínez A, Oteo M, Ibañez M, Santos M, Morcillo MÁ. Development and long-term evaluation of a new  $^{68}\text{Ge}/^{68}\text{Ga}$  generator based on nano-SnO<sub>2</sub> for PET imaging. *Sci Rep.* 2020;10(1):12756.
- Malyshev KV, Smirnov VV. Generator of sup  $^{68}\text{Ga}$  based on zirconium hydroxide. *Sov Radiochem. (Engl. Transl.); (United States).* 1975;17:1.
- de Blois E, Sze Chan H, Naidoo C, Prince D, Krenning EP, Breeman WAP. Characteristics of SnO<sub>2</sub>-based  $^{68}\text{Ge}/^{68}\text{Ga}$  generator and aspects of radiolabelling DOTA-peptides. *App Radiat Isot.* 2011;69(2):308-15.
- Rösch F, Knapp FF, Jr. Radionuclide Generators. In: *Handbook of Nuclear Chemistry.* Springer US, 2011:1935-76.
- Arino H, Skraba WJ, Kramer HH. A new  $^{68}\text{Ge}/^{68}\text{Ga}$  radioisotope generator system. *The International Journal of Applied Radiation and Isotopes.* 1978;29(2):117-20.
- Loc'h C, Mazière B, Comar D. A new generator for ionic gallium-68. *J Nucl Med.* 1980;21(2):171-3.
- Jeong JM, Hong MK, Chang YS, Lee YS, Kim YJ, Cheon GJ, et al. Preparation of a promising angiogenesis PET imaging agent:  $^{68}\text{Ga}$ -labeled c(RGDyK)-isothiocyanatobenzyl-1,4,7-triazacyclononane-1,4,7-triacetic acid and feasibility studies in mice. *J Nucl Med* 2008;49(5):830-6.
- Schultz M, McAlister D, Tewson T, Harvey J, Horwitz P. Evaluation of a new Ge-68/Ga-68 generator for preparing high-purity radiopharmaceuticals for PET imaging. *Journal of Nuclear Medicine.* 2008;49(1):301.
- Zhernosekov KP, Filosofov DV, Baum RP, Aschoff P, Bihl H, Razbash AA, et al. Processing of generator-produced  $^{68}\text{Ga}$  for medical application. *J Nucl Med.* 2007;48(10):1741-8.
- Asti M, De Pietri G, Fraternali A, Grassi E, Sghedoni R, Fioroni F, et al. Validation of  $^{68}\text{Ge}/^{68}\text{Ga}$  generator processing by chemical purification for routine clinical application of  $^{68}\text{Ga}$ -DOTATOC. *Nucl Med Biol.* 2008;35(6):721-4.
- Rösch F.  $^{68}\text{Ge}/^{68}\text{Ga}$  Generators and  $^{68}\text{Ga}$  Radiopharmaceutical Chemistry on Their Way into a New Century. *J Postgrad Med Edu Res.* 2013;47(1):18-25.
- Lin M, Ranganathan D, Mori T, Hagooley A, Rossin R, Welch MJ, et al. Long-term evaluation of TiO<sub>2</sub>-based  $^{68}\text{Ge}/^{68}\text{Ga}$  generators and optimized automation of [ $^{68}\text{Ga}$ ]DOTATOC radiosynthesis. *Appl Radiat Isot.* 2012;70(10):2539-44.
- Sudbrock F, Fischer T, Zimmermanns B, Guliyev M, Dietlein M, Drzezga A, et al. Characterization of SnO<sub>2</sub>-based  $^{68}\text{Ge}/^{68}\text{Ga}$  generators and  $^{68}\text{Ga}$ -DOTATATE preparations: radionuclide purity, radiochemical yield and long-term constancy. *EJNMMI Res.* 2014;4(1):36.
- Roesch F. Maturation of a Key Resource – The Germanium-68/ Gallium-68 Generator: Development and New Insights. *Curr Radiopharm.* 2012;5(3):202-11.
- Patrascu I, Niculae D. The purification and the quality control of  $^{68}\text{Ga}$  eluates from  $^{68}\text{Ge}/^{68}\text{Ga}$  generator. *Romanian Reports in Physics.* 2011;63(4):988-96.
- Roesch F, Filosofov DV. Chapter 3: Production, radiochemical processing, quality evaluation of Ge-68 in Production of long lived parent radionuclides for generators Ge-68, Sr-82, Sr-90 and W-188. IAEA Radioisotopes and radiopharmaceuticals Series. 2<sup>nd</sup> Edn., International Atomic Energy Agency, 2010.

# Comparison between Anal Dilatation Protocols Following an Endorectal Pull-through for Hirschsprung Disease

Ravit Ruangtrakool, M.D., FRCST., Jirarak Deepor, M.D.

Division of Pediatric Surgery, Department of Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.

## ABSTRACT

**Objective:** The purpose of this study was to compare the mechanical obstruction rate following a transanal endorectal pull-through (TERPT) in patients with Hirschsprung disease, between regular anal dilatation (AD) and selective anal dilatation (NAD) which meant that dilatation was only performed when an obstructive symptom occurred.

**Materials and Methods:** A retrospective chart review of patients with Hirschsprung disease who underwent TERPT/abdominal assisted TERPT at Siriraj Hospital between January 2009 and December 2021 was carried out. It was the surgeon's preference that the dilatation protocols between the 2 groups (AD or NAD) were assigned. Mechanical obstructions included evidence of stricture, a clinical symptom of constipation, presence of Hirschsprung-associated enterocolitis (HAEC), and/or requirement of re-operation.

**Results:** In total, 132 patients were included in this study, including 55 cases in the AD group (41.7%) and 77 cases in the NAD group (58.3%). Postoperative mechanical obstructions occurred in 84 patients (63.6%). Among the mechanical obstructions, there were 35 strictures (26.5%), 26 constipation (19.7%), 40 HAECs (30.3%), and 6 re-operation (4.5%). The mechanical obstruction rates in the AD [33/55 (60.0%)] and NAD [51/77 (66.2%)] groups were not significantly different ( $p = 0.582$ ). The AD group was of a significantly younger age ( $p = 0.022$ ) and lower body weight ( $p = 0.048$ ) than the NAD group; however, a younger age and lower body weight were not significantly related with any of the obstructive complications. AD had a rate of anastomosis/cuff stricture [13/55 (23.6%)] similar to NAD [22/77 (28.6%)] ( $p = 0.665$ ). When the statistics tests for the non-inferiority of the difference in stricture events between NAD and AD were performed, non-inferiority was demonstrated ( $p = 0.049$ ). AD and NAD also had similar rates of other mechanical obstructions, including constipation ( $p = 0.767$ ), HAEC ( $p = 0.224$ ), and re-operation ( $p = 0.234$ ), respectively.

**Conclusion:** Regular anal dilatation and selective anal dilatation had comparable rates of all types of mechanical obstruction.

**Keywords:** Hirschsprung; stricture; anal dilatation; endorectal pull-through; complication (Siriraj Med J 2024; 76: 97-105)

## INTRODUCTION

Surgery is the mainstay definitive treatment for Hirschsprung disease. The effective surgical treatment includes resection of the aganglionic portion of the bowel and identification of the proximal normally ganglionic

bowel, followed by a leveled coloanal anastomosis. Operative approaches to correct Hirschsprung disease are derived from the original concepts of Swenson, Duhamel, and Soave-Boley.<sup>1-3</sup> The standard Soave-Boley endorectal pull-through procedure used both abdominal

Corresponding author: Ravit Ruangtrakool

E-mail: sisuped@mahidol.ac.th

Received 14 December 2023 Revised 8 January 2024 Accepted 9 January 2024

ORCID ID: <http://orcid.org/0000-0001-8162-2941>

<https://doi.org/10.33192/smj.v76i2.266716>



All material is licensed under terms of the Creative Commons Attribution 4.0 International (CC-BY-NC-ND 4.0) license unless otherwise stated.

and transanal approaches.<sup>2,3</sup> The Soave-Boley endorectal pull-through procedure was transformed into a solely transanal approach named “transanal endorectal pull-through (TERPT)” by De la Torre and Ortega in 1988.<sup>4-6</sup> If the transanal approach cannot be performed perfectly, a combination of abdominal and transanal approaches, named “abdominal assisted transanal endorectal pull-through” (abdo + TERPT), is used.

On a transanal endorectal pull-through, mucosal traction sutures are placed to define the submucosal plane. A circular incision is made at 0.5 cm proximal to the anal dentate line. This submucosal plane is then developed using both blunt and sharp dissection. A submucosal dissection of the rectum after a circumferential incision of the rectal mucosa is performed. Following submucosal dissection, a seromuscular layer of the rectum is incised circumferentially. Mucosectomy of the rectum, leaving a muscular cuff, is also performed. The ganglionic colon is pulled through the aganglionic rectal cuff and a coloanal anastomosis is then carried out.<sup>4-6</sup>

An anastomotic stricture after a pull-through procedure is an important postoperative complication. Risk factors of an anastomotic stricture include anastomotic ischemia, muscular cuff ischemia, anastomotic leak, and small circular anastomosis.<sup>7</sup> In the author’s previously reported studies, anastomotic stricture was found to be the most common complication in this procedure.<sup>8,9</sup> In particular, anastomotic stricture was frequently found in those with a low transitional zone,<sup>8</sup> and it was found that the TERPT had a higher risk (12%) of anastomotic strictures than the abdominal assisted transanal endorectal pull-through (5%).<sup>8</sup> Following a TERPT, De la Torre, who has been a pioneer in this operation for more than 3 decades, still questions whether the anus should be routinely dilated or not.<sup>10</sup>

In the Division of Pediatric Surgery, Department of Surgery, Siriraj University Hospital, there are two strategies for anal dilatation in children: regular anal dilation (AD) and selective anal dilation (NAD). However, there is no definite criteria that determines which of the two options is appropriate for certain patients. The choice might depend on age and weight at operation, the attending surgeon’s preference, the operation technique, and the difficulty of the operation.

Although the AD method is a popular option, some argue that it can have a great impact on the mental health of both the patients or parents. The patients and parents are all affected mentally while the patient must be held still in order to dilate the anus for a long period of time.<sup>11,12</sup>

The aim of this study was to determine which option

of anal dilatation, whether AD or NAD, is the most appropriate option to prevent mechanical obstruction following a transanal endorectal pull-through or an abdominal assisted transanal endorectal pull-through. Individual mechanical obstructions were further studied and compared between these two options of anal dilatation.

## MATERIALS AND METHODS

After obtaining an approval from the Siriraj Institutional Review Board (COA. no. Si 969/2021), a retrospective study was carried out in children diagnosed with Hirschsprung disease who underwent either a transanal endorectal pull-through or abdominal assisted transanal endorectal pull-through at Siriraj Hospital between January 2009 to December 2021. In this study, mechanical obstruction was defined as: severe stricture (either coloanal anastomosis or seromuscular cuff stricture), constipation with the usage of laxatives, enemas, and/or bowel irrigation for more than 3 months<sup>13</sup>, Hirschsprung’s enterocolitis with a Hirschsprung-associated enterocolitis (HAEC) score<sup>14</sup> greater than or equal to 10, and re-operation related to coloanal anastomosis or seromuscular cuff stricture. Patients with Hirschsprung disease who previously underwent a definitive operation at another hospital, and those with incomplete medical information were excluded from the study.

In the AD group, the size of the anastomosis was measured and calibrated at 2-3 weeks following an operation by a finger or a Hegar dilator. In some cases, the operation was done under general anesthesia. Then, the anus was dilated with a Hegar dilator 1-2 times a day at home by the parents. The duration of dilatation was at least 6 months.<sup>15-18</sup> The main concept of the AD was to gradually expand the anus to the size equivalent to a normal anus, to prevent stricture, severe intractable constipation, and HAEC. It was believed that if a stricture has already occurred, most patients remained asymptomatic until the stricture became too severe to be dilated with ease.<sup>19</sup>

In the NAD approach, the size of the anastomosis was measured and calibrated at 2-3 weeks following surgery. Anal dilatation had not been started until a patient developed symptoms of mechanical obstruction or had a stricture documented while following up at the outpatient department (OPD) then the patient would be dilated daily by their parents.<sup>13,19,20</sup>

Patients’ demographic data, age and weight at operation, transitional zone level, types of operation, options of anal dilatation, and postoperative mechanical obstructions, such as stricture, constipation, Hirschsprung’s enterocolitis, and re-operation following a TERPT were collected. The collected data were analyzed using SPSS

software version 18 (SPSS Inc. Released 2009. PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc). Continuous data were expressed as the median and interquartile range (IQR) and categorical data were expressed as numbers and percentages. For the qualitative data, the chi-square test or Fisher's exact test was used to compare the difference in proportions between independent groups. For the quantitative data, the Mann-Whitney U test was used to compare the mean between each group. A *p*-value of <0.05 indicated statistical significance.

A statistics test for non-inferiority was also performed. Non-inferiority was demonstrated when the lower bound of the 95% one-sided confidence interval (CI) for the difference in the stricture rate between the AD and the NAD was lower than the pre-specified non-inferiority margin of 10%. The *p*-value of the non-inferiority test was considered when less than a significant level of 0.05.

## RESULTS

A total of 132 Hirschsprung patients were included in this study. Postoperative mechanical obstructions occurred in 84 patients (63.6%), including 35 cases

(26.5%) of stricture, 26 constipation (19.7%), 40 HAEC (30.3%), and 6 patients who required re-operation (4.5%). Fifty-five patients (41.7%) were in the AD group, whereas the other 77 patients (58.3%) were in the NAD group. The patient's demographic data in both regular anal dilatation group and selective anal dilatation group are demonstrated in Table 1. Median ages of the patients in the AD and the NAD group were 1.4 months and 2.8 months, respectively. The difference was statistically significant (*p* = 0.022). The median weight of the patients in the AD group and the NAD group were 4.2 and 4.9 kg, respectively. Although this seemed not clinically significant difference, the difference was statistically significant (*p* = 0.048). Other factors, including gender, level of transitional zone, and whether an abdominal assisted approach was used, between these two groups were not statistically different (*p* = 0.492, *p* = 0.212, and *p* = 0.084, respectively). On comparing the incidences of postoperative mechanical obstructions between the AD group and the NAD group (Table 2), the incidences of mechanical obstruction were 60.0% and 66.2%, respectively (*p* = 0.582).

**TABLE 1.** Patients' demographic data: Comparison between the regular anal dilatation group and selective anal dilatation group.

	Regular anal dilatation (n = 55)	Selective anal dilatation (n = 77)	Total (n = 132)	<i>p</i> -value
<b>Gender, n (%)</b>				0.492
Male	39 (70.9%)	49 (63.6%)	88 (66.7%)	
Female	16 (29.1%)	28 (36.4%)	44 (33.3%)	
<b>Age (months)</b> (median (IQR))	1.4 (3.9)	2.8 (12.8)	2.3 (7.3)	0.022
<b>Weight (kg)</b> (median (IQR))	4.2 (2.8)	4.9 (4.6)	4.5 (4.2)	0.048
<b>Transitional zone, n (%)</b>	(n = 53)	(n = 76)	(n = 129)	0.212
Rectum	12 (22.6)	17 (22.4%)	29 (22.5%)	
Rectosigmoid colon	31 (58.5%)	40 (52.6%)	71 (55.0%)	
Descending colon	9 (17.0%)	10 (13.2%)	19 (14.7%)	
Long segment Hirschsprung	1 (1.9%)	9 (11.8%)	10 (7.8%)	
<b>Operation, n (%)</b>				0.084
TERPT	41 (74.5%)	45 (58.4%)	86 (65.2%)	
Abdominal/Laparoscopic-assisted TERPT	14 (25.5%)	32 (41.6%)	46 (34.8%)	

**TABLE 2.** Comparison of the incidences of post-operative mechanical obstructions between the regular anal dilatation group and selective anal dilatation group.

Mechanical obstruction	Regular anal dilatation (n = 55)	Selective anal dilatation (n =77)	Total (n = 132)	p-value
Yes; n (%)	33 (60%)	51 (66.2%)	84 (63.6%)	0.582
No; n (%)	22 (40%)	26 (33.8%)	48 (36.4%)	

Because the demographic data of the patients in the AD group and the NAD group showed differences in median age at operation and weight at operation with statistical significance, it could not be directly concluded that the AD group and the NAD group had similar incidence of mechanical obstruction without considering the effects of the difference in age, weight, and types of operation. Therefore, further analysis was performed to assess whether each type of mechanical obstruction was a result of the protocol for anal dilatation and the identified factors (age, weight, type of operation).

*Factors associated with stricture:*

Comparisons between those with and without stricture are presented in Table 3. Anastomosis/cuff stricture was found in 35 patients (26.5%). The AD group had 13/55 patients with stricture, whereas the NAD group had 22/77 patients with stricture ( $p = 0.665$ ). The median age of the patients with and without stricture were 2.3 months and 2.3 months, respectively ( $p = 0.472$ ).

The median weight at operation of the stricture group (4.4 kg) was not significantly different from those without stricture (4.6 kg) ( $p = 0.560$ ).

The statistical analysis results for the non-inferiority test for stricture are shown in Table 4. Non-inferiority was demonstrated when the lower bound of the 95% one-sided CI for the difference in postoperative stricture rate between the selective anal dilatation group and regular anal dilatation group was lower than the pre-specified non-inferiority margin of 10%. When the statistics tests for the non-inferiority of the difference in stricture events between the selective anal dilatation group and regular anal dilatation group were performed, non-inferiority was demonstrated, as the difference in the stricture events between these two groups was 0.049. The  $p$ -value of the non-inferiority test was less than the level of 0.05 that would indicate significance, which indicated that selective anal dilatation was not worse than regular anal dilatation.

**TABLE 3.** Factors associated with stricture: Comparison between the regular anal dilatation group and selective anal dilatation group and between age, body weight, and type of operation.

Factors	Stricture (n = 35)	Non-stricture (n = 97)	p-value
<b>Anal dilatation, n (%)</b>			0.665
Regular anal dilatation (n = 55)	13 (37.1%)	42 (43.3%)	
Selective anal dilatation (n = 77)	22 (62.9%)	55 (56.7%)	
<b>Age (months)</b> (median (IQR))	2.3 (5.8)	2.3 (11.3)	0.472
<b>Body weight (kg)</b> (median (IQR))	4.4 (2.8)	4.6 (4.4)	0.560
<b>Operation, n (%)</b>			0.264
TERPT (n = 86)	26 (74.3%)	60 (61.9%)	
Abdominal/Laparoscopic-assisted TERPT (n = 46)	9 (25.7%)	37 (38.1%)	

**TABLE 4.** Non-inferiority test\* for postoperative stricture.

Factors	Stricture (n)	Non-stricture (n)	Total count (n)	Proportion
Selective anal dilatation	22	55	77	P1 = 0.286
Regular anal dilatation	13	42	55	P2 = 0.236

One-sided 95% Confidence Interval for the difference = -0.1169 to 0.2024.

Difference P1-P2 = 0.0494\*\*.

\* Non-inferiority was demonstrated when the lower bound of the 95% one-sided CI for the difference in the postoperative stricture rate between the selective anal dilatation group and regular anal dilatation group was lower than the pre-specified non-inferiority margin of 10%.

\*\* The *p*-value of the non-inferiority test was less than the significant level of 0.05 then non-inferiority was demonstrated.

#### *Factors associated with constipation:*

Comparisons between those with and without constipation are presented in Table 5. In our study, 26 patients (19.7%) had postoperative constipation, comprising 12 patients in the AD group and 14 patients in the NAD group. The difference was not statistically significant ( $p = 0.767$ ). There was no significant difference in age, weight and types of operation between those with and without constipation.

#### *Factors associated with HAEC:*

Comparisons between those with and without HAEC are presented in Table 6. In the AD group, 13 patients with HAEC were recorded, whereas 27 patients in the NAD group had this condition. There was no difference in the incidence of HAEC between each protocol of anal dilatation ( $p = 0.224$ ). Interestingly, the operative technique was the only factor associated with the incidence of HAEC, whereby 21/46 (45.7%) of the patients in the abdominal assisted TERPT group developed HAEC, which was significantly higher than that of the TERPT group [19/86 (22.1%)] ( $p = 0.009$ ).

#### *Factors associated with re-operation:*

In our series, re-operation for mechanical obstruction was performed in 6 cases, comprising 5 re-pull-through operations and one posterior myectomy done due to anastomotic stricture. Comparisons between those with and without re-operation are presented in Table 7 and revealed that the factors, including the protocol of anal dilatation (either regular anal dilatation or selective anal dilatation), age at operation, weight at operation, and type of operation, all showed no statistically significant differences.

## **DISCUSSION**

Mechanical obstruction<sup>10,21</sup> is an important postoperative complication following a pull-through operation for Hirschsprung disease. The obstructive symptoms are often accompanied by abdominal distention, bloating, borborygmic, increased constipation, and HAEC. Mechanical obstruction has many causes<sup>10,21</sup>, such as stricture of the anastomosis, rectal muscular cuff stricture, and twisting of the pull-through colon. An anastomotic stricture after the pull-through procedure is the most significant postoperative complication.<sup>8,9</sup> The risk factors include anastomotic ischemia, cuff ischemia, anastomotic leak, and small circular anastomosis.<sup>7</sup>

Apart from daily regular anal dilatation (AD) and selective anal dilatation (NAD) indicated by obstructive symptoms as described in our study, there are other practices. Weekly calibration and dilatation of the anus for 6 weeks by a pediatric surgeon is also recommended.<sup>22</sup> In this practice, the size of the anastomosis is first calibrated and dilated at 2–3 weeks after the surgery. The patient then makes an appointment every week for 6 weeks, during which the parents do not have to dilate the patient's anus at all.<sup>22</sup> At the follow-up appointment at the OPD, the pediatric surgeon will dilate the anus gently. If the pediatric surgeon feels that there is a stricture, whether an anastomotic stricture or cuff stricture, the pediatric surgeon will begin to regularly dilate the patient's anus and then the patient will be dilated daily by their parents.<sup>22</sup> Temple<sup>22</sup> reported that dilatation of the anastomosis every week without dilatation by the parents has a similar incidence of anastomotic strictures or leaks as cases involving daily anal dilatation by parents. In that study, only 3 of the 17 patients dilated by a doctor once a week were found to have a stricture later that caused the need

**TABLE 5.** Factors associated with constipation: Comparison between the regular anal dilatation group and selective anal dilatation group and between age, body weight, and type of operation.

Factors	Constipation (n = 26)	Non-constipation (n = 106)	p-value
<b>Anal dilatation, n (%)</b>			<b>0.767</b>
Regular anal dilatation (n = 55)	12 (46.2%)	43 (40.6%)	
Selective anal dilatation (n = 77)	14 (53.8%)	63 (59.4%)	
<b>Age (months)</b> (median (IQR))	2.1 (16.4)	2.3 (6.2)	0.786
<b>Body weight (kg)</b> (median (IQR))	5.2 (5.0)	4.5 (3.0)	0.238
<b>Operation, n (%)</b>			0.797
TERPT (n = 86)	18 (69.2%)	68 (64.2%)	
Abdominal/Laparoscopic-assisted pull-through (n = 46)	8 (30.8%)	38 (35.8%)	

**TABLE 6.** Factors associated with Hirschsprung-associated enterocolitis (HAEC): Comparison between the regular anal dilatation group and selective anal dilatation group and between age, body weight, and type of operation.

Factors	HAEC (n = 40)	Non-HAEC (n = 92)	p-value
<b>Anal dilatation, n (%)</b>			0.224
Regular anal dilatation (n = 55)	13 (32.5%)	42 (45.7%)	
Selective anal dilatation (n = 77)	27 (67.5%)	50 (54.3%)	
<b>Age (months)</b> (median (IQR))	2.2 (6.9)	2.3 (8.7)	0.551
<b>Body weight (kg)</b> (median (IQR))	4.4 (3.1)	4.6 (4.4)	0.652
<b>Operation, n (%)</b>			0.009
TERPT (n = 86)	19 (47.5%)	67 (72.8%)	
Abdominal/Laparoscopic-assisted TERPT (n = 46)	21 (52.5%)	25 (27.2%)	

to change to a protocol with dilation of the anus every day by parents. Another practice is anal calibration under general anesthesia at the 6<sup>th</sup> week postoperatively.<sup>23</sup> The first calibration will be scheduled in the 6<sup>th</sup> week postoperatively, and performed using a finger or a Hegar dilator. When a stricture is detected, anal dilatation will be immediately performed. Obermayr<sup>23</sup> studied 20 patients with Hirschsprung disease who were scheduled to have the size of the anus calibrated under general anesthesia at the end of the 6<sup>th</sup> week following the surgery. It was found that 12 patients had no stricture but 8 patients

had a stricture of the anus and serial anal dilatation was then further done on these. Two patients still required redo pull-through operation for an intractable rectal stricture.

In our study, postoperative mechanical obstructions occurred in 63.6%, comprising 26.5% patients with stricture, 19.7% with constipation, 30.3% with HAEC, and 4.5% needing a re-operation. From Table 2, the incidences of mechanical obstruction in the AD and NAD group were 60% and 66.2%, respectively. These two protocols of anal dilatation had similar results for postoperative

**TABLE 7.** Factors associated with re-operation: Comparison between the regular anal dilatation group and selective anal dilatation group and between age, body weight, and type of operation.

Factors	Re-operation (n = 6)	Non-reoperation (n = 126)	p-value
<b>Anal dilatation, n (%)</b>			0.234
<b>Regular anal dilatation (n = 55)</b>	4 (66.7%)	51 (40.5%)	
<b>Selective anal dilatation (n = 77)</b>	2 (33.3%)	75 (59.5%)	
<b>Age (months)</b> (median (IQR))	3.2 (30.3)	2.2 (7.2)	0.670
<b>Body weight (kg)</b> (median (IQR))	5.2 (7.0)	4.5 (4.1)	0.458
<b>Operation, n (%)</b>			0.420
TERPT (n = 86)	3 (50.0%)	83 (65.9%)	
Abdominal/Laparoscopic-assisted TERPT (n = 46)	3 (50.0%)	43 (34.1%)	

mechanical obstruction. Because the patients in the AD group had a younger mean age, lower body weight at the operative period and different operative approaches, compared to in the NAD group (Table 1), and so we could not conclude directly that regular anal dilatation and selective anal dilatation had similar incidence of mechanical obstruction without considering the effect of the difference in the patients' age, weight, and type of operation.

Anastomotic stricture following TERPT has been reported an incidence of between 4%-19%.<sup>24-27</sup> Anastomotic stricture may be caused by ischemia of the anastomosis, too much tension of the anastomosis, an incomplete suture technique at anastomosis<sup>27</sup>, and ischemia of the rectal muscular cuff.<sup>27</sup> Rectal muscular cuff stricture<sup>10,28</sup> may be caused by too long a rectal cuff.<sup>13</sup> It has been reported that the rectal muscular cuff should be cut at the posterior side to ensure a minimal length of the remaining rectal muscular cuff.<sup>13</sup> In the author's previous reported studies, anastomotic stricture was the most common complication,<sup>8,9</sup> representing 32.6% of all complications.<sup>8</sup> A high incidence of anastomotic stricture in the author's previous study may be the results of too tight coloanal anastomosis in patients without laparotomy and a minor degree of anastomotic ischemia.<sup>8</sup>

In the present study, anastomosis/cuff stricture was found in 26.5% patients. The AD group had a similar rate of anastomosis/cuff stricture to the NAD group ( $p = 0.665$ ). Although, regular anal dilatation practice tended to be used in patients with a younger age in the present study (median age = 1.4 months) than selective anal

dilatation (median age = 2.8 months), this confounding factor was not the factor influencing anastomosis/cuff stricture. The median age at the operation and median weight at the operation of the patients with stricture and those without stricture were the same (Table 3). It seems that surgeon tends to practice regular anal dilatation in endorectal pull-through in younger children to prevent anastomosis/cuff stricture, but actually the stricture rate here did not relate to which anal dilatation protocol was used, and selective anal dilatation had the similar stricture rate as regular anal dilatation. Among all the postoperative mechanical obstructions (stricture, constipation, Hirschsprung-associated enterocolitis, and re-operation), anastomosis/cuff stricture was the most relevant postoperative mechanical obstruction; therefore, a statistics test for non-inferiority for stricture was conducted. Non-inferiority was demonstrated when the lower bound of the 95% one-sided CI for the difference in the postoperative stricture rate between the NAD group and AD group was lower than the pre-specified non-inferiority margin of 10%. In this study, the one-sided 95% confidence interval (CI) for the difference ranged from -0.1169 to 0.2024. Non-inferiority was demonstrated as the difference in stricture events between these two groups was 0.049 and the  $p$ -value for the non-inferiority test was less than the significant level of 0.05, which indicated that selective anal dilatation was not worse than regular anal dilatation. Our study's results corresponded with those reported by Aworanti<sup>29</sup>, who revealed that the rate of anastomotic strictures was not reduced when anal dilatations were prescribed routinely. However, routine

dilatations will prevent early onset strictures. The mean durations between surgery and stricture of the routine anal dilatation group and selective anal dilatation group were 348 and 74 days, respectively.<sup>29</sup>

In our study, constipation was defined as the usage of laxatives, enemas, and/or bowel irrigation for more than 3 months. In the regular anal dilatation group, 21.8% patients had constipation, but in the selective anal dilatation group, 18.2% patients had constipation. The difference was not statistically significant. In order to reduce the confounding factors of age and weight at operation, the median age and weight at operation of the constipated group and non-constipated group were compared and there was no difference in age or weight found between the constipated group and non-constipated group. However, constipation following TERPT occurred from many conditions, such as neuronal intestinal dysplasia, retained aganglionosis<sup>8</sup>, and twisting of the pull-through colon<sup>10,21</sup>, therefore, the practices of anal dilatation would be only one factor among other factors.

There was no different rate of HAEC in each protocol of anal dilatation, i.e., regardless of whether regular anal dilatation or selective anal dilatation was used. The median age and weight at operation in the regular anal dilatation group and in the selective anal dilatation group were the same. Our results were similar to the findings in Aworanti's study<sup>29</sup>, which indicated that anal dilatation prescribed routinely could not reduce the risk of HAEC. Re-operations were performed in 6 cases in our series, comprising redo pull-through operations in 5 cases and one posterior myectomy, which was performed due to an anastomotic stricture.

Our study does have some limitations to note. First, it was a retrospective design, which meant some information may be missing. Patients with Hirschsprung disease who were referred back to other hospitals or who were lost to follow-up, and those who had incomplete medical information were excluded from the study. Only patients who still regularly followed up at Siriraj Hospital were included in the study. Second, there was no definite criteria about which patients should receive one of the two protocols: regular anal dilatation or selective anal dilatation. How to select which protocol of anal dilatation might be depended on each surgeon, the patient's age at operation, weight at operation, the operative technique, and the difficulty of the operation. This selection bias suggests that a prospective randomized controlled study should be performed in the future. Third, information about the duration of anal dilatation was lacking. Fourth, the generalizability of the results is restricted. The study

was conducted in one university hospital, and thus, the method and technique of the pull-through operations as well as anal dilatations may differ from those used in other institutions.

## CONCLUSION

Regular anal dilatation and selective anal dilatation had the same rates for all types of mechanical obstruction. However, in our study, selection bias in the patient selection for the protocol of anal dilatation should be further studied by performing a prospective randomized study.

## ACKNOWLEDGEMENTS

The researcher would like to thank Dr. Sasima Tongchai from the Division of Clinical Epidemiology, Department of Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University for her continuous help with the data processing and statistical analysis.

## Conflicts of interest

The authors have no conflicts of interest to declare.

## REFERENCES

- Swenson O. My early experience with Hirschsprung's disease. *J Pediatr Surg.* 1989;24:839-45.
- Soave F. A new surgical technique for the treatment of Hirschsprung's disease. *Surgery.* 1964;56:1007-14.
- Boley SJ. New modification of the surgical treatment of Hirschsprung's disease. *Surgery.* 1964;56:1015-7.
- De la Torre L, Ortega A. Transanal versus open endorectal pull-through for Hirschsprung's disease. *J Pediatr Surg.* 2000;35:1630-2.
- Morowitz MJ, Georgeson KE. Laparoscopic assisted pull-through for Hirschsprung's disease. In: Holcomb GW, Georgeson KE, Rothenberg SS, eds. *Atlas of pediatric laparoscopy and thoracoscopy*, Philadelphia, Elsevier, 2008.p.101-85.
- Teitelbaum DH, Cilley RE, Sherman NJ, Bliss D, Uitvlugt ND, Renaud EJ, et al. A decade of experience with the primary pull-through for Hirschsprung disease in the newborn period: A multicenter analysis of outcomes. *Ann Surg.* 2000;232:372-80.
- Teitelbaum DH, Coran AG. Long-term results and quality of life after treatment of Hirschsprung's disease and allied disorders. In: Holschneider AM, Puri P, eds. *Hirschsprung's disease and allied disorders*, 3<sup>rd</sup> ed., New York, Springer, 2008.p.389-97.
- Ruangtrakool R, Krajangjit P. Early surgical complications following transanal endorectal pull-through for Hirschsprung's disease. *Siriraj Med J.* 2023;75(6):445-53.
- Ruangtrakool R, Tiyaamornwong S. Incidence of infection-related complications and optimal saline irrigation volume for preoperative bowel preparation to reduce postoperative infections in Hirschsprung's Disease. *Siriraj Med J.* 2023;75(11):763-9.
- De la Torre L, Langer JC. Transanal endorectal pull-through

- for Hirschsprung disease: technique, controversies, pearls, pitfalls, and an organized approach to the management of postoperative obstructive symptoms. *Semin Pediatr Surg.* 2020; 19(2):96-106.
11. Diseth TH. Dissociation following traumatic medical treatment procedures in childhood: a longitudinal follow-up. *Dev Psychopathol.* 2006;18:233-51.
  12. Diseth TH, Egeland T, Emblem R. Effects of anal invasive treatment and incontinence on mental health and psychosocial functioning of adolescents with Hirschsprung's disease and low anorectal anomalies. *J Pediatr Surg.* 1998;33:468-75.
  13. Van de Van TJ, Stoots CEJ, Wijnen MHWA, Rassouli R, Rooij IV, Wijnena RM, et al. Transanal endorectal pull-through for classic segment Hirschsprung's disease: With or without laparoscopic mobilization of the sigmoid? *J Pediatr Surg.* 2013; 48:1914-8.
  14. Langer JC. Hirschsprung Disease. In: Coran AG, Caldamone A, Adzick NS, Krummel TM, Laberge JM, eds. *Pediatric Surgery*, 7<sup>th</sup> ed, Philadelphia, Mosby, 2012.p.1265-78.
  15. Aslanabad S, Ghalehgholab-Behbahan A, Zarrintan S, Jamshidi M, Seyyedhejazi M. Transanal one-stage endorectal pull-through for Hirschsprung's disease: a comparison with the staged procedures. *Pediatr Surg Int.* 2008;24:925-9.
  16. De la Torre L, Ortega A. Transanal endorectal pull-through for Hirschsprung disease. *J Pediatr Surg.* 1998;33:1283-6.
  17. Ergun O, Celik A, Dokumcu Z, Balik E. Submucosal pressure-air insufflations facilitate endorectal mucosectomy in transanal endorectal pull-through in patients with Hirschsprung's disease. *J Pediatr Surg.* 2003;38:188-90.
  18. Pratap A, Shakya VC, Biswas BK, Sinha A, Tiwari A, Agrawal CS, et al. Single-stage transanal endorectal pull-through for Hirschsprung disease: perspective from a developing country. *J Pediatr Surg.* 2007;42:532-5.
  19. Hollwarth ME, Rivorseccchi M, Scheef J, Deluggi S, Fasching G, Ceriati E, et al. The role of transanal endorectal pull-through in the treatment of Hirschsprung's disease - a multicenter experience. *Pediatr Surg Int.* 2002;18:344-8.
  20. Albanese CT, Jennings RW, Smith B, Bratton B, Harrison MR. Perineal one stage pull-through for Hirschsprung disease. *J Pediatr Surg.* 1999;34:377-80.
  21. Langer JC. Persistent obstructive symptoms after surgery for Hirschsprung disease: development of a diagnostic and therapeutic algorithm. *J Pediatr Surg.* 2004;39:1458-62.
  22. Temple SJ, Sawyer A, Langer JC. Is daily dilatation by parents necessary after surgery for Hirschsprung disease and anorectal malformations? *J Pediatr Surg.* 2012;47:209-12.
  23. Obermayr F, Szavay P, Beschomer R, Fuchs J. Outcome of transanal endorectal pull-through in patients with Hirschsprung's disease. *Eur J Pediatr Surg.* 2009;19(4):220-3.
  24. Langer JC, Durrant AC, De la Torre L. One - stage transanal Soave pullthrough for Hirschsprung's disease. *Ann Surg.* 2003;238(4): 569-83.
  25. Minford JL, Ram A, Turnock RR, Lamont GL, Kenny SE, Rintala RJ, et al. Comparison of functional outcomes of Duhamel and transanal endorectal coloanal anastomosis for Hirschsprung disease. *J Pediatr Surg.* 2004;39:161-3.
  26. Invised T, Vejchapipat P, Chiengkriwate P, Thepsuwan P, Tiansri K, Kiatipunsodsai S. Multicenter experience of primary transanal endorectal pull-through operation in childhood Hirschsprung's disease. *J Med Assoc Thai.* 2016;99(4):S59-63.
  27. Gosemann JH, Friedmacher F, Ure B, Lacher M. Open versus transanal pull-through for Hirschsprung disease: A systematic review of long-term outcome. *Eur J Pediatr Surg.* 2013;23(2): 94-102.
  28. Eisherbeny M, Addelhay S. Obstructive complications after pull-through for Hirschsprung's disease: different causes and tailored management. *Annals of Pediatric Surgery.* 2019;15(2): 1-5.
  29. Aworanti O, Hung J, McDowell D, Martin I, Quinn F. Are routine dilatations necessary post pull-through surgery for Hirschsprung Disease? *Eur J Pediatr Surg.* 2013;23(5):383-8.

# Beyond Vision: Potential Role of AI-enabled Ocular Scans in the Prediction of Aging and Systemic Disorders

Moez Osama Omar<sup>1</sup>, Muhammad Jabran Abad Ali<sup>1</sup>, Soliman Elias Qabillie<sup>1</sup>, Ahmed Ibrahim Haji<sup>1</sup>, Mohammed Bilal Takriti<sup>1</sup>, Ahmed Hesham Atif<sup>1</sup>, Rangraze Imran, M.D.<sup>2</sup>

<sup>1</sup>Medical Intern, RAK Medical and Health Sciences University, RAK, United Arab Emirates, <sup>2</sup>Associate Professor, Internal Medicine, RAK Medical and Health Sciences University, RAK, United Arab Emirates.

## ABSTRACT

In all medical subfields, including ophthalmology, the development of artificial intelligence (AI), particularly cutting-edge deep learning frameworks, has sparked a quiet revolution. The eyes and the rest of the body are anatomically related because of the unique microvascular and neuronal structures they possess. Therefore, ocular image-based AI technology may be a helpful substitute or extra screening method for systemic disorders, particularly in areas with limited resources. This paper provides an overview of existing AI applications for the prediction of systemic diseases from multimodal ocular pictures, including retinal diseases, neurological diseases, anemia, chronic kidney disease, autoimmune diseases, sleep disorders, cardiovascular diseases, and various others. It also covers the process of aging and its predictive biomarkers obtained from AI-based retinal scans. Finally, we also go through these applications existing problems and potential future paths.

**Keywords:** AI-Driven ocular scans, Area under the curve (AUC); deep learning; retinal age (RA); fundus autofluorescence; ROPtool; Convolutional Neural Network (CNN); Color Fundus Photography (CFP); Machine Learning (ML) (Siriraj Med J 2024; 76: 106-115)

## INTRODUCTION

In the realm of artificial intelligence (AI) within computer science, algorithms are trained to perform human-like tasks, spanning areas like robotics, natural language processing, and machine learning.<sup>1</sup> AI's rapid response, decision-making, and learning capabilities have led to its widespread use in recommendation algorithms, search engines, and autonomous vehicles. The eye's unique translucent refractive interstitium allows for the non-invasive assessment of blood vessels and nerves, making it a valuable diagnostic tool for systemic conditions such as diabetes and hypertension.<sup>2</sup> With advancements in AI techniques, ocular images have become crucial in diagnosing diseases like diabetic retinopathy, age-

related macular degeneration (AMD), and glaucoma.<sup>3-5</sup> AI enables the identification of previously unseen associations between ocular features and systemic illnesses, expanding diagnostic possibilities. Recent studies have linked ocular characteristics to diseases like diabetes, cardiovascular issues, Alzheimer's, and kidney disease. This review aims to summarize the latest developments in ocular image-based AI's applications in diagnosing various systemic diseases.

### Search strategy and article selection

A search strategy was implemented to identify and review the literature pertaining to the application of AI in ophthalmology and its relation to various other disorders

Corresponding author: Imran Rangraze

E-mail: [imranrashid@rakmhsu.ac.ae](mailto:imranrashid@rakmhsu.ac.ae)

Received 12 November 2023 Revised 12 December 2023 Accepted 17 December 2023

ORCID ID: <http://orcid.org/0000-0003-2786-9630>

<https://doi.org/10.33192/smj.v76i2.266303>



All material is licensed under terms of the Creative Commons Attribution 4.0 International (CC-BY-NC-ND 4.0) license unless otherwise stated.

via searching through engines like PubMed, MEDLINE, Scopus, and Google Scholar using the keywords “AI-enabled retinal scans,” “aging biomarkers”, “artificial intelligence,” “machine learning,” “deep learning,” “artificial neural networks,” “Retinal age,” and “natural language processing”.

### **Inclusion criteria**

Articles related to artificial intelligence in ophthalmology; Original articles of full-text length covering the diagnostic capabilities and AI in ophthalmology and its relation to various other disorders.

### **Exclusion criteria**

Abstracts, editorial comments, and chapters from books; Animal, laboratory, or cadaveric studies. Non-ophthalmic studies.

### **Artificial intelligence**

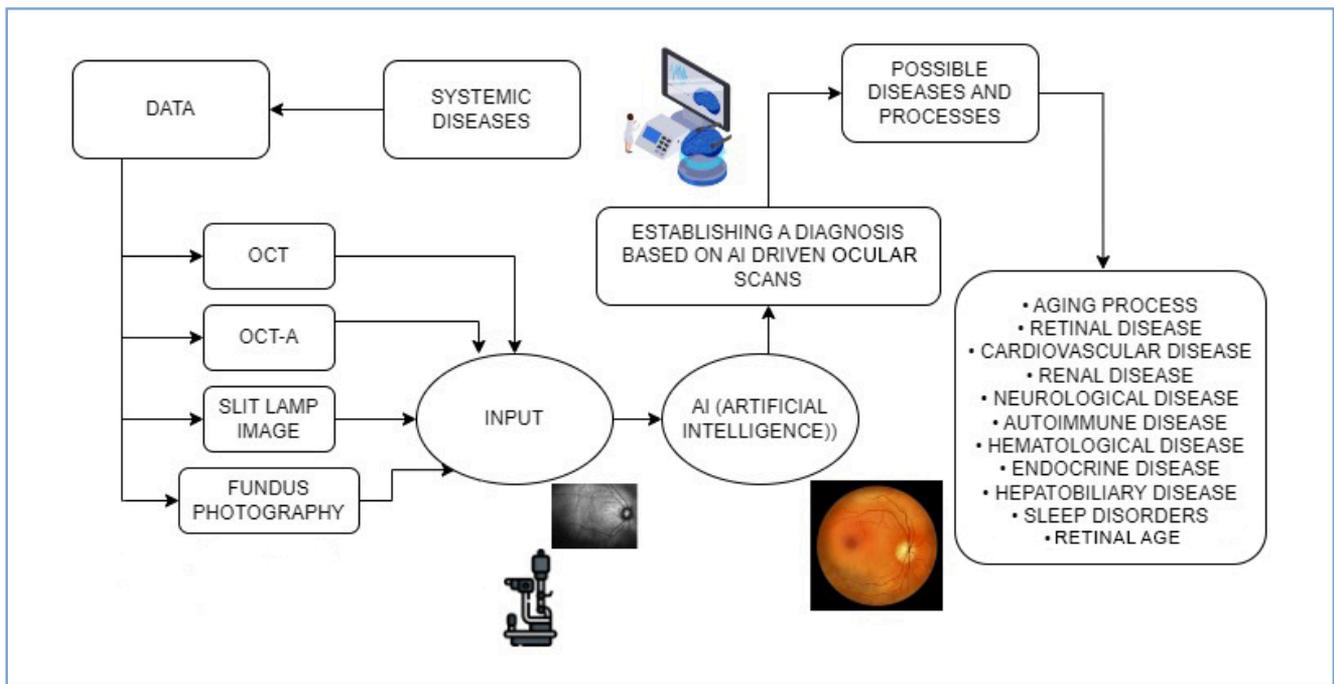
Artificial intelligence (AI) involves computer-based simulations of intelligent behavior with limited human intervention. The inception of robots marked the beginning of AI, with the term “robot” originating from the Czech word “robota”, denoting bio-engineered devices for forced labor.<sup>6</sup> AI in medicine encompasses virtual and physical domains. The former encompasses deep learning, information management, and decision support systems, while the latter involves robots assisting patients and physicians in an innovative engineering field addressing complex problems. Speed, capacity, and software advancements could eventually enable computers to match human intelligence. Modern cybernetics has significantly contributed to AI progress.<sup>6</sup> Medical AI tackles the challenge of assimilating and applying vast clinical knowledge. AI systems aid clinicians in diagnosing, treatment decisions, and outcome predictions. Techniques like deep learning, and non-neural networks are used. Deep learning (DL) has transformative potential in healthcare, mapping inputs to outputs across interconnected neuron layers. DL excels in clustering, regression, classification, and prediction tasks. However, it is more resource-intensive than traditional machine learning methods, particularly in imaging. Supervised and unsupervised learning, along with semi-supervised learning are also some of the training strategies that can be acquired through this mechanism. Area under the curve (AUC) is one of the metrics utilized by the AI, the receiver operating characteristic (ROC) area under the curve (AUC) quantifies the model’s overall ability to distinguish between positive and negative instances. Plotted on the ROC curve are the true positive and false positive rates at different categorization criteria.

AUC 1 indicates an error-free model, while AUC 0.5 indicates a random estimating model. Because AUC is a crucial metric for assessing an AI algorithm’s efficacy, a higher AUC suggests improved prediction accuracy and offers pertinent details regarding how well an AI model can differentiate between multiple classes. However, it does not fully capture the utility of a model in a clinical setting as different tasks, such as screening, may require separate sensitivity metrics.<sup>6,7</sup>

As healthcare data is so vital, data mining has emerged as a significant and challenging field in the healthcare industry. Recent developments in data mining techniques have established a solid basis for a multitude of uses, such as disease diagnosis, pattern recognition, enabling patient-friendly and affordable medical treatments, and intrusion detection. Artificial intelligence supports this process by functioning as a machine learning subfield to improve predictive capabilities. Three well-known supervised learning classifiers are used in the field of classification and prediction: Random Forests, Support Vector Machines (SVM), and Naive Bayes. Based on Bayes’ theorem, Naive Bayes is a probabilistic classifier that is independent of features. SVM is an effective classification method that finds the best hyperplane to divide classes. SVM and Naive Bayes both improve the precision of medical predictions. Robust ensemble learning techniques like the Random Forest algorithm make a substantial contribution to classification tasks by preventing overfitting, optimizing model performance, and utilizing insights from multiple decision trees. Furthermore, a variety of statistical and machine-learning methods are used by artificial intelligence to model complex data relationships. One of the most important metrics for evaluating regression models is the R-squared value, sometimes referred to as the coefficient of determination, which shows how well the model accounts for the variance in the dependent variable. Greater consistency between predictions and observed results and a more accurate depiction of the data are indicated by higher R-squared values. Medical AI has potential, but its acceptance among clinicians requires evidence through randomized controlled experiments. Medical AI is poised to enhance 21st-century healthcare, augmenting future clinicians’ medical intelligence.<sup>6,7</sup> A graphical overview of the process that AI goes through to achieve a diagnosis of systemic illness is illustrated in Fig 1.

### **Use of AI-based retinal scans as biomarkers for the aging process**

In recent estimations, the global elderly population aged 65 years and above reached approximately 750



**Fig 1.** A graphical overview of the process that Artificial Intelligence goes through in achieving diagnosis for various systemic illnesses by utilizing Ocular scans.

million and is projected to double in the future.<sup>8</sup> Aging significantly influences the pathophysiology of various diseases, making it a crucial risk factor.<sup>9</sup> This exploration focuses on the use of retinal scans and deep machine learning (DL) as an innovative method to predict aging and biomarkers, utilizing retinal age (RA) to calculate morbidity and mortality risk, as well as studying aging genetics.

Chronological age (CA) has long been associated with age-related morbidity and mortality; however, individual variations suggest that the rate of aging differs among people.<sup>10</sup> Biological age (BA) accounts for gradual cellular and physicochemical changes, offering a more accurate indicator of health status.<sup>11</sup> Current BA assessment methods, such as blood profiles and DNA methylation, are costly, invasive, and ethically concerning.<sup>12-15</sup> Retinal assessment provides a non-invasive, cost-effective, and user-friendly alternative, given the retina's physiological similarities with other organs and its responsiveness to aging-related changes.<sup>16</sup>

Research has demonstrated retinal microvascular variations linked to circulatory pathophysiological changes, as well as molecular alterations associated with neural retinal layers and neurodegenerative disorders.<sup>17</sup> DL models have been employed to predict RA, showing remarkable accuracy in determining retinal age compared to chronological age. Model performance was evaluated using samples of retinal images from two separate sets

from biobank databases. Upon completion of training, the DL model exhibited the ability to predict RA and CA ( $p < 0.001$ ) with a mean absolute error of 3.55 years. The difference between predicted RA and CA, termed the age gap, serves as a potential biomarker. Positive age gaps indicate older retinas, while negative gaps suggest younger retinas. Studies have revealed that an increase in the retinal age gap correlates with a significant rise in mortality, highlighting its potential as an independent predictor of age-related mortality.<sup>18</sup>

In a study conducted with participants from the Korean Health Screening, a DL algorithm called RetiAGE predicted BA accurately, demonstrating excellent performance (95% confidence interval [CI]: 0.965–0.970) and accurate predictions of mortality, especially in cancer and cardiovascular disease events.<sup>19</sup> Genetics significantly influence the aging process, with ALKAL2 identified as a key gene associated with age-related changes. This discovery sheds light on the molecular mechanisms governing aging, offering opportunities for therapeutic interventions and targeted research initiatives. Furthermore, predicting age using retinal images operates independently of existing methods, providing a unique perspective on aging. Integrating retinal imaging with other markers enhances understanding of an individual's BA. Unlike invasive blood tests, non-invasive retinal imaging facilitates actionable biological and behavioural interventions.<sup>20</sup>

In summary, the integration of retinal scans and DL techniques offers a ground-breaking approach to predicting aging and associated biomarkers. The non-invasive nature of retinal assessment, coupled with its accuracy and potential for genetic insights, positions it as a promising tool for understanding age-related conditions and developing targeted interventions in the future.

### Use of AI-based ocular scans in diabetic retinopathy

Since diabetic retinopathy (DR) is a major contributor to visual impairment in developed nations, innovative approaches to patient screening, complication avoidance, and care optimization are required. An emphasis on AI-based models, especially those that enable large-scale screening, has resulted from the increasing prevalence of DR. Starting with fundus pictures, these models are essential for identifying DR-related changes such as haemorrhages, exudates, cotton wool patches, and neovascularization. They determine whether DR is present or absent and provide a grade based on accepted DR grading schemes.<sup>21</sup>

Numerous artificial intelligence systems have been developed to achieve these goals. When the IDx-DR system was tested on a number of populations, including the 3,640 participants in the Kenyan Nakuru Eye Study<sup>22</sup>, it demonstrated a sensitivity of 87% and a specificity of 70%. Additionally, it demonstrated strong performance in tests utilizing the Messidor-2 dataset, yielding a 97% sensitivity and 59% specificity.<sup>23</sup> These encouraging results led to IDx-DR's approval by the US Food and Drug Administration in 2018.<sup>24</sup> The RetmarkerDR software showed a sensitivity of 73% for any DR, 85% for referable DR, and 98% for proliferative DR.<sup>25</sup> EyeArt performed well on the Messidor-2 dataset, achieving a sensitivity of 94% and a specificity of 72%.<sup>26</sup> Using a sensitivity of 96% for any DR, 99% for referable DR, and 99% for DR that posed a risk to vision, it was employed in the smartphone-based DR screening of 296 patients.<sup>27,28</sup> Additionally, EyeArt was applied to a dataset with over 30,000 images, achieving a sensitivity of 96% for referable DR.<sup>29</sup> In addition, other systems that have proven to be as reliable in DR screening are the Google Inc.-sponsored system, Retinalyze, EyeWisdom®, and the Bosch DR Algorithm.<sup>21</sup>

AI models are not only good at screening, but they also assist in grading and staging direct response content. Gulshan et al. demonstrated high sensitivity and specificity in identifying the presence of diabetic macular edema and the severity of DR.<sup>30</sup> Ting et al. supported these findings by looking at nearly 500,000 images.<sup>31</sup> Moreover, the high reliability of AI-based screening was validated by a

recent study that tested a deep learning algorithm using over 200,000 fundus images from 16 clinical settings.<sup>32</sup> Ultimately, promising strategies for combating DR are offered by AI-based models. Because of their remarkable sensitivity and specificity, these systems enhance patient care, facilitate early intervention, and greatly aid in the widespread prevention of DR-related complications and visual impairment.

### Use of AI-based ocular scans in macular degeneration

In developed nations, age-related macular degeneration (AMD) is a major cause of visual impairment, which calls for the use of AI-based techniques for precise analysis. The main source of data is optical coherence tomography (OCT) images, which need to be precisely segmented in order to identify retinal structures. During follow-up, AI-based segmentation algorithms, utilizing unsupervised learning techniques, have demonstrated remarkable success in identifying retinal features and measuring retinal fluids.<sup>33,34</sup> These algorithms operate autonomously, eliminating the need for human interpretation of the images. They excel in fluid localization and quantification, as well as in evaluating retinal integrity. Advances in predicting visual outcomes and evaluating treatment responses have been made possible by AI in AMD research. The prevalence of AMD-related lesions and the progression of the disease varied between AMD eyes treated initially and twice, according to AI models.<sup>35</sup> AI also measured the number of drusen, evaluated their distribution, and examined hyper-reflective foci on OCT scans to forecast the likelihood of disease progression and the beginning of complications.<sup>36</sup>

Schmidt-Erfurth et al. made a substantial contribution by estimating the risks of AMD progression, highlighting the prognostic significance of intra-retinal cystoid fluid, and creating automated techniques for fluid volume calculation.<sup>37</sup> By examining fluid changes following injections, AI forecasted visual results for a treat-and-extend regimen. AI also measured leakage on angiography and segmented macular neovascularizations.<sup>38,39</sup> AI proved helpful in conjunction with new treatments like pegcetacoplan, as it accurately identified atrophic margins and tracked their expansion in the context of geographic atrophy.<sup>40</sup> Self-monitoring AI systems, like the Notal Vision Home OCT and ForeseeHome, have proven accurate and feasible.<sup>41</sup> Patients who used these systems for daily self-imaging demonstrated good agreement with expert-based grading, and over a 3-year period, ForeseeHome successfully identified changes in visual acuity and indicated the likelihood that a disease would progress in 2,123 patients.<sup>41,42</sup> These uses highlight

AI's critical contribution to improving patient care and AMD research.

### Use of AI-based ocular scans in glaucoma

Artificial intelligence has been applied in glaucoma diagnosis using various imaging techniques, such as fundus photographs, optical coherence tomography (OCT), and visual field (VF) tests. Deep convolutional neural network models have shown high accuracy in distinguishing normal and glaucomatous VF, as well as diagnosing glaucoma based on Retinal Nerve Fiber Layer (RNFL) thickness and Optic Nerve Head (ONH) parameters. AI can effectively learn complex glaucoma features from fundus photographs and holds promise in OCT-based glaucoma assessment using RNFL or Ganglion Cell-Inner Plexiform Layer (GCIPL) thickness parameters.<sup>5</sup>

### Use of AI-based ocular scans in cardiovascular diseases

Given that cardiovascular illnesses are one of the leading causes of death worldwide, early detection is vital to patient health.<sup>43</sup> Because fundus vessels are directly visible in hypertensive retinopathy, it is a useful biomarker for hypertension in ophthalmology. Researchers have studied cardiovascular and ocular diseases in greater detail thanks to AI. By taking advantage of the rare opportunity to observe fundus vessel parameters directly through the eye, researchers studying cardiovascular disease have investigated parameters such as diameter, density, and tortuosity.<sup>44</sup> Based on retinal images, artificial intelligence (AI) has made it possible to identify key cardiovascular risk factors like age, gender, blood pressure, and smoking status.<sup>45</sup> In contrast to conventional methods, risk factors can be directly acquired through AI analysis; however, there is a tendency to overuse AI in the prediction of these factors. This over-reliance has affected the information's accuracy and could hinder the advancement of AI-based retinal image screening.<sup>46</sup>

Furthermore, using fundus images to detect the coronary artery calcification fraction has been made possible by AI. Son et al. compared the predictive power of fundus images and clinical data by classifying subjects according to age interval and coronary artery calcification fraction. The values of the Area Under the Curve (AUC) for age, bilateral images, and unilateral images were 0.828, 0.832, and 0.823, respectively. Interestingly, the AI model concentrated on the blood vessels in the retina, highlighting atherosclerosis and hypertension as important indicators of cardiovascular disorders.<sup>47</sup> In order to predict hypertension, Kim et al. created an AI system with an astounding AUC of up to 0.961,

highlighting the significance of determining the risk of cardiovascular events based on vascular status as reflected in the eyes.<sup>48</sup> Additionally, using clinical data and retinal images, artificial intelligence has been used to predict the frequency of cardiovascular events. Researchers developed prediction models using the atherosclerosis score and coronary artery calcification fraction identified by AI as predictors in long-term studies. Subject grouping and the prediction of cardiovascular events in different groups were made possible by the prediction of coronary artery calcification or atherosclerosis scores using fundus images.<sup>49,50</sup> These findings highlight the possibility of using fundus images to identify pertinent biomarkers for cardiovascular disease and to forecast the course of the condition in the future.

### Use of AI-based ocular scans in diabetes

Early and effective screening techniques are required due to the significant health burden posed by the increasing prevalence of diabetes worldwide. Even though they are accurate, traditional oral glucose tolerance tests are intrusive and have limited applicability. Because chronic hyperglycemia affects the retinal microvasculature, there is a well-established correlation between diabetes and ocular changes, including retinopathy.<sup>2</sup> Researchers have investigated AI-based screening with retinal images by taking advantage of this relationship. In 2020, an artificial intelligence (AI) system examined 1222 retinal fundus photos from rural Chinese citizens, detecting hyperglycemia with 78.7% accuracy and an area under the curve (AUC) of 0.880.<sup>51</sup> Expanding on this, Zhang et al. combined fundus images with patient metadata in 2021 to predict the incidence of type 2 diabetes within five years, using over 100,000 images. The method was inventive, but the non-standardized risk score casts doubt on its applicability in a wider context. AUCs for diabetes detection in external datasets were higher than 0.80, and the prediction model's AUC was 0.824. The AI system was incorporated into smartphones for cloud-based retinal image analysis to improve accessibility, increase screening options, and lowering healthcare inequities.<sup>51</sup> The fact that diabetic complications go beyond eye problems highlights the need for all-encompassing AI-based strategies.

### Use of external eye images in the prediction of laboratory results

Another study which was done on diabetic patients proved to be useful in detecting systemic parameters through the use of external eye images. This study developed and evaluated a deep learning system (DLS) using external eye photographs to predict systemic parameters related

to liver, kidney, bone, thyroid, and blood. Trained on 123,130 images from 38,398 diabetic patients, the DLS outperformed baseline models in predicting abnormalities such as elevated AST, low calcium, decreased eGFR, low hemoglobin, low platelets, elevated ACR, and low WBC in validation sets. The DLS demonstrated superior performance by achieving absolute AUC improvements of 5.3–19.9%. Notably, the study suggests that external eye photographs could serve as a non-invasive screening tool for systemic diseases, showcasing potential applications for accessible and widespread disease detection. The results indicate promising performance, even with low-resolution images, opening possibilities for the use of consumer-friendly devices like smartphones. The study emphasizes the importance of further research to explore the generalizability and practical implications of this approach in diverse populations and clinical settings.<sup>52</sup>

### **Use of AI-based scans in neurological diseases**

#### **Alzheimer's disease**

It is imperative to conduct early screening for Alzheimer's disease (AD), particularly in light of the rapidly aging population. By using fundus images from AD patients and healthy individuals from 11 different studies conducted in different countries, researchers such as Cheung et al. have made significant progress. They produced impressive results when building and validating a model for AD diagnosis, with AUCs in external validation sets ranging from 0.73 to 0.91. Their AI model performed better in patients with ocular diseases and was able to distinguish between patients who tested positive and those who tested negative for beta-amyloid.<sup>53</sup>

Furthermore, retinal thickness is a useful parameter for AI-based detection as it indicates the progression of AD.<sup>54</sup> Retinal thickness images from optical coherence tomography (OCT) have been successfully used in AD detection algorithms with an AUC of 0.795.<sup>55</sup> AI systems perform even better when multiple imaging modalities and clinical data are combined. To create sophisticated AI models for AD detection, multimodal retinal images—including OCT, OCT-Angiography, and Ultra-widefield scanning laser ophthalmoscopy were combined with patient specific data. With parameters such as the OCT-derived ganglion cell-inner plexiform layer thickness map, these combined models produced remarkable outcomes with AUCs greater than 0.8.<sup>56</sup> These developments highlight AI's potential for early AD screening.

### **Use of AI-based scans in renal diseases**

#### **Chronic kidney disease**

Innovative methods for identifying chronic kidney

disease (CKD) through ocular manifestations have been made possible by the complex relationship between the kidney and the eye, which share similarities in structure, development, physiology, and pathogenic pathways.<sup>57</sup> It has been discovered by researchers that renal disease can be linked to ocular abnormalities like those seen in tubulointerstitial nephritis uveitis syndrome (TINUS) and that retinal microvascular parameters can predict the onset of chronic kidney disease (CKD).<sup>58</sup> Interstitial nephritis, a disorder that frequently precedes or coexists with ocular symptoms, is diagnosed in conjunction with symptoms of TINUS in children, which include fever, pain, photophobia, and acute bilateral non-granulomatous anterior uveitis.<sup>59</sup> According to a study on the relationship between CKD and age-related macular degeneration (AMD), patients with moderate CKD had three times the frequency of early AMD without geographic atrophy and choroidal neovascularization than patients with mild or no CKD.<sup>60</sup> Kidney function tests, urine analysis, and kidney puncture biopsy have historically been used in the diagnosis of kidney disease. Although these techniques work well, they can be laborious and could use more succinct screening methods. Artificial intelligence (AI) advances in the last few years have completely changed early detection techniques, especially when it comes to retinal imaging.

A deep learning model that uses retinal images to predict early renal functional impairment is a groundbreaking development. With an astounding AUC of more than 0.81, the model performed better in patients with higher HbA1c levels, the researchers found.<sup>61</sup> Furthermore, compared to the images-only model, the combination of retinal images and clinical data greatly improved the detection of CKD. The combined model had an AUC of about 0.8 in the entire population.<sup>61</sup> Notably, the AUC exceeded 0.9 in patients with hypertension or diabetes. This demonstrates the AI's capacity to classify CKD patients according to their estimated glomerular filtration rate (eGFR) and to differentiate between healthy people and CKD patients.

AI models based on retinal images were used to predict the course of CKD in cohort studies.<sup>62</sup> To predict the likelihood of developing CKD and advanced CKD in healthy subjects, researchers built predictive models using metadata, fundus images, or a combination of both. Cox proportional hazards analysis was used to evaluate these models, and the combined model produced impressive results: it had a C-index of 0.719 and an impressive prediction accuracy of up to 0.844 on the internal validation set. These studies do have certain limitations, though. Researchers used a high-sensitivity but low-specificity AI model to

improve screening performance, which may increase the number of CKD misdiagnoses. Furthermore, there may be limitations to the model's applicability in different research contexts due to the lack of universal acceptance of the risk stratification criteria used in these studies.<sup>62</sup> Additionally, there is room for improvement because the emphasis is on predicting the risk of progression in healthy subjects rather than patients with early-stage CKD. These studies highlight the promise of AI-driven retinal imaging in revolutionizing early CKD detection, despite these drawbacks.

### Use of AI-based ocular scans in hematological diseases

#### Anemia

Deep learning algorithms utilizing ocular imaging have emerged as a promising approach to forecasting anemia, the most prevalent hematological disorder. Researchers have explored subtle retinal changes and conjunctival symptoms as potential markers of anemia.<sup>63</sup> A notable study by Chen et al. presented a novel framework that combined semantic segmentation and a convolutional neural network to predict eyelid hemoglobin levels, achieving a promising R2 value of 0.512.<sup>64</sup> However, conjunctival imaging-based models encounter challenges concerning acquisition criteria, image extraction, and algorithm selection. To overcome these limitations, Mitani et al. developed a deep learning system based on Color Fundus Photography (CFP) for anemia screening, which showed significant promise. Utilizing data from the UK Biobank, the integrated AI system effectively determined hemoglobin levels and anemia, and it also performed well for diabetes patients (AUC = 0.89).<sup>65</sup>

Zhao et al. extended the focus to retinal features and employed Ultra-Widefield (UWF) retinal images as input, achieving an impressive AUC of 0.93 for accurate anemia prediction.<sup>66</sup> Recent research has investigated the relationship between anemia and alterations in capillary plexus density and retinal microvascular perfusion observed in optical coherence tomography angiography (OCTA). A lightweight network utilizing OCTA images achieved an excellent AUC of 0.99, demonstrating respectable performance. However, validation on a larger and more diverse dataset is essential to establish its reliability.<sup>67</sup>

In a different approach, Wu et al. devised a method to detect anemia in pregnant individuals by combining metadata and quantitative OCTA measurements, achieving an AUC of 0.874.<sup>68</sup> In conclusion, deep learning systems based on ocular imaging hold enormous potential for predicting anemia. Despite encouraging results, further validation and improvement are required to address issues related to data size and external validation. If these challenges are overcome, these techniques have

the potential to revolutionize anemia screening and management by offering non-invasive, effective, and reliable diagnostic tools for this common hematological illness.

### Use of AI-based ocular scans in autoimmune conditions

#### Multiple sclerosis

A number of autoimmune diseases, such as multiple sclerosis, inflammatory bowel disease, and Sjögren syndrome, are examples of the complex relationship between the immune system and the eyes. These conditions can cause symptoms that affect the eyes, such as uveitis, optic neuritis, and dry eyes. Retinal thickness may be a useful biomarker for the advancement of multiple sclerosis, according to studies examining the connection between the disease and the eyes.<sup>69</sup> In this context, optical coherence tomography (OCT) images have become a popular source for artificial intelligence (AI) diagnosis. Researchers that have gathered OCT images from multiple sclerosis patients and controls include Cavaliere et al. They examined various retinal and choroid regions in detail using the OCT ETDRS and TNSIT scan modes. Through the application of a support vector machine algorithm, they developed a diagnostic model by identifying variables with the highest area under the curve (AUC) of 0.97. This novel method showcased the ability of AI to detect multiple sclerosis in its early stages and the effectiveness of OCT-based diagnostic systems in disease detection and tracking.<sup>69,70</sup>

Martin et al. similarly examined OCT pictures from 48 patients with early-stage multiple sclerosis and 48 healthy people. They established an efficient classifier by precisely measuring the thickness of the retinal and choroidal layers, which allowed them to identify regions with significant discriminant capacity. The top-performing classifier demonstrated remarkable 0.98 measurements for both specificity and sensitivity. Their research clarified the various layers of the eye and suggested that the papillomacular bundle may be the first region to be affected in the early phases of multiple sclerosis. The significance of accurate layer analysis in early disease detection is highlighted by this discovery, however it's crucial to acknowledge the limitations, considering the high AUC scores, and to be cautious about potential overfitting, emphasizing the need for further validation on new data.<sup>70</sup> All of these studies highlight how OCT images can revolutionize AI-driven multiple sclerosis diagnosis. AI systems can provide invaluable insights into disease progression by utilizing the detailed information provided by OCT scans, which can help clinicians diagnose and intervene early in patients' lives.

### Use of AI-based ocular scans in Hepatobiliary conditions

A ground-breaking study has unveiled a strong correlation between major hepatic diseases and ocular features, paving the way for automated screening and identification of these conditions through fundus or slit-lamp images. The study's models demonstrated impressive performance in detecting liver cirrhosis and cancer, even in cases where these conditions manifested subtly, such as through yellowing of the sclera and conjunctiva due to elevated bilirubin accumulation.<sup>71</sup> Surprisingly, the fundus models performed as effectively as the slit-lamp models, revealing minute retinal alterations imperceptible to the human eye. Researchers speculated that these modifications were linked to advanced liver disease characteristics, including hyperammonaemia, hypoalbuminemia, imbalanced estrogen, and pathological changes like splenomegaly and portal hypertension.<sup>72</sup>

Hyperammonaemia, a common liver disease condition, damages retinal Müller cells, leading to hepatic retinopathy, while hypoalbuminemia causes fluid leakage into retinal tissues, forming retinal exudates.<sup>73</sup> Imbalanced estrogen can induce retinopathy<sup>74</sup>, and complications of decompensated cirrhosis result in thinning retinal arteries and tortuous vessels.<sup>75</sup> Splenomegaly leads to observable blood cell sequestration in fundus images, aiding hepatobiliary disease diagnosis, even in mild cases. These ocular changes offer valuable diagnostic insights, enhancing disease identification and understanding, even for milder hepatobiliary diseases like chronic viral hepatitis and non-alcoholic fatty liver disease.<sup>72</sup>

The study employed deep neural networks (ResNet-101) to develop screening models for hepatobiliary diseases using OCT and fundus images.<sup>72</sup> Notably, the iris, an unexplored area in hepatobiliary diseases, emerged as a significant diagnostic contributor. The models enable early detection and extensive, non-invasive screening, outperforming traditional approaches based on serum markers or systemic risk factors. These models can be integrated into existing fundus camera or slit-lamp systems, offering practical and effective opportunistic screening tools as quick and extensive screening is necessary because major hepatobiliary diseases are thought to be the cause of about two million deaths globally each year.<sup>76</sup> While the study acknowledges limitations such as sample size and potential biases, the authors emphasize the need for larger, diverse datasets to enhance accuracy and generalizability.<sup>72</sup>

### CONCLUSION

The utility of AI in medical diagnosis is growing as more links between ocular and systemic disorders are

discovered. Ocular pictures are being employed in the identification of endocrine, cardiovascular, neurological, renal, hematological, and many other disorders thanks to the development of AI, ML, DL, and medical big data. Although there is still much to learn about the fundamental connections between the eyes and other diseases, doing so will require continuing advancements in AI algorithms and our understanding of physiological and pathological mechanisms. Only then will we be able to fully comprehend the relationships between ocular and systemic health. AI is expected to revolutionize illness identification and patient treatment in the medical industry in the future.

### REFERENCES

1. He J, Baxter SL, Xu J, Xu J, Zhou X, Zhang K. The practical implementation of artificial intelligence technologies in medicine. *Nat Med.* 2019;25(1):30-6.
2. Zhang B, Chou Y, Zhao X, Yang J, Chen Y. Early detection of microvascular impairments with optical coherence tomography angiography in diabetic patients without clinical retinopathy: a meta-analysis. *Am J Ophthalmol.* 2021;222:226-37.
3. Moraes G, Fu DJ, Wilson M, Khalid H, Wagner SK, Korot E, et al. Quantitative analysis of OCT for neovascular age-related macular degeneration using deep learning. *Ophthalmology.* 2021;128(5):693-705.
4. Campbell JP, Kim SJ, Brown JM, Ostmo S, Chan RVP, Kalpathy-Cramer J, et al. Evaluation of a deep learning-derived quantitative retinopathy of prematurity severity scale. *Ophthalmology.* 2021;128(7):1070-6.
5. Xiangyu C, Yanwu X, Damon Wing Kee W, Tien Yin W, Jiang L. Glaucoma detection based on deep convolutional neural network. *Annu Int Conf IEEE Eng Med Biol Soc.* 2015;2015:715-8.
6. Hamet P, Tremblay J. Artificial intelligence in medicine. *Metabolism* 2017;69S:S36-S40.
7. Ramesh AN, Kambhampati C, Monson JR, Drew PJ. Artificial intelligence in medicine. *Ann R Coll Surg Engl.* 2004;86(5):334-8.
8. Chamie J. Population ageing: An inescapable future. *Global Issues.* [cited 2023 July 23]. Available from: <https://www.globalissues.org/news/2022/01/05/29746>
9. Childs BG, Durik M, Baker DJ, van Deursen JM. Cellular senescence in aging and age-related disease: from mechanisms to therapy. *Nat Med.* 2015;21(12):1424-35.
10. Lowsky DJ, Olshansky SJ, Bhattacharya J, Goldman DP. Heterogeneity in healthy aging. *J Gerontol A Biol Sci Med Sci* 2014;69(6):640-9.
11. Jylhävä J, Pedersen NL, Hägg S. Biological Age Predictors. *EBioMedicine.* 2017;21:29-36.
12. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat Rev Genet.* 2018;19:371-84.
13. Wang J, Knol MJ, Tiulpin A, Dubost F, de Bruijne M, Vernooij MW, et al. Gray matter age prediction as a biomarker for risk of dementia. *Proc Natl Acad Sci U S A.* 2019;116:21213-8.
14. Xia X, Chen X, Wu G, Li F, Wang Y, Chen Y, et al. Three-

- dimensional facial-image analysis to predict heterogeneity of the human ageing rate and the impact of lifestyle. *Nat Metab* 2020;2:946-57.
15. Cole JH, Ritchie SJ, Bastin ME, Valdes Hernandez MC, Munoz Maniega S, Royle N, et al. Brain age predicts mortality. *Mol Psychiatry*. 2018;23:1385-92.
  16. Country MW. Retinal metabolism: A comparative look at energetics in the retina. *Brain Res*. 2017;1672:50-57.
  17. Ikram MK, Ong YT, Cheung CY, Wong TY. Retinal vascular caliber measurements: clinical significance, current knowledge and future perspectives. *Ophthalmologica*. 2013;229(3):125-36.
  18. Zhu Z, Shi D, Guankai P, Tan Z, Shang X, Hu W, et al. Retinal age gap as a predictive biomarker for mortality risk. *Br J Ophthalmol*. 2023;107:547-54.
  19. Nusinovi S, Rim TH, Yu M, Lee G, Tham YC, Cheung N, et al. Retinal photograph-based deep learning predicts biological age, and stratifies morbidity and mortality risk. *Age Ageing*. 2022; 51(4):afac065.
  20. Ahadi S, Wilson KA, Babenko B, McLean CY, Bryant D, Pritchard O, et al. Longitudinal fundus imaging and its genome-wide association analysis provide evidence for a human retinal aging clock. *Elife*. 2023;12:e82364.
  21. Arrigo A, Aragona E, Bandello F. Artificial intelligence for the diagnosis and screening of retinal diseases. *touchReview in Ophthalmology*. 2023;17(2):9-14. [https://doi.org/10.17925/ usor.2023.17.2.1](https://doi.org/10.17925/usor.2023.17.2.1)
  22. Hansen MB, Abramoff MD, Folk JC, Mathenge W, Bastawrous A, Peto T. Results of automated retinal image analysis for detection of diabetic retinopathy from the Nakuru study, Kenya. *PLoS One*. 2015;10(10):e0139148.
  23. Abramoff MD, Folk JC, Han DP, Walker JD, Williams DF, Russell SR, et al. Automated analysis of retinal images for detection of referable diabetic retinopathy. *JAMA Ophthalmol*. 2013;131(3):351-7.
  24. US Food & Drug Administration. FDA permits marketing of artificial intelligence-based device to detect certain diabetes-related eye problems. News release, April 2018. DOI: 10.31525/fda2-ucm604357.htm.
  25. Ribeiro L, Oliveira CM, Neves C, Ramos JD, Ferreira H, Cunha-Vaz J. Screening for diabetic retinopathy in the central region of Portugal. Added value of automated 'disease/no disease' grading. *Ophthalmologica*. 2014 Nov 26.
  26. Tufail A, Kapetanakis VV, Salas-Vega S, Egan C, Rudisill C, Owen CG, et al. An observational study to assess if automated diabetic retinopathy image assessment software can replace one or more steps of manual imaging grading and to determine their cost-effectiveness. *Health Technol Assess*. 2016;20(92): 1-72.
  27. Solanki K, Ramachandra C, Bhat S, Bhaskaranand M, Nittala MG, Sadda SR. Eyeart: Automated, high-throughput, image analysis for diabetic retinopathy screening. *Investigative Ophthalmology & Visual Science*. 2015;56:1429.
  28. Rajalakshmi R, Subashini R, Anjana RM, Mohan V. Automated diabetic retinopathy detection in smartphone-based fundus photography using artificial intelligence. *Eye (Lond)*. 2018;32(6): 1138-44.
  29. Heydon P, Egan C, Bolter L, Chambers R, Anderson J, Aldington S, et al. Prospective evaluation of an artificial intelligence-enabled algorithm for automated diabetic retinopathy screening of 30,000 patients. *Br J Ophthalmol*. 2021;105(5):723-8.
  30. Gulshan V, Peng L, Coram M, Stumpe MC, Wu D, Narayanaswamy A, et al. Development and validation of a deep learning algorithm for detection of diabetic retinopathy in retinal fundus photographs. *JAMA*. 2016;316(22):2402-10.
  31. Ting DSW, Cheung CY-L, Lim G, Tan GSW, Quang ND, Gan A, et al. Development and validation of a deep learning system for diabetic retinopathy and related eye diseases using retinal images from multiethnic populations with diabetes. *JAMA*. 2017;318(22):2211-23.
  32. Lin D, Xiong J, Liu C, Zhao L, Li Z, Yu S, et al. Application of comprehensive artificial intelligence retinal expert (CARE) system: A national real-world evidence study. *Lancet Digit Health*. 2021;3(8):e486-e95.
  33. Lee CS, Baughman DM, Lee AY. Deep learning is effective for the classification of OCT images of normal versus age-related macular degeneration. *Ophthalmol Retina*. 2017;1(4):322-7.
  34. Lee CS, Tyring AJ, Deruyter NP, Wu Y, Rokem A, Lee AY, et al. Deep-learning based, automated segmentation of macular edema in optical coherence tomography. *Biomed Opt Express*. 2017;8(7):3440-8.
  35. Moraes G, Fu DJ, Wilson M, Khalid H, Wagner SK, Korot E, et al. Quantitative analysis of OCT for neovascular age-related macular degeneration using deep learning. *Ophthalmology*. 2021;128(5): 693-705.
  36. Waldstein SM, Vogl W-D, Bogunovic H, Sadeghipour A, Riedl S, Schmidt-Erfurth U. Characterization of drusen and hyperreflective foci as biomarkers for disease progression in age-related macular degeneration using artificial intelligence in optical coherence tomography. *JAMA Ophthalmol*. 2020;138(7):740-7.
  37. Schmidt-Erfurth U, Reiter GS, Riedl S, Seebock P, Vogl WD, Blodi BA, et al. AI-based monitoring of retinal fluid in disease activity and under therapy. *Prog Retin Eye Res*. 2022;86:100972.
  38. Bogunovic H, Mares V, Reiter GS, Schmidt-Erfurth U. Predicting treat-and-extend outcomes and treatment intervals in neovascular age-related macular degeneration from retinal optical coherence tomography using artificial intelligence. *Front Med (Lausanne)*. 2022;9:958469.
  39. Holomcik D, Seeböck P, Gerendas BS, Mylonas G, Najeeb BH, Schmidt-Erfurth U, et al. Segmentation of macular neovascularization and leakage in fluorescein angiography images in neovascular age-related macular degeneration using deep learning. *Eye (Lond)*. 2023;37(7):1439-44.
  40. Vogl W-D, Riedl S, Mai J, Reiter GS, Lachinov D, Bogunovic H, et al. Predicting topographic disease progression and treatment response of pegcetacoplan in geographic atrophy quantified by deep learning. *Ophthalmology Retina*. 2023;7(1):4-13.
  41. Liu Y, Holekamp NM, Heier JS. Prospective, Longitudinal study: Daily self-imaging with home OCT for neovascular age-related macular degeneration. *Ophthalmol Retina*. 2022;6(7): 575-85.
  42. Mathai M, Reddy S, Elman MJ, Garfinkel RA, Ladd B, Wagner AL, et al. Analysis of the long-term visual outcomes of ForeseeHome remote telemonitoring: The ALOFT study. *Ophthalmol Retina*. 2022;6(10):922-9.
  43. Wang H, Abbas KM, Abbasifard M, Abbasi-Kangevari M, Abastabar H, Abd-Allah F, et al. Global age-sex-specific fertility, mortality, healthy life expectancy (HALE), and population estimates in 204 countries and territories, 1950-2019: a comprehensive demographic analysis for the global burden of disease study 2019. *Lancet*. 2020;396(10258):1160-203.

44. Hannappe MA, Arnould L, Méloux A, Mouhat B, Bichat F, Zeller M, et al. Vascular density with optical coherence tomography angiography and systemic biomarkers in low and high cardiovascular risk patients. *Sci Rep.* 2020;10(1):16718.
45. Poplin R, Varadarajan AV, Blumer K, Liu Y, McConnell MV, Corrado GS, et al. Prediction of cardiovascular risk factors from retinal fundus photographs via deep learning. *Nat Biomed Eng.* 2018;2(3):158–64.
46. Kim YD, Noh KJ, Byun SJ, Lee S, Kim T, Sunwoo L, et al. Effects of hypertension, diabetes, and smoking on age and sex prediction from retinal fundus images. *Sci Rep.* 2020;10(1):4623.
47. Son J, Shin JY, Chun EJ, Jung KH, Park KH, Park SJ. Predicting high coronary artery calcium score from retinal fundus images with deep learning algorithms. *Transl Vision Sci Technol.* 2020;9(2):28.
48. Kim YD, Noh KJ, Byun SJ, Lee S, Kim T, Sunwoo L, et al. Effects of hypertension, diabetes, and smoking on age and sex prediction from retinal fundus images. *Sci Rep.* 2020;10(1):4623.
49. Son J, Shin JY, Chun EJ, Jung KH, Park KH, Park SJ. Predicting high coronary artery calcium score from retinal fundus images with deep learning algorithms. *Transl Vision Sci Technol.* 2020;9(2):28.
50. Chang J, Ko A, Park SM, Choi S, Kim K, Kim SM, et al. Association of cardiovascular mortality and deep learning-funduscopy atherosclerosis score derived from retinal fundus images. *Am J Ophthalmol.* 2020;217:121-30.
51. Zhang K, Liu X, Xu J, Yuan J, Cai W, Chen T, et al. Deep-learning models for the detection and incidence prediction of chronic kidney disease and type 2 diabetes from retinal fundus images. *Nat Biomed Eng.* 2021;5(6):533-45.
52. Babenko B, Traynis I, Chen C, Singh P, Uddin A, Cuadros J, et al. A deep learning model for novel systemic biomarkers in photographs of the External Eye: A Retrospective Study. *Lancet Digit Health.* 2023;5(5):e257-64.
53. Cheung CY, Ran AR, Wang S, Chan VTT, Sham K, Hilal S, et al. A deep learning model for detection of Alzheimer's disease based on retinal photographs: a retrospective, multicentre case-control study. *Lancet Digit Health.* 2022;4(11):e806-15.
54. O'Bryhim BE, Lin JB, Stavern GPV, Apte RS. OCT angiography findings in preclinical Alzheimer's disease: 3-year follow-up. *Ophthalmology.* 2021;128(10):1489-91.
55. Nunes A, Silva G, Duque C, Januario C, Santana I, Ambrosio AF, et al. Retinal texture biomarkers may help to discriminate between Alzheimer's, Parkinson's, and healthy controls. *PLoS ONE.* 2019;14(6):e0218826.
56. Wisely CE, Wang D, Henao R, Grewal DS, Thompson AC, Robbins CB, et al. Convolutional neural network to identify symptomatic Alzheimer's disease using multimodal retinal imaging. *Br J Ophthalmol.* 2022;106(3):388-95.
57. Wong CW, Wong TY, Cheng CY, Sabanayagam C. Kidney and eye diseases: common risk factors, etiological mechanisms, and pathways. *Kidney Int.* 2014;85(6):1290-302.
58. Wong TY, Coresh J, Klein R, Muntner P, Couper DJ, Sharrett AR, et al. Retinal microvascular abnormalities and renal dysfunction: the atherosclerosis risk in communities study. *J Am Soc Nephrol.* 2004;15(9):2469-76.
59. Pakzad-Vaezi K, Pepple KL. Tubulointerstitial nephritis and uveitis. *Curr Opin Ophthalmol.* 2017;28(6):629-35.
60. Liew G, Mitchell P, Wong TY, Iyengar SK, Wang JJ. CKD increases the risk of age-related macular degeneration. *J Am Soc Nephrol.* 2008;19(4):806-11.
61. Kang EY, Hsieh YT, Li CH, Huang YJ, Kuo CF, Kang JH, et al. Deep learning-based detection of early renal function impairment using retinal fundus images: model development and validation. *JMIR Med Inform.* 2020;8(11):e23472.
62. Sabanayagam C, Xu D, Ting DSW, Nusinovic S, Banu R, Hamzah H, et al. A deep learning algorithm to detect chronic kidney disease from retinal photographs in community-based populations. *Lancet Digit Health.* 2020;2(6):e295-302.
63. Bauskar S, Jain P, Gyanchandani M. A Noninvasive Computerized Technique to Detect Anemia Using Images of Eye Conjunctiva. *Pattern Recognit. Image Anal.* 2019;29:438-46.
64. Chen Y, Zhong K, Zhu Y, Sun Q. Two-Stage Hemoglobin Prediction Based on Prior Causality. *Front Public Health.* 2022;10:1079389.
65. Mitani A, Huang A, Venugopalan S, Corrado GS, Peng L, Webster DR, et al. Detection of Anaemia from Retinal Fundus Images via Deep Learning. *Nat Biomed Eng.* 2020;4(1):18-27.
66. Zhao X, Meng L, Su H, Lv B, Lv C, Xie G, et al. Deep-Learning-Based Hemoglobin Concentration Prediction and Anemia Screening Using Ultra-Wide Field Fundus Images. *Front Cell Dev Biol.* 2022;10:888268.
67. Wei H, Shen H, Li J, Zhao R, Chen Z. AneNet: A Lightweight Network for the Real-Time Anemia Screening from Retinal Vessel Optical Coherence Tomography Images. *Optics & Laser Technology.* 2020;136(1):106773.
68. Wu Y, Wang D, Wu X, Shen L, Zhao L, Wang W, et al. Optical Coherence Tomography Angiography for the Characterisation of Retinal Microvasculature Alterations in Pregnant Patients with Anaemia: A Nested Case-control Study. *Br J Ophthalmol.* 2022;bjophthalmol-2022-321781.
69. Graves J, Balcer LJ. Eye disorders in patients with multiple sclerosis: natural history and management. *Clin Ophthalmol.* 2010;4:1409-22.
70. Garcia-Martin E, Ortiz M, Boquete L, Sánchez-Morla EM, Barea R, Cavaliere C, et al. Early diagnosis of multiple sclerosis by OCT analysis using Cohen's d method and a neural network as classifier. *Comput Biol Med.* 2021;129:104165.
71. Peng CY, Chien RN, Liaw YF. Hepatitis B virus-related decompensated liver cirrhosis: benefits of antiviral therapy. *J Hepatol.* 2012;57(2):442-50.
72. Xiao W, Huang X, Wang JH, Lin DR, Zhu Y, Chen C, et al. Screening and identifying hepatobiliary diseases through deep learning using ocular images: A prospective, multicentre study. *Lancet Digit Health.* 2021;3(2):e88-e97.
73. Reichenbach A, Fuchs U, Kasper M, el-Hifnawi E, Eckstein AK. Hepatic retinopathy: morphological features of retinal glial (Müller) cells accompanying hepatic failure. *Acta Neuropathol.* 1995;90(3):273-81.
74. Onder C, Bengur T, Selcuk D, Bulent S, Belkis U, Ahmet M, et al. Relationship between retinopathy and cirrhosis. *World J Gastroenterol.* 2005;11(14):2193-6.
75. Iwakiri Y. Pathophysiology of portal hypertension. *Clin Liver Dis.* 2014;18(2):281-91.
76. Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. *J Hepatol.* 2019;70(1):151-71.