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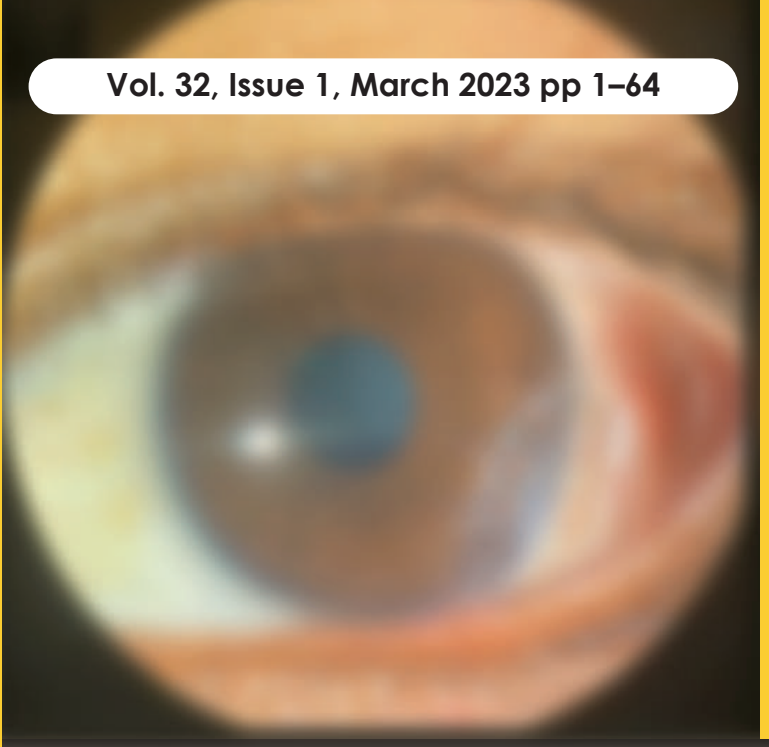

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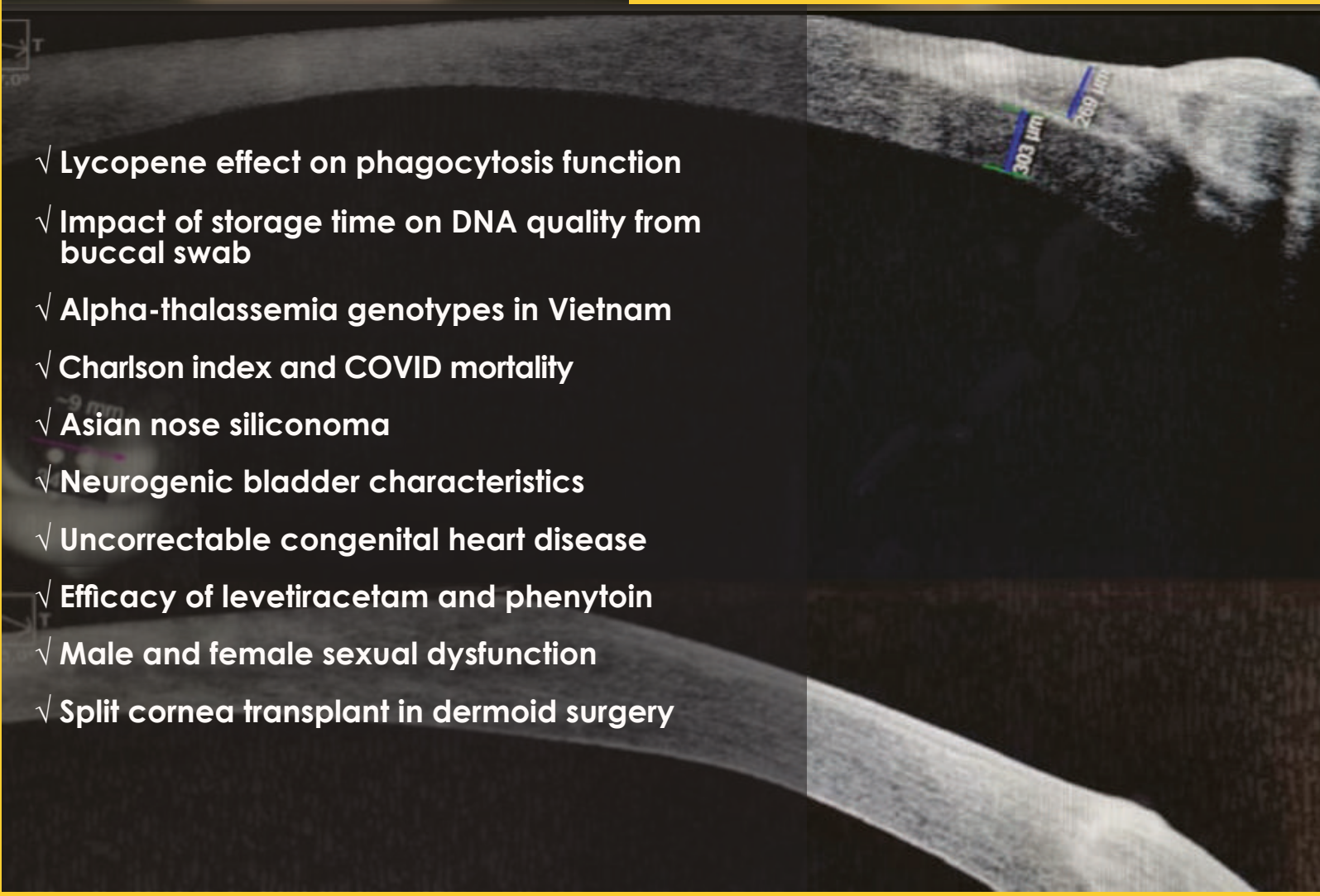

- √ Lycopene effect on phagocytosis function
- √ Impact of storage time on DNA quality from buccal swab
- √ Alpha-thalassemia genotypes in Vietnam
- √ Charlson index and COVID mortality
- √ Asian nose siliconoma
- √ Neurogenic bladder characteristics
- √ Uncorrectable congenital heart disease
- √ Efficacy of levetiracetam and phenytoin
- √ Male and female sexual dysfunction
- √ Split cornea transplant in dermoid surgery

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Medical Journal of Indonesia

The **Medical Journal of Indonesia** (abbr: Med J Indones) was founded in 1991 as the Medical Journal of the University of Indonesia (abbr: Med J Univ Indon). Since the first issue in 1992, it has been published quarterly, consistently, and continuously, covering a wide range of medical subject and issues from every medical specialist aspect. In 1995 the name was changed to Medical Journal of Indonesia which reflected the widening of its coverage beyond Universitas Indonesia. The mission of our journal is to provide biomedical scientist, clinical, and public health researchers as well as other health care professionals with the media to published their research works. Starting from 2011, Deutsch-Indonesische Gesellschaft für Medizin (DIGM) Medical Journal has been merged into Medical Journal of Indonesia. This also means that DIGM is officially collaborating with Medical Journal of Indonesia and Faculty of Medicine Universitas Indonesia as the owner and publisher. The mission of the Medical Journal of Indonesia is to improve health care management by providing high-quality articles from Indonesia and worldwide.

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Medical Journal of Indonesia focuses on promoting medical sciences generated from **basic sciences**, **clinical**, and **community or public health research** to integrate researches in all aspects of human health.

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Brief communications containing short features of medicine, latest developments in diagnostic procedures, treatment, or other health issues that is important for the development of health care system are also acceptable.

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The paper meets the requirements for acid free paper.

Editorial Board

Medical Journal of **Indonesia**

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Contents

Case Report/Series

Correction

***Front Page*:** Slit-lamp examination of the patient's left eye 4 weeks after re-surgery showed a well-apposed host-graft junction, and AS-OCT examination revealed a mass in the corneal-limbal-conjunctiva with half the depth of the stroma, as reported by Kurniawardhani et al. These are published in this issue.

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Contents

See the journal website for contents.

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Submission

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All identifiers, including but not limited to names, initials, dates of birth, medical record numbers, must be removed from the patient's data or information published on the manuscript.

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Acknowledgments

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   Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. The genetic basis of human cancer. New York: McGraw-Hill; 2002. p. 93–113.

3. ***Homepage/Web site*** **[Edited 12 May 2009]**

   Cancer-Pain.org [Internet]. New York: Association of Cancer Online Resources, Inc.; c2000-01 [updated 2002 May 16; cited 2002 Jul 9]. Available from: http://www.cancer-pain.org/.

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Medical Journal of **Indonesia**

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Clinical Research

Charlson comorbidity index to predict 28-day mortality in critically ill COVID-19 patients

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ABSTRACT

BACKGROUND Severe COVID-19 patients may become critically ill and require treatment in the intensive care unit (ICU). As intensive care resources are limited, mortality predictors should be used to guide resource allocation. This study aimed to validate the Charlson comorbidity index (CCI) as the mortality predictor of critical COVID-19 patients in the ICU.

METHODS A retrospective cohort study was done in adult patients admitted to the ICU with severe COVID-19 at Cipto Mangunkusumo Hospital and Universitas Indonesia Hospital from March to August 2020. We extracted the subject's CCI score from the medical records and the 28-day mortality after ICU admission. The CCI score was validated by the Hosmer–Lemeshow calibration test, determination of area under the curve (AUC), and optimal cut-off point for the critical patients in the ICU. We used the chi-square test to examine the association of comorbidities with mortality.

RESULTS Mortality was higher in CCI scores >4 (odds ratio [OR]: 8.83; 95% confidence interval [CI] = 1.81–43.01). The CCI score had moderate discrimination ability (AUC 76.1%; 95% CI = 0.661–0.881). Chronic kidney disease (CKD) (OR: 18.00, 95% CI = 2.19–147.51), congestive heart failure (CHF) (OR: 4.25, 95% CI = 1.23–14.75), and uncontrolled diabetes mellitus (DM) (OR: 18.429, 95% CI = 2.19–155.21) increased the risk of 28-day mortality.

CONCLUSIONS The CCI score could predict the 28-day mortality of critical COVID-19 patients. The coexistence of CKD, CHF, DM, peripheral vascular disease, and peptic ulcer in COVID-19 patients should be considered for patient management.

KEYWORDS Charlson comorbidity index, COVID-19, critical illness, mortality

Coronavirus disease 2019 (COVID-19) was declared a pandemic by the World Health Organization (WHO) on March 11, 2020;[1] it has since evolved into an unprecedented global health crisis. As of May 12, 2020, the global case fatality rate (CFR) of COVID-19 was 6.95%, and the corresponding Indonesian CFR was 6.93%.[2] COVID-19 is associated with high mortality rates in the intensive care units (ICUs). Considering the high demand for ICU beds and the impact on hospital capacity preparedness, it is important to establish a prediction tool for ICU mortality to enable early prognosis screening. Such a tool will help identify patients with better outcomes and lower mortality risks, which will subsequently benefit ICU admission screening and management.[3]

The most frequently used ICU scoring systems, Acute Physiology and Chronic Health Evaluation II and Simplified Acute Physiology Score II, have yielded unusually low scores in non-survivors and underestimated the actual disease severity and

mortality risk in critically ill COVID-19 patients.[1] The Charlson comorbidity index (CCI) is a well-known scoring system that considers 19 comorbidities, weighted 1–6 depending on their severity; from these, a single numerical score (ranging from 0 to 33) is obtained, which enables the prediction of the 1-year mortality. Christensen et al[4] reported that when controlled for age and sex, a CCI score of >0 was associated with an increased risk of severe COVID-19 and death. Although the CCI was initially used to predict long-term mortality, it has recently been used for ICU admission screening and to assess short-term mortality in high-risk patients.[4–6] In clinical practice, the CCI helps stratify patients into subgroups based on severity, develops care models, and targets resource allocation.[7] A mortality predictor can be used to assess the course of a particular disease and disease management strategies, including resource allocation. Therefore, this study aimed to validate the CCI as a predictor of mortality in critically ill COVID-19 patients admitted to the ICU.[7]

METHODS

Research design

This retrospective cohort study was conducted at Cipto Mangunkusumo Hospital, Jakarta, Indonesia and Universitas Indonesia Hospital, Depok, Indonesia from September to October 2020. The study population comprised COVID-19 patients admitted to the ICU between March and August 2020. This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (No: KET-745/UN2.F1/ETIK/PPM.00.02/2020). The minimum sample size for this study was estimated to be 95 patients with the following parameters: predefined type-1 error (α), 5%; precision, 10%; and expected area under the curve (AUC), 80%.

Inclusion and exclusion criteria

The inclusion criteria were aged >18 years, positive tests for COVID-19, and ICU admission. The exclusion criteria were death before admission to the ICU, incomplete medical records, and unknown mortality outcomes before 28 days of treatment.

Patient selection

Data of patients who met the criteria for the diagnosis and surveillance of COVID-19, according to the WHO COVID-19 case definition (updated in Public Health Surveillance for COVID-19 [December 16, 2020]), were collected from a registry book and electronic medical records maintained in the ICU. Confirmed case patients should be patients who fulfilled the following criteria: 1) COVID-19 diagnosis confirmed via positive nucleic acid amplification tests or reverse transcription-polymerase chain reaction assays; 2) presence of severe acute respiratory illness, chest imaging findings suggestive of COVID-19, recent onset of anosmia or ageusia in the absence of other etiologies, and respiratory distress prior to death; and 3) history of contact with a probable or confirmed case or of links with a COVID-19 cluster.[8]

Comorbidity diagnostic criteria

Data for CCI score analysis were extracted from electronic health records. Each comorbidity was defined based on the International Classification of Diseases codes. The comorbidities were diagnosed by the physician-in-charge after considering the laboratory findings obtained within the first 24 hours of ICU admission.[9]

Myocardial infarction was categorized as definite or probable with electrocardiogram changes and/or enzymatic changes. Congestive heart failure (CHF) was characterized by exertional dyspnea or paroxysmal nocturnal dyspnea. Peripheral vascular disease (PVD) was characterized by the presence of intermittent claudication or chronic arterial insufficiency, a history of gangrene or acute arterial insufficiency, or an untreated thoracic or abdominal aorta aneurysm (≥6 cm). Cerebrovascular disease was characterized by a cerebrovascular accident with minor or no residual symptoms and transient ischemic attacks. Dementia was defined as a chronic cognitive deficit. Chronic obstructive pulmonary disease was characterized by the presence of chronic respiratory disorder with a history of passive or active smoking. Peptic ulcer disease was characterized by a history of ulcer bleeding or any history of treatment for ulcer disease. Liver disease was categorized as severe (cirrhosis and portal hypertension with variceal bleeding history), moderate (cirrhosis and portal hypertension without a variceal bleeding history), and mild (chronic hepatitis or cirrhosis without portal hypertension). Diabetes mellitus (DM) was categorized as non-DM, controlled DM without complications, and uncontrolled DM with chronic complications. Hemiplegia was defined by the

presence of weakness as a sequela of a cerebrovascular accident. Chronic kidney disease (CKD) was categorized as moderate (creatinine >3 mg/dl) to severe (on dialysis therapy). Solid tumors were identified as with or without metastasis. Leukemia was classified as acute or chronic myelogenous leukemia, acute and chronic lymphocytic leukemia, and polycythemia vera. Lymphomas comprised Hodgkin's lymphoma, lymphosarcoma, Waldenstrom's macroglobulinemia, myeloma, and other lymphomas.[9] AIDS was defined as patients included with definite or probable AIDS. Connective tissue disease was included as systemic lupus erythematous, polymyositis mixed connective tissue disease, polymyalgia rheumatica, and moderate to severe rheumatoid arthritis.[9]

Mortality outcome

The survival or mortality outcomes within 28 days after ICU admission were recorded by a research assistant blinded to the comorbidity data. Data on patients discharged home before 28 days of hospitalization were obtained by contacting them directly. All of participants could be contacted, and the informed consents were complete. The participants were excluded if the data were incomplete or they could not be contacted.

Statistical analysis

All data were analyzed using SPSS software version 25.0 (IBM Corp., USA). The patient characteristics were subjected to a univariate analysis. Categorical data are expressed as proportions, whereas numerical data are expressed as means if normally distributed or as medians if non-normally distributed.

The CCI scores were classified into two groups (≤4 and >4 points) to predict 28-day mortality. The cut-offs are based on previous studies suggesting that patients with CCI scores of ≤4 points are at a lower risk of mortality.[10] A bivariate analysis was performed to evaluate the association of the CCI scores of ≤4 and >4 with the 28-day ICU mortality in patients with COVID-19. Categorical variables were analyzed using a chi-square test or Fisher's exact test. Numerical variables were analyzed using an unpaired or Mann–Whitney tests. $p<0.05$ was considered statistically significant. The predictive validity of the CCI score was evaluated using multivariate logistic regression analysis based on the discrimination and calibration values; the calibration values were calculated using the Hosmer–Lemeshow test.

RESULTS

Among the 213 patients isolated in the ICU, 2 and 3 patients were excluded for missing medical records and incomplete data, respectively. Among the remaining 208 patients, 95 (45.7%) were confirmed to have COVID-19. Table 1 shows the characteristics and CCI scores between the COVID-19 survivors and non-survivors.

The proportion of men was higher than that of women in both the survivor and non-survivor groups. The Hosmer–Lemeshow calibration test showed that the CCI score can predict the outcomes of COVID-19 (chi-square = 1.242 and $p = 0.743$). The AUC value was 76.1% (95% confidence interval [CI] = 0.661–0.881), showing that the CCI score has moderate discrimination quality in predicting the outcome of COVID-19 critically ill patients (Figure 1). The cut-off on the CCI score was 2.5 with sensitivity at 72.5% and specificity at 67.3% in predicting the 28-day mortality of COVID-19 patients in the ICU. Table 1 presents data on the association between CCI scores and patient outcomes. The mortality risk was higher in the group with CCI scores >4 than in the group with CCI scores ≤4.

Some comorbidities were significantly associated with mortality in COVID-19 patients; significant associations were found between mortality and patients with moderate-to-severe CKD (odds ratio [OR]: 18.00, 95% CI = 2.19–147.51, $p = 0.001$), CHF (OR: 4.25, 95% CI = 1.23–14.75, $p = 0.016$), DM (controlled DM OR: 1.86, 95% CI = 0.68–5.07; uncontrolled DM OR: 18.43, 95% CI = 2.19–155.21; $p = 0.003$), PVD (OR: 0.0402, 95% CI = 0.31–0.52, $p = 0.039$), and peptic ulcer (OR: not available [NA], 95% CI = NA, $p = 0.029$). These patients were at a relatively high risk of mortality; among patients with DM, the mortality risk was higher in those with uncontrolled DM than in those without DM (OR: 18.429, 95% CI = 2.19–155.21, $p = 0.003$).

DISCUSSION

We found a significant association between the CCI and short-term ICU mortality in patients with COVID-19. A higher CCI score was associated with poor outcomes in the acute setting. Moreover, the CCI score had good discrimination and calibration values for predicting the outcomes of patients with confirmed COVID-19 admitted to the ICU. With an AUC of 76.1%, the CCI can predict mortality in the ICU

Table 1. Outcomes on COVID-19 patients based on comorbid variables

Variables	Outcomes		OR (95% CI)	*p*
	Non-survivors, n (%) (N = 40)	Survivors, n (%) (N = 55)		
Age (years), mean (SD)	59.03 (14.07)	46.60 (15.05)	-	-
Male sex	27 (37)	45 (63)	-	-
Confirmed status	40 (42)	55 (58)	-	-
CCI score				**0.003**
Score ≤4	30 (32)	53 (56)	1.00	
Score >4	10 (11)	2 (2)	8.83 (1.81–43.01)	
CKD moderate–severe	10 (91)	1 (9)	18.00 (2.19–147.51)	**0.001**
DM				**0.003**
Non-DM	21 (33)	43 (67)	1.00	
Controlled DM	10 (48)	11 (52)	1.86 (0.68–5.07)	
Uncontrolled DM	9 (90)	1 (10)	18.43 (2.19–155.21)	
CHF	10 (71)	4 (29)	4.25 (1.23–14.75)	**0.016**
MI	12 (48)	13 (52)	1.39 (0.55–3.47)	0.487
COPD	11 (44)	14 (56)	1.11 (0.44–2.79)	0.823
Liver disease mild–severe	2 (40)	3 (60)	0.92 (0.15–5.73)	1.000
PVD	3 (100)	0 (0)	0.04 (0.31–0.52)	**0.039**
Solid tumor	2 (33)	4 (67)	0.67 (0.12–3.86)	1.000
CVA or TIA	2 (100)	0 (0)	NA*	0.175
Dementia	2 (67)	1 (33)	NA*	0.571
AIDS	0 (0)	1 (100)	NA*	1.000
Connective tissue disease	0 (0)	1 (100)	NA*	1.000
Peptic ulcer	4 (100)	0 (0)	NA*	**0.029**

CCI=Charlson comorbidity index; CHF=congestive heart failure; CI=confidence interval; CKD=chronic kidney disease; COPD=chronic obstructive pulmonary disease; COVID-19=coronavirus disease 2019; CVA=cerebrovascular accident; DM=diabetes mellitus; MI=myocardial infarction; NA=not available; OR=odds ratio; PVD=peripheral vascular disease; SD=standard deviation; TIA=transient ischemic attack

*Unable to be analyzed due to absence of patient in one or more cells. Categorical data were analyzed using chi-square or Fisher's exact test. There was no patient with leukemia, hemiplegia, and lymphoma in the survivor and non-survivor groups

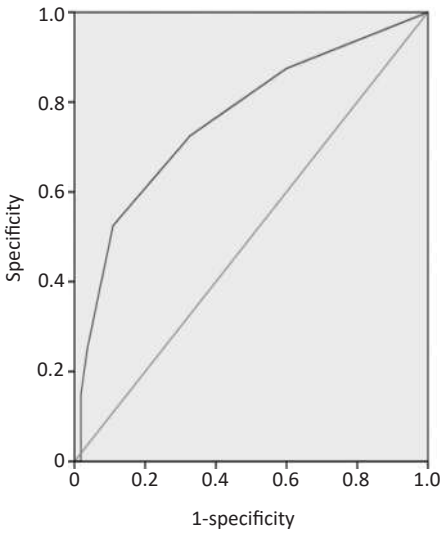

Figure 1. Charlson comorbidity index (CCI) score's receiving operating characteristic curve (ROC) in predicting outcome of confirmed case

with moderate quality and good match-model results using the Hosmer–Lemeshow goodness of fit test. This is in line with the findings reported by Zhou et al[11] who stated that with an AUC of 81.6%, the CCI score had a good discrimination ability to predict the outcome of confirmed COVID-19 patients in ICU. Our study revealed a predictive cut-off CCI score of 2.5 for mortality (sensitivity: 72.5%, specificity: 67.3%) and the risk of mortality increased by 1.89 times for every increase in the CCI score.

COVID-19 patients with comorbidities are believed to be at a higher risk of severe COVID-19. This study showed that compared to patients with COVID-19 but without CKD, those with both COVID-19 and CKD were at an 18-fold higher mortality risk. A previous meta-analysis also revealed a significant association between CKD and severe COVID-19, which affected the mortality

rate.[9] CKD modifies the immune system via persistent systemic inflammation and immunosuppression secondary to dysfunctional B cells and phagocytic T cells, along with increased proinflammatory cytokine production and monocyte activation. These changes are worsened by decreased kidney function. Neutrophil function decreases in patients before and on dialysis. In patients with CKD, impaired B and T cells undergo apoptosis; this causes T lymphopenia, progressive immunodeficiency, and a high risk of infection.[9,12]

CHF was significantly associated with mortality outcomes. Patients with COVID-19 and CHF were at a 4.25-fold higher risk of mortality than those without CHF. In a previous study, compared with patients with COVID-19 but without CHF, those with COVID-19 and CHF were associated with an approximately 2-fold higher risk of mortality, 3 times higher risk of the need of mechanical ventilation, and a longer hospital stay length despite controlling for relevant clinical factors.[13] CHF induces monocytes to produce many tumor necrosis factor alpha and lesser interleukin-10, thereby weakening immunity. Acute COVID-19 causes tissue damage and cardiac depression, leading to the onset of acute decompensated heart failure. The resultant decreased hemodynamic stability is more likely to fail to overcome the severe inflammation common in critically ill COVID-19 patients.[14,15] Moreover, coagulation dysfunction triggered by sepsis worsens CHF.[16]

PVD was significantly correlated with COVID-19-associated ICU mortality. A case study revealed PVD onset without a prior history; unfortunately, few studies have examined the association between peripheral arterial disease and COVID-19. However, inflammatory cells, such as polymorphonuclear neutrophils, T lymphocytes, histiocytes, and macrophages in the lining of blood vessels, are correlated with endothelial proliferation, angiogenesis with collagen deposition degree, myofibroblast proliferation, and thrombosis.[17]

DM is significantly associated with COVID-19 outcomes. It worsens the risk of COVID-19 through several mechanisms wherein the viral load and high angiotensin-converting enzyme 2 (ACE2) receptor expression serves as a gateway for viral infection in the lung tissues. Viral replication increases blood sugar levels.[18] Patients with DM present with mild-to-chronic elevations in inflammation-inducing macrophage, monocyte, and T-cell recruitment levels, which worsen the already excessive production of proinflammatory cytokines and further damage the lung tissues. This damage is associated with some structural changes in the lungs, including vascular permeability changes and alveolar dysfunction, which reduce gaseous exchange. These respiratory impairments complicate lung function and result in a higher risk of mechanical ventilator requirement.[18–20] Severe acute respiratory syndrome coronavirus 2 infects the endothelial cell directly via the ACE2 receptor. Patients with DM with highly elevated cytokine levels suffer from systemic hypercoagulation, tissue edema, and microcirculatory vasoconstriction, all of which worsen organ ischemia in severe COVID-19. Hypercoagulation, along with increased platelet activation and adhesion to the endothelial wall, promotes thromboembolic events in COVID-19.[18,21]

Only a few studies have evaluated the association between peptic ulcers and COVID-19. The present study revealed a significant association between peptic ulcer incidents and mortality in patients with COVID-19. A case study by Melazzini et al[22] revealed that gastrointestinal bleeding secondary to peptic ulcers was common in patients with severe COVID-19. The mechanism underlying COVID-19-associated peptic ulcer development may involve acute gastric mucosa inflammation, direct gastric epithelial damage by the causative virus, and cytokine-activated inflammation.[22]

This study had several limitations. Considering the urgent need for short-term mortality predictors to develop effective management strategies, this study was retrospective in nature and was conducted over a short duration. Secondary data were extracted from medical records, incurring a risk of selection, recall, or misclassification bias; furthermore, causation could not be determined. The included patients received multidisciplinary care; this increased the potential bias in establishing the operational definition of each comorbidity according to personal interpretation. Limited diagnostic test availability may have increased the bias in the determining confirmed cases, which may have influenced the patient's disease progression, prognosis, and mortality. Moreover, the small sample size for the CCI cut-off resulted in disproportionate data that affected its validity as a predictive tool. We suggest further prospective observational studies to assess the relationships between the risk factors (adjusted for comorbidities) and the CCI score for predicting disease severity and mortality in critically ill COVID-19 patients.

In conclusion, the CCI score could predict the 28-day mortality in patients with critical COVID-19. Critical

COVID-19-associated mortality rates remain high; thus, mortality risk stratification of patients with COVID-19 based on their underlying comorbidities may improve clinicians' ability to identify those who require more intensive care and greater hospital financial allocation for improved outcomes. Moreover, the presence of comorbidities, including CKD, CHF, DM, PVD, and peptic ulcers in these patients, should be considered during patient management.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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Clinical Research

Photogrammetrics and clinical features of nasal siliconoma in Asians

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ABSTRACT

BACKGROUND Nasal silicone injections have been a common procedure among Asians. However, this procedure can lead to severe complications. Unfortunately, there are limited data available on the distortive characteristics of nasal siliconoma in the Asian population. This study aimed to provide objective data on the distortive characteristics of nasal siliconoma to be a reference for a treatment outcome.

METHODS This cross-sectional study was conducted at Cipto Mangunkusumo Hospital from June 2017 to March 2018 involving 30 Asian females with nasal siliconoma. Nasal photogrammetric measurements were taken using a portable mirror stand device and analyzed to formulate the distortive characteristics.

RESULTS The mean (standard deviation) of intercanthal width was 3.33 (0.25) cm, nasal root width was 2.70 (0.30) cm, alar width was 4.48 (0.31) cm, two tip-defining points (TDP) distance was 2.09 (0.22) cm, nasofrontal angle was 141.10 (8.40)°, length of the nose was 3.10 (0.48) cm, nasofacial angle was 32.94 (4.51)°, nasion projection was 0.64 (0.36) cm, pronasion projection was 2.00 (0.25–2.46) cm, tip angle was 122.7 (4.52)°, nasolabial angle was 78.81 (15.93)°, columella length (n = 20) was 0.64 (0.20) cm, tip lobular portion length was 1.12 (0.20) cm, the extend of extended columella was 0.47 (0.31) cm, and base of the nasal width was 3.98 (0.25) cm.

CONCLUSIONS Nasal siliconoma in Asians had certain characteristics such as a wider nasal root, wider two TDP distance, wider nasion projection, acute nasolabial angle, hanging columella, and a long lobular portion of the tip.

KEYWORDS Asia, face, nose, photogrammetry, silicones

Asian noses are typically boxy with wide alae and a lack of tip projection and dorsal height. Many Asians seek out nose beautification for strong aesthetic reasons.[1] However, some individuals still opt for injectable silicone to augment soft tissue, despite its ban by the Food and Drug Administration in 1940.[2,3] The lure of cheaper costs and 'instant' results can be tempting without realizing the potential for disfiguring complications such as siliconoma.[4]

Surgery remains the primary treatment for correcting aesthetic distortions caused by nasal siliconomas. Unfortunately, achieving the desired aesthetic outcome can be challenging, particularly when attempting to achieve a relatively slimmer nasal dorsum and a defined tip. Surgical correction often results in a broadened nasal dorsum and poor tip definition. Despite the best efforts of surgeons, patients may still be dissatisfied with their appearance after surgery.[5]

Objective data on the distortion characteristics and clinical features of nasal siliconomas have not yet been reported. Hence, to improve the outcomes of nasal reconstruction, this study aimed to provide data as benchmarks for reconstruction that will help surgeons and patients agree on achievable targets for reconstruction and better appreciate the post-surgical results.

METHODS

This cross-sectional study was conducted on 30 females with nasal siliconoma at Cipto Mangunkusumo Hospital, Jakarta, Indonesia, from June 2017 to March 2018. The sample size was calculated based on the standard deviation (SD) of a previous study on the morphometry of the Deutero-Malay female nose.[6] Deutero-Malay was selected because it was the only specific subrace with available published data on nasal morphometry related to Mongoloid Asians among the various other Asian subraces in Indonesia.

The participants were of Mongoloid Asian origin and had a history of previous injections of materials such as silicone, paraffin solution, and mineral oil in the nasal region. The collected data included age, injection time, service provider, and type of injectable material used. All participants voluntarily participated in the study and provided informed consent and a photo release consent. The Ethics Committee of the Faculty of Medicine, Universitas Indonesia, approved this study (No: 892/UN2.F1/ETIK/2017).

The participants completed a questionnaire regarding their aesthetic perception of their nose and expectations for their future appearance after surgical correction. After receiving a brief information on the available surgical options, they expressed their opinions on nasal reconstructive techniques, including the open technique and the use of autografts and/or implants. They were also asked about their nasal shape before the injection and the reasons for the injection.

Four anatomical landmarks including the maxillofrontal (mf), tip-defining points (TDP), highest point of the columella (c), and subnasal were marked on the subjects' faces using makeup pencils. Photographs were taken with a mirror stand (MirS) device by adopting the technique as described earlier,[7] using a Canon IXUS 500 HS digital camera (Canon Inc., Japan) (Figure 1). The MirS device provided consistent and unbiased photographs as a constant formula developed by inventors to convert photographic values into anthropometric values that are consistent with actual facial measurements.[7]

Measurements were taken using the method described by Prasetyono et al[6] and categorized according to the universal one-third division of the face. The measurements included the intercanthal width, nasal root width (the distance between two mf points), alar width, distance between two TDP, nasofrontal angle, length of the nose (radix to pronasion), nasofacial angle, nasion projection, pronasion projection, tip angle, nasolabial angle, columella length, extension of the columella, and base of the nasal width (Figure 2). From the lateral view, the columella was assessed by measuring the distance between the long axis of the nostril and the columellar edge, as well as the distance from the long axis to the superior nostril rim. Furthermore, the lobular portion of the nose (tip lobule length) and the ratio of the columella to the lobular portion of the nose were measured based on the basal view (Figure 2f). The presence of long-standing inflammation of the nose, which manifested as persistent redness after injection, was also assessed.

Photographs were analyzed using the ImageJ software (LOCI, USA) to obtain morphometric measurements. In addition, the lengths of the upper face (trichion to glabella), middle face (glabella to subnasal), and lower face (subnasal to menton) were measured to obtain the horizontal one-third of the facial parts. Differences between the non-chin-injected and chin-injected groups were analyzed using one-way analysis of variance and Kruskal-Wallis tests. SPSS software version 22.0 (IBM Corp., USA) was used for the statistical analysis.

RESULTS

Thirty females were enrolled in the study, with a mean age of 46.6 (9.01) years. Table 1 shows the demographics of the participants, and Table 2 shows the morphometric data of the nasal siliconomas.

Of the 30 patients, 15 had a low nasal dorsum before the injection, 12 had a low nasal root width, 2 had a round or broad nasal tip, and 1 had broad alae. Eleven participants desired a higher nasal dorsum, and eight wanted to improve their appearance. When asked about the liquid material injected into their noses, 19 participants mentioned "collagen,"

Figure 1. Photograph of the patient with nose siliconoma taken with mirror stand

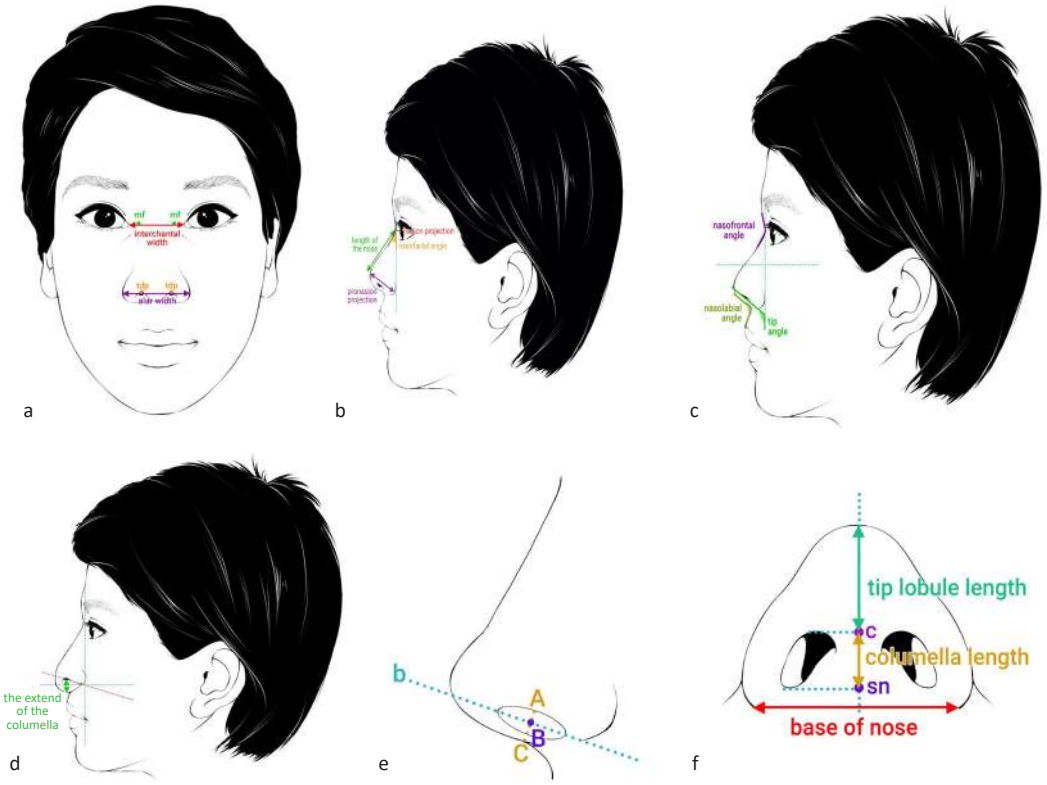

Figure 2. Measurements based on one-third division of the face. (a) frontal; mf–mf was nasal root width, and TDP–TDP was tip-defining points width, (b–e) left lateral; point B to point C was the distance between the long axis of the nostril and the columellar edge. Point A to point B was the distance from the long axis to the superior nostril rim. Point B was the intersection of line b and line AC. Line b was drawn through the most anterior and posterior portions of the oval-shaped nostril, and (f) basal views. mf=maxillofrontal; TDP=tip-defining points; c=highest point of the columella; sn=subnasal

10 mentioned "liquid silicone", and one mentioned "vitamin". However, the terms used by the participants may not reflect the actual materials used, as the nonprofessional injectors explained the terms to them based on their own understanding. Likely, the liquid used was silicone.

There were significant differences between the horizontal facial thirds of the participants. The upper part of the face was significantly different from the middle and lower parts. Additionally, significant differences were observed between the middle and lower parts in the non-chin-injected group. However,

Table 1. Demography of the participants

Variables	n (%) (N = 30)
Education	
Elementary school	18 (60)
Junior high school	4 (13)
Senior high school	8 (27)
Marriage status	
Single	2 (7)
Married	28 (93)
Social reason for taking injection	
Lured by provider	7 (23)
Lured by friend	17 (57)
Lured by family	3 (10)
Lifestyle	3 (10)
Provider of injection	
Beautician in beauty salon	2 (7)
Allied health worker	2 (7)
Individual door-to-door salesperson	26 (87)

Table 2. Morphometrics of nasal siliconoma in Asians

Parameters	Nasal siliconoma, mean (SD)	Normal nose, mean (SD)
Intercanthal width (cm)	3.33 (0.25)	3.56 (0.27)[6]
Nasal root width (cm)	2.70 (0.30)	1.9 (0.2)[8]
Alar width (cm)	4.48 (0.31)	4.14 (0.28)[6]
Two TDP width (cm)	2.09 (0.22)	2.09 (0.22)[6]
Nasofrontal angle (°)	141.10 (8.40)	134.6 (7.3)[6]
Length of the nose (cm)	3.10 (0.48)	4 (0.21)[6]
Nasofacial angle (°)	32.94 (4.51)	36.3 (4.3)[6]
Nasion projection (cm)	0.64 (0.36)	0.43 (0.22)[6]
Pronasion projection (cm)	2.00 (0.25–2.46)	2.29 (0.26)[6]
Tip angle (°)	122.7 (4.52)	111.5 (4.4)[6]
Nasolabial angle (°)	78.81 (15.93)	90.4 (8.3)[6]
The extend of extended columella (cm)	0.47 (0.31)	-
Base of the nose width (cm)	3.98 (0.25)	3.69 (0.29)[6]
Columella length (cm)*	0.64 (0.20)	0.78 (0.15)[8]
Tip lobular portion length	1.12 (0.20)	-

SD=standard deviation; TDP=tip-defining points
*There are 20 data available; [6]Prasetyono TOH, Karina. Morphometry of Deutero Malay female nose. Med J Indones. 2009;18(2):120–3; [8]Leong SC, White PS. A comparison of aesthetic proportions between the Oriental and Caucasian nose. Clin Otolaryngol Allied Sci. 2004;29(6):672–6

there was no significant difference between the middle and lower parts in the chin-injected group. The measurements of the horizontal thirds of the faces of the participants are presented in Table 3.

The mean (SD) distance between the long axis of the nostril and the columellar edge was 0.36 (0.22) cm; and 0.14 (0.13) cm from the long axis to the superior nostril rim on the lateral view. On the basal view, the ratio of the columella to the lobular portion of the nose was 1:1.75, with a mean (SD) lobular portion length of 1.12 (0.20) cm and columella length of 0.64 (0.20) cm. Figure 3 illustrates the characteristics of Asian females with nasal siliconoma based on the data above.

Long-standing inflammation was defined as persistent skin redness. Only two of the 30 participants had long-standing inflammation, with no signs of infection.

In terms of anatomical regions, most participants (60%) received injections only in the nasal region, whereas the remaining received injections in other areas such as the chin, lower lip, eyelid, temple, and forehead. After the injection, 19 participants had a higher nasal root, while 11 had a higher nasal dorsum. None of the participants underwent any procedure to remove the injected material.

When asked about their aesthetic perception of their noses, 25 participants rated it as average, four rated as poor, and one as good. Most participants (20) were unaware of the available reconstructive techniques, whereas 10 were aware of several open techniques involving autografts and/or implants. In terms of expectations for future nose reconstruction, 22 (73%) participants expected a higher nasal root, followed by 4 (13%) participants who wished a higher nasal dorsum and a slimmer nasal tip at the same number of responses.

DISCUSSION

The normal ranges of morphometry for the Asian nose are represented by the Oriental[8,9] and Deutero-Malay[6] populations. This study showed that nasal siliconoma is characterized by a wider nasal root width, alar width, nasion projection, nasofrontal angle, and tip angle, as well as more acute nasofacial and nasolabial angles, compared with a normal Asian nose. Meanwhile, the intercanthal width and nasofacial angles were similar to what is obtained in a normal nose.

Table 3. Lengths of horizontal facial one-third of the participants

Horizontal one-third of the face	Non-chin-injected group			Chin-injected group		
	Mean (cm) (n = 21)*	Post-hoc multiple comparison Bonferroni	*p*	Mean (cm) (n = 9)	Post-hoc multiple comparison	*p*
Upper face	5.77 (0.59)	Upper vs. middle	<0.001	5.44 (0.49)†	Upper vs. middle	<0.001
Middle face	6.50 (0.52)	Upper vs. lower	<0.001	6.87 (6.34–8.28)‡	Upper vs. lower	<0.001
Lower face	6.96 (0.56)	Middle vs. lower	0.030	6.89 (0.49)	Middle vs. lower	>0.99

*Analysis of variance (ANOVA) before post-hoc multiple comparison Bonferroni showing $p<0.001$; †Kruskal-Wallis test before post-hoc multiple comparison showing $p<0.001$; ‡mean (range)

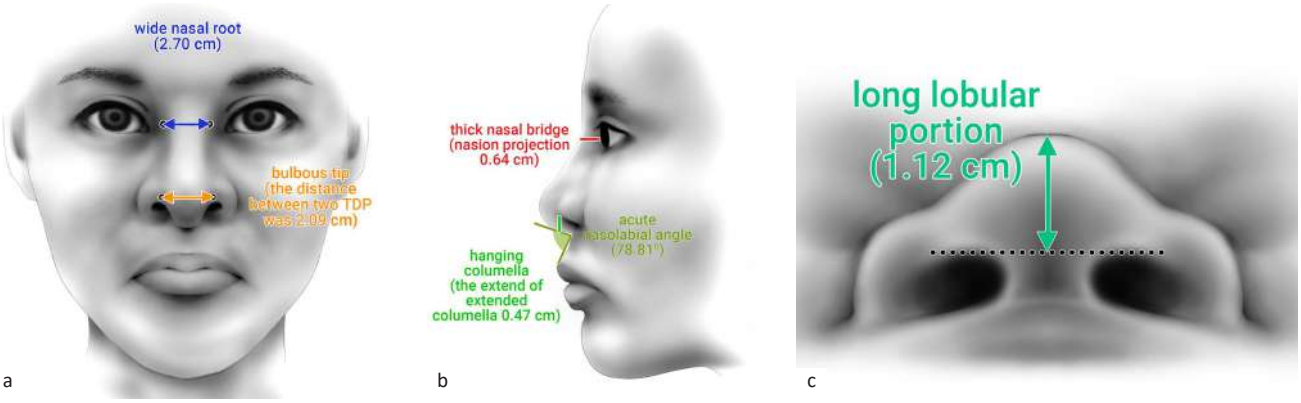

Figure 3. Schematic drawing in the (a) frontal,* (b) lateral,† and (c) basal views‡ of the average siliconoma nose in Asian females. *Siliconoma nose had a wide nasal root (2.70 [0.30] cm) and two TDP distance of 2.09 (0.22) cm; †the nasion projection was 0.64 (0.36) cm; nasolabial angle was 78.81 (15.93)°; the extend of extended columella was 0.47 (0.31) cm; ‡the lobular portion of the tip was 1.12 (0.20 cm). TDP= tip-defining points

This study showed that a face with nasal siliconoma did not fit the neoclassical facial proportion.[10] Distortion of the nose was demonstrated by significant elongation of the middle face compared to the upper face. This was because the subnasal point interfered with the hanging columella. Interestingly, the lower face was significantly longer than the upper face, even in participants who did not receive chin injections, creating an unnatural appearance.

Compared with to the normal Oriental nose, the nasal root of the nasal siliconoma was 1.42 times wider, and the two TDP were 0.6 cm wider, creating a more bulbous appearance.[11] The nasion projection or nasal bridge of nasal siliconoma was also 0.21 cm thicker than that of the Asian Deutero-Malay nose[6] and 0.14 thicker than the Oriental nose.

The distance between the long axis of the nostril and the columellar edge in nasal siliconoma was 0.36 cm, and the distance from the long axis to the superior nostril rim was 0.14 cm. Based on the classification by Gunter et al,[12] this study showed a true hanging columella in the alar-columellar relationship of nasal siliconomas. As a result of this hanging columella, it is not surprising that the nasolabial angle in nasal siliconomas is more acute than the Asian norm.

From the basal view, the ratio of the columella to the lobular portion of the nose was 1:1.75, with the lobular portion being larger than the columella portion. Unfortunately, there was no reference for this ratio. Among the normal Western population, the ratio is approximately 2:1.[13]

Most participants had an "average" aesthetic perception of their nose, although they knew that their nose differed from the norm. This response may be related to their subjective coping mechanisms for the distortions. Interestingly, most participants with nasal siliconoma demanded a higher nasal root than what was believed to be the higher nasal dorsum.

This study had limitations, including a small sample size and the subject population being from the middle and lower social classes. The invitation for the study did not draw the interest of people from a higher social class who might have different aesthetic perceptions and expectations for their future nose appearance after surgical correction. Moreover, this study had no controls, and it would be better to conduct a case-control study in the future. Nevertheless, there was no comparison between the original features and the features after injection to provide objective data on nasal siliconoma. This study is expected to aid in appreciating reconstructive surgery by referring to the characteristics of the disfigurements identified in this study. Additionally, the questionnaire used in this study was developed without validation.

In conclusion, nasal siliconomas in Asian females were characterized by a wider nasal root and greater width of the two TDP, creating a more bulbous appearance, a thickened nasal bridge, and a hanging columella with a longer lobular portion of the tip.

Conflict of Interest

Theddeus Octavianus Hari Prasetyono is the editorial board member but was not involved in the review or decision making process of the article.

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Clinical Research

Factors associated with the uncorrectable congenital heart disease in children with pulmonary arterial hypertension

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ABSTRACT

BACKGROUND Pulmonary arterial hypertension associated with congenital heart disease (PAH-CHD) is a common complication of uncorrected left-to-right shunt defects in acyanotic CHD and a frequent type of pulmonary hypertension in youth. The standards for operability in left-to-right shunts with increased pulmonary vascular resistance are not universally agreed upon. This study aimed to identify variables associated with uncorrectable lesion in children with PAH-CHD.

METHODS This retrospective study used a database of all children who underwent cardiac catheterization at Sanglah Hospital, Bali, from May 2009 to April 2021. Pulmonary hypertension was defined as pulmonary artery pressure of >25 mmHg, while correctability was a fall of >20% in the pulmonary arterial resistance index (PARI) with final value of <6 WU/m^2 when doing an acute vasoreactivity test using 100% oxygen. The analyses were carried out using SPSS software version 22.0 (IBM Corp., USA).

RESULTS A total of 104 children were included. Cardiac catheterization showed that the uncorrectable group had a higher PARI (14.4 [8.88] WU/m^2 *versus* 8.43 [3.85] WU/m^2) and lower flow ratio (1.27 [0.83] *versus* 1.47 [0.77]) at baseline. In terms of correctability, pre-tricuspid lesions (OR = 0.05; 95% CI = 0.01–0.47; p = 0.01) and younger age group (OR = 0.32; 95% CI = 0.12–0.85; p = 0.01) were protective variables, whilst high baseline PARI (OR = 4.54; 95% CI = 1.64–12.57; p = 0.01) was unfavorable.

CONCLUSIONS High baseline PARI was the most significant variable in predicting uncorrectable left-to-right shunt defects in PAH-CHD.

KEYWORDS children, congenital heart disease, heart septal defects, pulmonary hypertension

Patients with uncorrected congenital heart disease (CHD) are more likely to develop pulmonary arterial hypertension (PAH), which is diagnosed as pulmonary arterial hypertension associated with congenital heart disease (PAH-CHD) and typically results from a significant systemic-to-pulmonary shunt that is present from infancy.[1] The remodeling of the pulmonary vascular system leads to increased pulmonary arterial pressure (PAPs). PAH-CHD accounts for over 40% of all PAH cases and is the second most prevalent form among children. This form is also prevalent in developing and impoverished regions.[2] PAH-CHD has a significant negative impact on health-related quality of life across several physical and mental domains, including physical functioning, symptoms at rest and during physical activity, and social functioning. Other impacts of PAH-CHD are adverse effects from medication, uncertainty about prognosis, anxiety, and depression in patients and caregivers.[3]

Patients with uncorrected PAH-CHD may not have undergone corrective surgery or transcatheter device closure. Moreover, late repair of CHD with

intra- or extracardiac shunt is an important etiology of pulmonary hypertension. Therefore, prompt repair of such pathology is crucial since, in most cases, early shunt closure can halt the progression of pulmonary hypertension in the future.[4] However, procedures to fix the defect might increase morbidity in certain circumstances.

Cardiac catheterization is required to plan a surgical repair when an intervention to close a cardiac defect during catheterization is impossible. Cardiac catheterization can capture information such as pressure measurements, blood oxygen saturation, estimated cardiac output, and pulmonary vascular resistance (PVR), as well as angiographic images using fluoroscopic imaging during contrast injection. The integrated cardiac center at Sanglah Hospital, Bali, Indonesia, a tertiary healthcare facility that receives regional referrals from Bali, Nusa Tenggara, and some parts of the East Java province, has performed cardiac catheterizations and cardiac surgeries since 2009. In Sanglah Hospital, correctability in CHD is defined during an acute vasoreactivity test (AVT) using 100% oxygen.[5] However, there is currently no consensus on the criteria for correctability in left-to-right shunts with elevated PVR. The practical definition of correctability may also vary between centers. Therefore, this study aimed to determine the variables predicting uncorrectable lesion in children with PAH-CHD.

METHODS

This retrospective study used a database comprising the medical data of all children who underwent cardiac catheterization at Sanglah Hospital, Bali, Indonesia, from May 2009 to April 2021. Variables regarding age, sex, anthropometric measurements, related to clinical condition and hemoglobin (Hb) level, as well as anatomy and hemodynamic status from cardiac catheterization of all children aged 3 months and above were extracted from the database. The Research Ethics Committee of the Faculty of Medicine Universitas Udayana, Bali, Indonesia approved this study (Approval No: 2230/UN14.2.2.VII.14/LT/2021). The need for informed consent was waived by the ethics committee.

During cardiac catheterization, anatomical defects were identified, and intracardiac pressures were measured before and after an AVT using 100% oxygen. Defect size was classified according to the type of defect. Ventricular septal defects (VSDs) were considered small if they had a diameter <5 mm, moderate VSD had a diameter ≥5 and <10 mm; while large VSD had a diameter ≥10 mm.[6] Small atrial septal defects (ASDs) in infancy had a diameter >3 mm to ≤5 mm, moderate defects measured >5 mm to 9 mm, and large defects were ≥10 mm. After infancy, the ASD size was interpreted as follows: <10 mm as small, 10–20 mm as moderate, and >20 mm as large.[7] Patent ductus arteriosus (PDA) was considered small at 1.5 to 3 mm, moderate between 3 and 5 mm, and large if it exceeded 5 mm.[8] Mean pulmonary arterial pressure (mPAP) was the pressure in the pulmonary artery measured during a catheterization procedure. Mean systemic arterial pressure was defined as the aortic pressure measured during the catheterization procedure. Pulmonary arterial resistance index (PARI) was defined as the absolute value of pulmonary artery resistance against the patient's body surface area, representing body size's effect on blood flow. The flow ratio was the total pulmonary blood flow to the total systemic blood flow. Pulmonary hypertension was defined as PAP greater than 25 mmHg, as determined by cardiac catheterization. Correctability as a dependent variable was defined as a fall in the PARI of >20% with final values of <6 WU/m^2 during the AVT using 100% oxygen. These values were the basis for classifying PAH-CHD as correctable or uncorrectable.

Data were analyzed using SPSS software for Windows version 22.0 (IBM Corp., USA). Descriptive data are summarized as means and percentages. The data in this study were not normally distributed. For the univariate analysis, the chi-square test was used to analyze the association between independent and dependent variables. Post-hoc analysis using the logistic regression test was conducted to determine the most significant predictors of uncorrectable PAH-CHD. The results are reported as odds ratios (ORs) and 95% confidence intervals. *p*-values lower than 0.05 were deemed statistically significant.

RESULTS

A total of 104 pediatric patients with PAH-CHD identified during cardiac catheterization were included in this study. They were divided into three age groups: infants (3–11 months 29 days old), children (1–12 years 11 months old), and adolescents (13–18 years old). Most of the participants in the uncorrectable group were

Table 1. Variables associated with uncorrectable CHD in children with PAH (N = 104)

Variables	Uncorrectable (N = 26)	Correctable (N = 78)	Univariate analysis*		Multivariate analysis†	
			OR (95% CI)	*p*	OR (95% CI)	*p*
Age (months), n, mean (SD)			-	-	-	-
Infants	11, 6.91 (3.21)	15, 6.4 (2.41)				
Children	9, 81.1 (43.89)	56, 52.7 (41.89)				
Adolescents	6, 187.33 (23.56)	7, 189.86 (24.59)				
Infant, n (%)	11 (42)	15 (19)	0.32 (0.12–0.85)	**0.01**‡	0.25 (0.07–0.89)	**0.03**‡
Male sex, n (%)	9 (35)	31 (40)	1.25 (0.49–3.15)	0.64	1.39 (0.42–4.61)	0.58
Height (cm), mean (SD)			-	-	-	-
Infants	61.18 (8.39)	57.8 (4.83)				
Children	105.1 (18.16)	93.52 (23.16)				
Adolescents	156.5 (3.73)	148.57 (12.83)				
Weight (kg), mean (SD)			-	-	-	-
Infants	5.09 (1.55)	4.88 (0.96)				
Children	17.02 (6.98)	13.04 (8.25)				
Adolescents	41.58 (6.89)	38.86 (8.91)				
Pneumonia, n (%)	6 (23)	8 (10)	-	-	-	-
Clinical Down syndrome, n (%)	4 (15)	8 (10)	1.59 (0.44–5.79)	0.48	1.14 (0.21–6.29)	0.88
Hb (g/dl), mean (SD)	13.79 (2.07)	12.27 (1.61)				
Defect types						
Simple defect, n				**0.01**‡		**0.01**‡
Pre-tricuspid shunt	5	1	0.05 (0.01–0.47)		0.04 (0.0–0.41)	
Post-tricuspid shunt	19	74	1.00		1.00	
Combined defects, n			-	-	-	-
VSD + PDA	2	3				
Defect size, n				0.28		0.35
Small to moderate	11	24	0.61 (0.24–1.5)		0.55 (0.16–1.9)	
Large	15	54	1.00		1.00	
Hemodynamic status at baseline, mean (SD)			-	-	-	-
mPAP (mmHg)	47.92 (14.69)	46.1 (14.34)				
mSAP (mmHg)	64.44 (14.73)	66.58 (12.81)				
PARI (WU/m^2)	14.4 (8.88)	8.43 (3.85)				
FR	1.27 (0.83)	1.47 (0.77)				
PARI of >8, n (%)	20 (77)	33 (42)	4.54 (1.64–12.57)	**0.01**‡	3.76 (1.08–13.14)	**0.04**‡
FR of <1.5, n (%)	20 (77)	49 (63)	1.97 (0.71–5.48)	0.19	0.79 (0.21–2.96)	0.72
Hemodynamic status after AVT, mean (SD)			-	-	-	-
PARI, WU/m^2	8.95 (5.67)	1.69 (1.09)				
FR	1.67 (1.23)	6.27 (9.29)				

AVT=acute vasoreactivity test; CHD=congenital heart disease; CI=confidence interval; FR=flow ratio; Hb=hemoglobin; mPAP=mean pulmonary arterial pressure; mSAP=mean systemic arterial pressure; PAH=pulmonary arterial hypertension; PARI=pulmonary arterial resistance index; OR=odds ratio; PDA=patent ductus arteriosus; SD=standard deviation; VSD=ventricular septal defect

*Chi-square test; †logistic regression test; ‡significant if $p<0.05$

infants (42%), and most in the correctable group were children (72%). Both groups had a female predominance with a male-to-female ratio of 1:1.8. The uncorrectable group had a higher proportion of patients with Down syndrome and pneumonia and higher Hb levels. From the baseline pressure measurement during cardiac catheterization, the correctable group had lower mPAP, lower PARI, and higher flow ratio (Table 1).

The most frequent anatomical cardiovascular disorders were VSD (48 of 102) and PDA (42 of 102), with the most frequent defect size being large (69 of 102). Hemodynamic status was measured before and after the AVT using 100% oxygen. A baseline PARI of >8 WU/m^2 was found in 42% of patients in the correctable group (33 of 78) compared with 77% in the uncorrectable group (20 of 26).

Variables hypothesized to correlate with the uncorrectable PAH-CHD were analyzed. Post-hoc analysis showed that a type of CHD, age group, and baseline PARI were independent variables associated with uncorrectable defects. Furthermore, a baseline PARI of >8 WU/m^2 had the highest OR for predicting uncorrectable defects in children with PAH-CHD (Table 1).

DISCUSSION

This study found that age at presentation were correlated with the correctability of pulmonary hypertension. Infants had more severe pulmonary hypertension than older children. The most common defect in the uncorrectable group was a large PDA, in which Eisenmenger's physiology usually develops in early infancy. These results are similar to those of a study by Chinawa et al[9] who reported that infants with PDA were more likely to have pulmonary hypertension than older children.

The female predominance in the present study is similar to that in a study by the Tracking Outcomes and Practice in Pediatric Pulmonary Hypertension registry, which involved 456 children aged 3 months to 18 years. Females were more prevalent in populations with pulmonary hypertension due to any cause (59%) and in those with PAH-CHD (62%).[10]

Pulmonary hypertension is a complication of CHD in individuals with Down syndrome. According to Bush et al,[11] children with Down syndrome and CHD are 5 times more likely to be diagnosed with pulmonary hypertension than those with Down syndrome only. They also have a higher prevalence of pulmonary hypertension than patients with non-syndromic CHD.[12] Various studies have reported that patients with Down syndrome and CHD tended to develop pulmonary hypertension faster than patients without Down syndrome. The pathophysiology of Down syndrome is thought to be influenced by genetic expression, which leads to poor lung vascular development and signaling.[13] In this study, the uncorrectable group, which was associated with a more severe form of pulmonary hypertension, had a higher proportion of patients with Down syndrome.

Children with CHD are prone to recurrent pneumonia and are more likely to develop severe pneumonia, including respiratory failure. In this study, pneumonia was more common in the uncorrectable group; however, a single-center retrospective cohort study by Inrianto et al[14] reported that pneumonia was not associated with pulmonary hypertension.

Hb, a protein in red blood cells, contains iron and is responsible for carrying oxygen. In this study, the uncorrectable group had a higher Hb level. This result is consistent with a Taiwanese cohort study that found that children with Eisenmenger syndrome who had high Hb levels also had worse outcomes.[15]

In this study, the most common cardiac defects identified in patients with pulmonary hypertension were VSD and PDA, with mostly large defect sizes. The blood in each ventricle of a person with a VSD has two alternative systolic routes: it can exit through the normal ventricular outflow tract or escape through the VSD and exit through the other ventricular outflow tract. The volume and direction of systolic flow over the defect are both determined by the relative resistance of each conduit. The sum of resistance from the left ventricle to the pulmonary artery, for instance, is very low compared with the resistance of flow to the systemic circulation, resulting in a large left-to-right systolic flow across the defect in the presence of normal PVR and a large, nonrestrictive VSD. Small VSDs have higher resistance at the defect site, restricting the left-to-right shunt.[16]

The clinical histories of VSD and PDA are identical. The patient mostly shows signs of congestive heart failure in infancy if the PDA is large. If the abnormality is not fixed, this condition frequently advances into Eisenmenger's physiology. At the PDA, a right-to-left shunt forms as PVR increases.[17] The PDA cannot be closed because of the same physiological reasons as

those because of which patients with Eisenmenger syndrome cannot tolerate VSD repair.

From the baseline pressure measurement, the correctable group had lower mPAP, lower PARI, and higher flow ratio. These findings are consistent with the pathophysiology of shunt through a defect in CHD. The left ventricular volume and pressure spread to the pulmonary vascular bed in patients with moderate-to-large defects. If the problem is not treated, Eisenmenger's physiology develops. In this process, the medial layer of the pulmonary arterioles hypertrophies in response to the stimulus of the left ventricle's volume and pressure. PVR starts to increase as pulmonary artery lumens are reduced. The tiny vessels eventually become utterly obliterated as the hypertrophy advances. Resistant levels in the lungs increase until they surpass systemic resistance. At this point, the patient's blood flow is reversed into a right-to-left shunt, which makes the patient cyanotic. Since this process is irreversible, the severity of the clinical symptoms corresponds with the PAP readings. Any procedure performed to repair the defect at this point would result in abrupt right ventricle failure, venous stasis, reduced cardiac output, and lower life expectancy than if left open.

PAP, shunting, and AVT are performed during cardiac catheterization to assess the impact of short-acting pulmonary vasodilators on PVR. Although the AVT remains the gold standard for diagnosing and prognosticating pulmonary hypertension,[18,19] no standardized guideline for the pediatric population with pulmonary hypertension has been established, and referral facilities typically follow different practices.[20] The differences include the vasodilators (nitric oxide ± oxygen *versus* inhaled iloprost *versus* other orally or intravenously administered substances [sildenafil and treprostinil]),[21–26] definition of a positive AVT response, and mode of anesthesia (general anesthesia with mechanical ventilation *versus* local anesthesia). These differences make it challenging to compare test results, and they may lead to misunderstandings and, eventually, incorrect decisions concerning the most appropriate intervention.[27]

The definition of a positive acute vasodilator test remains controversial. In children with PAH-CHD, an acute vasodilator test is used to assess operability. A favorable response to the acute vasodilator test should be defined as a >20% decrease in PARI with a final value of <6 WU/m^2.[28,29] Nevertheless, surgical safety cannot be ensured (the gray zone is defined as a PARI of 6–8 WU/m^2). In this study, both groups showed a decrease of >20% in PARI after the acute vasodilator test, but the PARI in the uncorrectable group remained >6 WU/m^2. This result is consistent with a diagnostic study by Day,[30] who found that a PARI of <8 WU/m^2 had a sensitivity of 100% and specificity of 35% in predicting correctability, with a 46% accuracy. Adding 100% oxygen to the acute vasodilator test increases specificity to 88% and accuracy to 88%.[30] A cohort study by Jarutach et al[31] of patients with completely repaired CHD found a decreased 10-year survival rate from 98.6 ± 0.8% in patients with a PARI of <4 WU/m^2 to 76.5 ± 11.2% in patients with a PARI of >8 WU/m^2.

Age at which the surgery is performed, in addition to the type or size of the lesion and concomitant syndromes, is one of the most crucial variables for long-term survival in a child with PAH-CHD. The American Heart Association and American Thoracic Society recommend cardiac catheterization to evaluate the PARI and flow ratio if the repair had not been performed by the age of 2 years.[2] In that case, repair should be considered if the PARI is <6 WU/m^2 or the AVT reveals reversibility when the PARI is ≥6 WU/m^2. However, the European Pediatric Pulmonary Vascular Disease Network suggested that there is a gray zone group whose PARI is between 6 and 8 WU/m^2. In this group, individual patient evaluation is necessary, and clinical subjectivity is inevitable.[29] In contrast, Saudi guidelines suggested repair when the PARI is between 4 and 8 WU/m^2.[32] Despite the variation, all guidelines do not recommend repair if the PARI value exceeds 8 WU/m^2. Measurement of PARI is emphasized as an important predictor to determine correctability in children with PAH-CHD.

This study had several limitations. This was a single-center study in which the patient's sociodemographic characteristics might differ from those in other centers. There was also a difference in sample size and proportion between groups, which might affect the results. Moreover, the patients were not observed following the cardiac catheterization procedure. A multicenter cohort study is needed to confirm the results of this study.

In conclusion, pre-tricuspid lesions, young age, and high baseline PARI were significant predictors of uncorrectable defects in children with PAH-CHD. A baseline PARI of >8 WU/m^2 had the highest OR as the

predictor of uncorrectable defects in children with PAH-CHD. This study not only provides the clinical relevance of PARI measurement in assessing operability, but also additionally supplies clinical data regarding children with PAH-CHD in Indonesia.

Conflict of interest

The authors affirm no conflict of interest in this study.

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Clinical Research

Characteristics of neurogenic lower urinary tract dysfunction patients at Cipto Mangunkusumo Hospital

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ABSTRACT

BACKGROUND Neurogenic lower urinary tract dysfunction (NLUTD) is an abnormal function of the bladder, urethra (and/or prostate in males) in patients with a clinically confirmed relevant neurologic disorder. Hence, accurate diagnosis and management of NLUTD is crucial. This study aimed to recognize the characteristics of NLUTD to identify, manage, and prevent the associated complications.

METHODS This retrospective study was conducted at the Outpatient Clinic of the Department of Urology, Cipto Mangunkusumo Hospital, Jakarta, Indonesia, from January 2011 to December 2021. The study analyzed data collected from voiding dysfunction patients with upper motor neurological disorders who underwent urodynamic studies during the study period. Incomplete data in the medical records were excluded.

RESULTS Mean age of the participants was 50.7 (18–95) years old. The most common cause of NLUTD was stroke (26.6%), followed by unspecified groups and spinal cord injury. Patients under 20 years old were affected by trauma and congenital defects. Of the patients, 34.0% had urinary retention, and 18.1% had incontinence. Small bladder capacity occurred in patients with stroke, Parkinson's disease, and spinal/cerebral tumors, leading to decreased bladder compliance.

CONCLUSIONS NLUTD was associated with aging, with upper motor neurological lesions such as trauma, stroke, and spinal/cerebral injury being the most common etiologies. Most patients with NLUTD had small bladder capacity and decreased compliance based on urodynamic result.

KEYWORDS nervous system diseases, population characteristics, urinary incontinence, urinary tract infection

According to the International Continence Society (ICS), neurogenic lower urinary tract dysfunction (NLUTD) is defined as an "abnormal or difficult function of the bladder, urethra (and/or prostate in men) in mature individuals in the context of clinically confirmed relevant neurologic disorder." NLUTD can lead to urinary tract problems, such as incontinence and infection, which greatly affect a person's quality of life.[1,2] Its most common etiologies include spinal cord injury (SCI) secondary to trauma-induced spinal column fractures, vascular ischemia, and infections. The incidence of NLUTD also increases in some neurological diseases affecting children and adults, such as multiple sclerosis, Parkinson's disease, stroke, and spina bifida.[3,4]

NLUTD is commonly diagnosed based on urodynamic examinations in high-risk patients; these examinations involve invasive or non-invasive assessments of lower urinary tract parameters regarding function and dysfunction. Invasive urodynamic investigations may be challenging in

patients with neurourological problems and require the evaluation of several technical sources of artifacts. Maintaining good urodynamic recordings and interpretation based on the good urodynamic practice guidelines by ICS is critical to determine the existence of a malfunction and comprehend its clinical ramifications in such a manner. Thus, non-invasive urodynamic investigations are more suitable for diagnosing NLUTD, with repeated studies performed in a single session aiding clinical decision-making and enabling significantly improved outcomes.[3]

To improve the diagnosis and management of NLUTD, exploring the characteristics of patients with NLUTD-associated neurological disorders is essential. However, studies on NLUTD have yet to be conducted in Indonesia. Therefore, this study aimed to evaluate the prevalence of NLUTD and the characteristics of affected patients (including clinical and urodynamic parameters) at a tertiary care center in Indonesia.

METHODS

This study was conducted at Cipto Mangunkusumo Hospital, a single tertiary care center in Indonesia. The medical records of patients with NLUTD who visited the outpatient clinic of the Department of Urology were evaluated retrospectively. Individual patient data were collected and reviewed through accidental sampling between 2011 and 2021. This study was approved by the institutional review board (No: KET-328/UN2.F1/ETIK/PPM.00.02/2022). Informed consent was not needed as this study used secondary data from medical records.

All patients with voiding dysfunction who had undergone a urodynamic study between January 2011 and December 2021 and had upper motor neurological disorders were included without any restrictions on age, sex, and other demographics. Patients with incomplete medical records were excluded. We assessed the patients' demographic data (age and sex), chief complaints, diagnoses of neurological disorders, and urodynamic findings obtained at the onset of neurological disease.

The symptom because of which the patient visited the urologist was considered the chief complaint and was evaluated based on storage and voiding symptoms. Neurological disorders were classified as cerebrovascular accidents, tumors, spinal cord surgery, SCI, Parkinson's disease, or spina bifida. Bowel problems were evaluated based on fecal incontinence or defecation problems, and male patients were assessed for sexual dysfunction (erectile dysfunction [ED] and retrograde ejaculation).

Urodynamic findings were obtained during the filling and voiding phases. Patients were evaluated for bladder capacity, bladder compliance, urodynamic stress incontinence (USI), detrusor overactivity (DO), and detrusor overactivity incontinence (DOI) during the filling phase, and for bladder outlet obstruction index (BOOI), detrusor underactivity (DU), acontractile bladder, and detrusor sphincter dyssynergia (DSD) during the voiding phase. Bladder capacity was categorized as small (<250 ml), normal (250–500 ml), or large (>500 ml).

Bladder compliance was determined by dividing the change in volume by the change in detrusor pressure (Pdet); it was classified as either normal (Pdet: 12.5–40 ml/cmH_2O) or low (end-filling Pdet ≥17.5 cmH_2O). Increased Pdet and poor urine flow during the voiding phase were considered indicative of BOO. In men, the BOOI was calculated using the following formula: BOOI = PdetQmax–2Qmax. BOOIs of <20, 20–40, and >40 indicated no obstruction, equivocal obstruction, and infravesical obstruction, respectively.[4] In women, BOO was diagnosed if the maximal flow rate (Qmax) was <12 ml/sec and the Pdet at Qmax (PdetQmax) was >20 cmH_2O.[5]

The bladder contractility index (BCI) was calculated to diagnose DU using the following formula: BCI = PdetQmax + 5Qmax. For both men and women, DU was defined by a BCI of <100, Qmax of 12 ml/sec, and PdetQmax of 10 cmH_2O.[6] An acontractile detrusor was indicated by undetectable bladder contraction during voiding.[7] DSD was referred to BOO caused by simultaneous detrusor muscle contraction and involuntary urethral sphincter activation.

Categorical data are presented as absolute values and percentages. Normally distributed data are presented as means and standard deviations, whereas non-normally distributed data are presented as medians and ranges.

RESULTS

A total of 1,351 patients underwent urodynamic examinations at the study center between January 2011 and December 2021. Among these, 35.1% had an underlying neurological disease, and 48.5% had already

Table 1. Characteristics of the patients

Variables	n (%) (N = 474)
Male sex	269 (56.8)
Bowel problems	193 (40.7)
Male sexual dysfunction	102 (37.9)
Duration of urological complaints (>1 years)	230 (48.5)
Age (years)	
≤20	74 (15.6)
21–30	62 (13.1)
31–40	50 (10.5)
41–50	45 (9.5)
51–60	65 (13.7)
>60	178 (37.6)
Neurological disease (n = 570)*	
Cerebrovascular accident	131 (26.6)
SCI	84 (17.0)
DM, n = 474	77 (16.2)
Spinal cord surgery	77 (15.6)
Medulla spinalis/cerebral tumor	33 (6.7)
HNP	18 (3.7)
Congenital disease	15 (3.2)
Parkinson's disease	10 (2.0)
Infection	10 (2.0)
Autoimmune disease	5 (1.0)
Others†	110 (22.4)
Complaints	
LUTS	182 (38.4)
Frequency	67 (14.1)
Hesitancy	31 (6.5)
Urgency	20 (4.2)
Incomplete emptying	18 (3.8)
Weak stream	17 (3.6)
Nocturia	12 (2.5)
Straining	9 (1.9)
Dribbling	8 (1.7)
Urinary retention	161 (34.0)
Urinary incontinence	86 (18.1)
Dysuria	10 (2.1)
Others†	35 (7.4)

DM=diabetes mellitus; LUTS=lower urinary tract symptoms; HNP=herniated nucleus pulposus; SCI=spinal cord injury
*Some patients had multiple diseases; †others were hypertension, low back pain, and history of seizure

experienced urological problems for over a year. The patient characteristics were relatively balanced between the sexes, with more males included in the present study. In the entire cohort, the proportion of patients aged >60 years was the highest, followed by that of patients aged ≤20 years, with the mean age of 50.7 (19.5) years (Table 1). Furthermore, 25%, 6%, and 4% of the patients had a history of alpha-blocker usage, electrical stimulation, and bladder training, respectively; these treatments were discontinued 1 week before the urodynamic examination.

The most common neurological disease was stroke (26.6%), followed by other diseases presented as "others" in Table 1. The most common symptom was LUTS (38.4%), followed by urinary retention (34.0%). Most patients with stroke had a small bladder capacity but normal bladder compliance. Only a few patients had DO, DOI, or USI (Table 2).

DISCUSSION

This study found that up to 35.1% of the patients with neurological diseases had NLUTD, emphasizing the importance of NLUTD screening in this patient population. The prevalence of NLUTD increases with age; while trauma, stroke, and spinal/cerebral injuries are the most common etiologies of upper motor neurological lesions. NLUTD has a relatively high prevalence and affects both men and women equally, as indicated by the present study. However, Hamid et al[8] reported a higher prevalence of NLUTD in women than in men, which could be because women are more likely to seek medical help than men.

To make therapeutic decisions and to effectively tailor the management of NLUTD, invasive urodynamic procedures are essential for identifying changes in bladder function; urodynamic findings may vary based on the culprit lesion location. A previous study found that after stroke, DO with unrestrained bladder contraction was the most common urodynamic finding (68–90%).[9] Conversely, the present study found DU to be the most common urodynamic finding based on the weak BCI results, suggesting the need for further investigation into lesion location and other underlying comorbidities to further understand the etiology of low bladder contractility, hence enabling early management. Lee et al[10] found that in patients with chronic pontine stroke, there was a greater frequency of DU, a lower

Table 2. Urodynamic findings according to clinical diagnosis (N = 570)

Urodynamic findings	Neurological diseases										
	Stroke, n (%) (N = 131)	Medulla spinalis/ cerebral umor, n (%) (N = 33)	SCI, n (%) (N = 84)	Parkinson's, n (%) (N = 10)	Spinal cord surgery, n (%) (N = 77)	HNP, n (%) (N = 18)	Congenital, n (%) (N = 15)	Infection, n (%) (N = 10)	Autoimmune, n (%) (N = 5)	DM, n (%) (N = 77)	Not otherwise specified, n (%) (N = 110)
Bladder capacity (ml)											
Small (<250)	64 (48.9)	17 (51.5)	40 (47.6)	6 (60.0)	37 (48.0)	11 (61.1)	10 (66.7)	4 (40.0)	2 (40.0)	33 (42.9)	62 (56.4)
Normal (250–500)	52 (39.7)	10 (30.3)	35 (41.7)	3 (30.0)	30 (39.0)	6 (33.3)	5 (33.3)	6 (60.0)	3 (60.0)	35 (45.4)	43 (39.1)
Large (>500)	15 (11.4)	6 (18.2)	9 (10.7)	1 (10.0)	10 (13.0)	1 (5.6)	0	0	0	9 (11.7)	5 (4.5)
Compliance											
Normal	55 (42.0)	5 (15.2)	25 (29.8)	3 (30.0)	22 (28.6)	0	0	0	0	26 (33.8)	37 (33.6)
Low	32 (24.0)	20 (60.6)	27 (32.1)	5 (50.0)	39 (50.6)	9 (50.0)	11 (73.3)	1 (10.0)	1 (20.0)	22 (28.6)	42 (38.2)
Increased	44 (34.6)	8 (24.2)	32 (38.1)	2 (20.0)	16 (20.8)	9 (50.0)	4 (26.7)	9 (90.0)	4 (80.0)	29 (37.7)	31 (28.2)
USI	4 (3.1)	5 (15.2)	10 (11.9)	1 (10.0)	10 (13.0)	0	4 (26.7)	1 (10.0)	0	4 (5.2)	9 (8.2)
DO	19 (14.5)	13 (39.4)	22 (26.2)	1 (10.0)	24 (31.2)	4 (22.2)	8 (53.3)	6 (60.0)	0	14 (18.2)	26 (23.6)
DOI	9 (6.9)	5 (15.2)	13 (15.5)	1 (10.0)	15 (19.5)	1 (5.6)	5 (33.3)	1 (10.0)	0	4 (5.2)	9 (8.2)
BOOI											
Infravesical obstruction (>40)	41 (31.3)	11 (33.3)	17 (20.2)	0	17 (22.1)	6 (33.3)	10 (66.7)	2 (20.0)	1 (20.0)	22 (28.6)	34 (30.9)
Equivocally obstructed (20–40)	40 (30.5)	8 (24.2)	22 (26.2)	5 (50.0)	15 (19.5)	4 (22.2)	0	0	0	25 (32.5)	20 (18.2)
Unobstructed (<20)	50 (38.2)	14 (42.4)	45 (53.6)	5 (50.0)	45 (58.4)	8 (44.4)	5 (33.3)	8 (80.0)	4 (80.0)	30 (39.0)	56 (50.9)
BCI											
Strong (>150)	14 (10.7)	3 (9.1)	5. (6.0)	0	2 (2.6)	0	0	2 (20.0)	0	3 (3.9)	11 (10.0)
Normal (100–150)	25 (19.1)	3 (9.1)	16 (19.0)	0	9 (11.7)	7 (38.9)	2 (13.3)	3 (30.0)	4 (80.0)	8 (10.4)	25 (22.7)
Weak (<100)	92 (70.2)	27 (81.8)	63 (75.0)	10 (100.0)	66 (85.7)	11 (61.1)	13 (86.7)	5 (50.0)	1 (20.0)	66 (85.7)	74 (67.3)
DSD	2 (1.5)	0	1 (1.2)	1 (10.0)	0	0	0	0	0	3 (3.9)	4 (3.6)
Acontractile bladder	11 (8.4)	14 (42.4)	17 (20.2)	2 (20.0)	19 (24.7)	2 (11.1)	1 (9.1)	0	0	0	6 (5.5)

BCI=bladder contractility index; BOOI=bladder outlet obstruction index; DM=diabetes mellitus; DO=detrusor overactivity; DOI=detrusor overactivity incontinence; DSD=detrusor sphincter dyssynergia; HNP=herniated nucleus pulposus; SCI=spinal cord injury; USI=urodynamic stress incontinence

maximum detrusor pressure, and greater compliance, compared with patients with upper cervical SCI. Burney et al[11] further revealed that compared with patients with acute pontine infarction (lasting for <3 months), patients with chronic pontine infarction (lasting for >3 months) exhibited DU more frequently. Patients who have experienced a stroke may exhibit different urodynamic findings over time.

Based on the lesion level in patients with SCI in this study, neurogenic DO accounted for 95% of the storage abnormalities, and DU occurred in 83% of the cases. Kulaklı et al[12] found that traumatic brain injuries caused storage dysfunction (44%) and voiding dysfunction (38%). Regarding DU, it is theorized that severe neurological injury or disease disrupts efferent neuronal pathways. Notably, in this study, most patients with SCI had DU; 75.0% of the patients with SCI had a low BCI (similar to patients with stroke), with 70.2% exhibiting DU. Given that the S2–S4 segments of the sacral plexus control micturition, the urinary symptoms associated with SCI can be roughly divided into suprasacral, sacral, and infrasacral based on the level of injury. Suprasacral lesions (or upper motor neuron lesions) cause detrusor hyperactivity, leading to DO. Furthermore, individuals with lesions at or above this level may develop a neurogenic acontractile detrusor. The detrusor muscle and external sphincter show simultaneous hyperactivity in LUTD. The type of bladder dysfunction that develops after a spinal shock is determined by the level of the cord lesion.[13] Unfortunately, data on the level of spinal cord lesions were unavailable at our center.

Patients with cervical or thoracic tumors or lesions of different etiologies frequently report urinary problems, which typically develop later and rarely present as the initial symptoms. In these patients, compared with storage symptoms, voiding symptoms are more common and appear earlier.[13] In this study, 15.2% of the patients presented with acontractile bladders from all etiologies. Poor flow and residual urine volumes are closely associated with detrusor areflexia during voiding. Detrusor hyperreflexia, often caused by lesions in the inhibitory centrifugal circuit of the detrusor or by irritation of the neural pathways within the spinal cord, was observed in 28% of the patients. Low bladder compliance is commonly observed in patients with conus medullaris and cauda equina injuries and is considered a sign of parasympathetic decentralization,[14] as observed in 61.1% of patients in the current study.

Spina bifida is the most common cause of NLUTD in children and adolescents, particularly in developing countries, and can cause bladder problems[3,15] of severity depending on the type of spina bifida. A herniated nucleus pulposus (HNP) can cause bowel and bladder problems in approximately 1% of patients and is associated with a poor prognosis.[16] Bartolin et al[17] reported that voiding difficulties and acontractile detrusors were present in 26% of cases; they also reported that DU was observed in up to 83% of cases of disc problems, such as HNP. Abnormal urodynamic examination findings are common in patients with lumbar disc degeneration, with detrusor areflexia being the most frequent finding along with a high percentage of voiding complaints. Electromyography can detect anomalies in perineal floor muscle innervation. Chronic nerve injuries can lead to detrusor atrophy and bladder sensitivity.[18]

Patients with spinal tuberculosis (TB) who experience LUTD may exhibit various detrusor functions, ranging from normal urodynamic findings to an areflexic bladder, DO, DO combined with DSD, and a variable Qmax. Interestingly, one study revealed that the level of spinal cord involvement observed on magnetic resonance imaging did not correspond with the urodynamic results; however, some individuals with spinal TB developed DO and DSD with poor bladder compliance.[19] In the present study, only a small proportion of the patients had DSD or DO; however, a majority of these presented with a decreased bladder compliance.

Approximately 16.2% of the patients in the current study had a history of diabetes, as opposed to the 8.3% observed in another study that revealed a lower global prevalence of NLUTD.[20] In 2019, Indonesia had around 10.7 million individuals with diabetes, making it one of the countries with the highest absolute prevalence of diabetes.[21] Notably, approximately 66% of patients with DU also have diabetes.[7] The most common urological complications of diabetes are urgency and incontinence, with hyposensitivity and DU potentially developing at a later stage. The classic symptoms of diabetic cystopathy include reduced bladder sensation, increased bladder capacity, and reduced bladder emptying with increased post-void residual volume.[22] However, the current study found that patients with diabetes exhibited a variety of urological dysfunctions,

such as an overactive bladder (18.2%), low bladder compliance (28.6%), and DSD (3.9%). Over half of these patients had a blocked bladder outlet, while 85.7% experienced weak contractions. These findings are similar to those of Kaplan et al,[23] who reported detrusor hyperreflexia and abnormal detrusor contractility in 55% and 23% of the patients, respectively. Detrusor hyperactivity may be a compensatory response to increased urine production in the early stages of diabetes. Chronic hyperglycemia leads to myogenic degeneration and sensory/autonomic diabetic nerve impairment, resulting in bladder decompensation (such as DU) in the later stages of diabetes, indicating that DO begins early in the disease.[23]

Patients with neurological disorders frequently experience bladder and bowel problems; men, in particular, also experience sexual dysfunction due to obstruction of the long spinal pathways between the brain and the sacral cord or the pelvic autonomic nerves, which interferes with genital swelling, erection, ejaculation, and orgasm due to motor and sensory control loss.[24,25] In this study, 40.7% of patients experienced bowel problems (such as incontinence or constipation), whereas 37.9% of male patients reported sexual dysfunction, with ED being the most common issue among men. Unfortunately, corresponding data in women were lacking because women are not routinely evaluated for sexual dysfunction at our center. Martinez et al[25] reported that ED, ejaculation dysfunction, and problems with sexual desires in young men could be caused by various neurological disorders, such as SCIs and degenerative diseases. The incidence of sexual dysfunction among men with NLUTD is as high as 80%, with symptoms varying depending on the etiology.

The limitation of this study was the retrospective design; thus, some data were missing from the databases. In conclusion, urodynamic examinations in this study showed that most patients with NLUTD had a small bladder capacity and decreased bladder compliance. Recognizing the characteristics of NLUTD and conducting regular urodynamic examinations allow for early detection and tailored management of the condition. These findings can also be the base of new scheme in the hospital to conduct urodynamic study in patients diagnosed with neurological disorders to diagnose bladder dysfunction earlier. This study can also emphasize the importance of NLUTD screening. In the future, a prospective multicenter study is required to evaluate NLUTD and its urodynamic outcomes.

Conflict of Interest

Harrina Erlianti Rahardjo is the editorial board member but was not involved in the review or decision making process of the article.

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Alpha-thalassemia genotypes in Vietnam: a report of 12,030 pregnant women and their husbands performing prenatal screening for alpha-thalassemia

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ABSTRACT

BACKGROUND Alpha (α)-thalassemia is a global health concern, and improving screening methods is crucial for disease prevention. This study aimed to assess α-thalassemia genotypes and evaluate the effectiveness of various thresholds for mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) in prenatal screening for α-thalassemia.

METHODS This cross-sectional study included pregnant women and their husbands who underwent prenatal screening for thalassemia at the National Hospital of Obstetrics and Gynecology, Vietnam from January 2012 to August 2021. Blood samples were collected and analyzed using the strip assay technique, which can detect 21 common mutations in the α-globin gene and 22 common mutations in the beta-globin gene.

RESULTS Of the 12,030 participants, 931 were identified as having α-thalassemia, with $--^{SEA}$, $-\alpha^{3.7}$, and $-\alpha^{4.2}$ being the most common mutations. When examining different thresholds of MCV and MCH, MCV <85 fL and MCH <28 pg had a lower missing rate than MCV <80 fL and MCH <27 pg, respectively. MCH <28 pg showed the highest sensitivity in screening for α-thalassemia. MCV <85 fL showed the lowest positive predictive value (PPV). The combination of MCV <80 fL and MCH <27 pg showed the lowest sensitivity in screening for α-thalassemia but the highest PPV among all thresholds.

CONCLUSIONS Optimizing the screening methods for α-thalassemia is important for preventing and managing the disease in the community. These findings have important implications for thalassemia prevention and management programs and may contribute to reducing the burden of thalassemia in the global population.

KEYWORDS alpha-thalassemia, hemoglobin, mutation, prenatal diagnosis

Thalassemia refers to a hereditary group of blood disorders or hemoglobinopathies that cause anemia and hemolysis. According to the 2013 Thalassemia International Federation (TIF) report, approximately 7% of the world's population carries the hemoglobin (Hb) disease gene, with over 200 countries and territories affected by the disease, including Vietnam, where an estimated 5–7% of the population are carriers of thalassemia gene mutations.[1]

There are two primary types of thalassemia: alpha (α)- and beta (β)-thalassemia. α-thalassemia is caused by mutations in the α-globin genes, resulting in a deficiency in the production of the α-globin chain, with a carrier frequency of approximately 20% of the population worldwide. The phenotypes of α-thalassemia vary from asymptomatic to severe, depending on the number of α-globin gene deletions/inactivations. Among these, hemoglobin H (HbH)

and hemoglobin Bart's (Hb Bart's) hydrops fetalis syndrome are two clinically significant forms. Hb Bart's, the most severe form of α-thalassemia, is caused by deletion/inactivation of all four α-globin genes (--/--), and fetuses with Hb Bart's usually die in pregnancy or shortly after birth.[2] Meanwhile, HbH is caused by the deletion/inactivation of three α-globin genes (--/-α), with patients typically producing less than 30% of the normal α-globin chains.[3] Severe HbH patients have hepatosplenomegaly, mild jaundice, and sometimes thalassemia-like bone changes and require a blood transfusion.[4] β-thalassemia is caused by mutations in the β-globin genes, resulting in a deficiency in β-globin chains. There are three phenotypes of β-thalassemia: β-thalassemia trait, β-thalassemia intermediate, and β-thalassemia major, which are distinguished by recurrent or regular blood transfusions.

Regular blood transfusion and continual iron chelation are the leading therapeutic strategies for transfusion-dependent thalassemia treatment; however, the accumulation of excess iron can adversely affect organ functions and cause morbidity and mortality. This treatment also imposes a significant economic burden, with treatment costs ranging from 629 to 2,300 USD per patient depending on age groups, and the lifetime healthcare costs reaching approximately USD 606,665.[5,6]

Prenatal screening for thalassemia based on complete blood count (CBC) and serum iron and ferritin levels is the most effective and common method to prevent severe forms of thalassemia. Screening aims to identify asymptomatic individuals whose offspring are at risk of thalassemia and provide appropriate counseling for couples to reduce the number of children born with severe thalassemia. A good understanding of genotype characteristics and indices in the prenatal diagnosis of thalassemia is essential for the precise and accurate analysis of laboratory results and for providing proper medical indications and advice to patients, thereby minimizing the birth of infants with the fatal Hb Bart's hydrops fetalis syndrome.

The mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) are common thalassemia screening parameters used in Vietnam, as recommended by TIF. The Vietnamese Ministry of Health raised the MCV threshold to 85 fL in 2020 to increase the sensitivity of thalassemia screening and expand the screening population. This study aimed to evaluate the effectiveness of the new MCV threshold for thalassemia screening in Vietnam, along with the potential benefits and challenges of this policy change.

METHODS

Subjects and sampling

This descriptive cross-sectional study used convenience sampling technique in pregnant women and their husbands undergoing thalassemia screening at the National Hospital of Obstetrics and Gynecology, Vietnam, from January 2012 to August 2021. This hospital is the central hospital of northern Vietnam, where pregnant women and their husbands from various provinces with pregnancy-related conditions are examined. This study included 12,030 pregnant women and their husbands with a family history of thalassemia who underwent CBC tests. Those with normal MCV and MCH values did not require further assessment. However, if either of these indices were reduced, both the participants and their partners underwent molecular diganostic testing. High-risk participants were defined as those with at least one of the following: (i) MCV <80 fL and/or MCH <28 pg; (ii) a history of Hb Bart's hydrops fetalis syndrome; and (iii) a history of children diagnosed with thalassemia. In total, 1,344 high-risk participants underwent thalassemia mutation testing (Figure 1). The exclusion criteria were patients with other diseases affecting CBC and Hb variant analysis results, which included iron deficiency anemia, chronic kidney failure, aplastic anemia, systemic lupus erythematosus, Crohn's disease, and other chronic diseases.

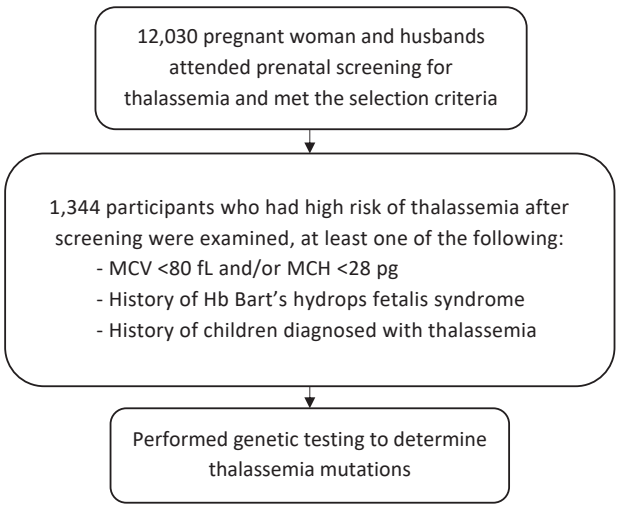

Figure 1. Participant recruitment process

Blood sample collection and analysis

To perform the CBC test, 3 ml of venous blood was collected in K2 EDTA tubes and analyzed using automated cell counters. Hb variant analysis was performed using high-performance liquid chromatography. The strip assay technique was used to detect 21 common mutations ($-\alpha^{-3.7}$, $-\alpha^{-4.2}$, $--^{MED}$, $--^{SEA}$, $--^{THAI}$, $--^{FIL}$, $-\alpha^{-20.5}$, anti $-\alpha^{-3.7}$, α^{-1} CD14 [G>A], α^{-1} CD59 [G>A], α^{-2} int CD [T>C], α^{-2} CD19 [-G], α^{-2} IVS1 [-5nt], α^{-2} CD59 [G>A], α^{-2} CD125 [CTG>CCG] Qs, α^{-2} CD142 [TAA>CAA] Cs, α^{-2} CD142 [TAA>AAA], α^{-2} CD142 [TAA>TAT], α^{-2} CD142 [TAA>TCA], α^{-2} polyA-1 [AATAAA>AATAAG], and α^{-2} polyA-2 [AATAAA>AATGAA]) in the α-globin gene and 22 common mutations ($^{-31}$ [A>G], $^{-29}$ [A>G], $^{-28}$ [A>G], Cap+1 [A>C], initiation CD [ATG>AGG], CD8/9 [+G], CD15 [TGG>TAG], CD17 [A>T], CD19 [A>G] Malay, CD26 [G>A] HbE, CD 27/28 [+C], IVS 1.1 [G>T], IVS 1.5 [G>C], CD41/42 [-TTCT], CD43 [G>T], CD71/72 [+A], CD89/90 [-GT], CD90 [G>T], CD95 [+A], IVS 2.1 [G>A], IVS 2.654 [C>T], and CD121 [G>T]) in the β-globin gene, including both heterozygous and homozygous classifications.

Ethical considerations

This study was approved by the Ethics Committee of the National Hospital of Obstetrics and Gynecology (register number 1042/CN-PSTW), and all participants provided written informed consent.

Data analysis

Data were analyzed using the SPSS software version 20 (IBM Corp., USA), and descriptive statistics were used to summarize the data.

RESULTS

Of 12,030 participants, 1,344 were at high risk of thalassemia, with 931 (7.7%) had α-thalassemia. The most frequent genotype observed was $--^{SEA}/\alpha\alpha$, accounting for 91.9% of the total patients, followed by $-\alpha^{3.7}/\alpha\alpha$ (1.7%) and $-\alpha^{4.2}/\alpha\alpha$ (1.5%). Nineteen patients had mutations in three genes, mainly $--^{SEA}$ mutation, that can cause HbH. The $--^{SEA}$ mutation was the most common mutation (91.8%), followed by $-\alpha^{3.7}$ (2.8%), $-\alpha^{4.2}$ (2.0%), and α^{CS} (1.1%) (Table 1).

Table 2 shows α-thalassemia genotypes that could be undetected if using only one of the two cut-off values of MCV: <80 fL and <85 fL. Most patients had MCV <80 fL, but some had values above, suggesting the presence of other factors affecting red blood cell size. The difference between the two MCV thresholds was clear. By using MCV <80 fL, 19 cases of α-thalassemia were missed. However, by using MCV <85 fL, only five cases were missed.

Table 1. Distribution of genotypes and mutations in α-thalassemia

	n (%)
Genotypes, n = 931	
$--^{SEA}/\alpha\alpha$	856 (91.9)
$-\alpha^{3.7}/\alpha\alpha$	16 (1.7)
$-\alpha^{4.2}/\alpha\alpha$	14 (1.5)
$--^{SEA}/-\alpha^{3.7}$	9 (1.0)
$\alpha^{CS}\alpha/\alpha\alpha$	5 (0.5)
$--^{SEA}/\alpha^{CS}\alpha$	5 (0.5)
$--^{SEA}/\alpha\alpha\alpha^{anti\text{-}3.7}$	5 (0.5)
$--^{THAI}/\alpha\alpha$	4 (0.4)
$\alpha\alpha\alpha^{anti\text{-}3.7}/\alpha\alpha$	4 (0.4)
$\alpha^{QS}\alpha/\alpha\alpha$	4 (0.4)
$--^{SEA}/-\alpha^{4.2}$	3 (0.3)
$\alpha^{init\ CD\ [T>C]}\alpha/\alpha\alpha$	2 (0.2)
$-\alpha^{3.7}/-\alpha^{4.2}$	2 (0.2)
$--^{SEA}/\alpha^{CD14\ [G>A]}\alpha$	1 (0.1)
$--^{THAI}/\alpha^{CS}\alpha$	1 (0.1)
Mutations, n = 957	
$--^{SEA}$	879 (91.8)
$-\alpha^{3.7}$	27 (2.8)
$-\alpha^{4.2}$	19 (2.0)
α^{CS}	11 (1.1)
$\alpha\alpha\alpha^{anti\text{-}3.7}$	9 (0.9)
$--^{THAI}$	5 (0.5)
α^{QS}	4 (0.4)
$\alpha^{init\ CD\ [T>C]}$	2 (0.2)
$\alpha^{CD14\ [G>A]}$	1 (0.1)

Table 3 shows the MCH values in patients with different α-thalassemia genotypes based on two different MCH thresholds (MCH <27 pg and MCH <28 pg). Most patients had MCH values <27 pg, which is consistent with the expected hypochromic phenotype in thalassemia. Nine α-thalassemia cases had an MCH ≥27 pg, and MCH ≥28 pg was seen in only three cases.

The highest number of detected α-thalassemia patients had MCH <28 pg (686/689 patients), followed by MCV <85 fL (684/689 patients). The lowest number comprised patients with a combination of MCV <80 fL and MCH <27 pg (668/689 patients). When looking at specific α-thalassemia genotypes, MCH <28 pg also detected most types of detectable mutations, and missed only three cases, including $-\alpha^{3.7}/\alpha\alpha$, $--^{SEA}/\alpha\alpha$, and $\alpha^{CS}\alpha/\alpha\alpha$. Meanwhile, the combination of MCV <80

Table 2. MCV in α-thalassemia patients

Genotypes	MCV ≥80	MCV ≥85
-α$^{3.7}$/αα	3	1
-α$^{4.2}$/αα	6	0
--^{SEA}/αα	5	2
--^{THAI}/αα	1	1
ααα$^{anti-3.7}$/αα	2	0
α^{CS}α/αα	1	1
--^{SEA}/α^{CS}α	1	0
Others	0	0
Total	19	5

MCV=mean corpuscular volume

Table 3. MCH in α-thalassemia patients

Genotypes	MCH ≥28	MCH ≥27
-α$^{3.7}$/αα	1	2
-α$^{4.2}$/αα	0	3
--^{SEA}/αα	1	2
--^{THAI}/αα	0	0
ααα$^{anti-3.7}$/αα	0	1
α^{CS}α/αα	1	1
Others	0	0
Total	3	9

MCH=mean corpuscular hemoglobin

fL and MCH <27 pg only detected 3/9 cases of -α$^{4.2}$/αα and no case of ααα$^{anti-3.7}$/αα. All thresholds of MCV and/or MCH showed detection rates of α-thalassemia above 70%, of which the lowest was MCV <85 fL (71.3%), whereas the highest was with MCV <80 fL combined with MCH <27 pg (73.3%) (Table 4).

DISCUSSION

α-thalassemia is a common cause of hydrops fetalis, accounting for 60–90% of cases in Southeast Asia.[7] The geographical distribution of α-thalassemia mutations is particularly concentrated in Africa, Southeast Asia, the Mediterranean region, and Middle East, with up to 40% of carriers.[8,9] Vietnam also has a high frequency of α-thalassemia because of its geographical location. Diagnosing α-thalassemia is important in patients with unsolved hypochromic microcytic anemia. This study found a high prevalence of the α-thalassemia gene carriers (69.3%), consistent with the findings of Nguyen et al[10] in 2015. This high prevalence may be attributed to the large number of women with a history of Hb Bart's hydrops fetalis syndrome or those whose babies with Hb Bart's hydrops fetalis syndrome during their current pregnancy visited the hospital for

Table 4. Number of detected thalassemia patients by different thresholds

		MCV <85	MCH <28	MCV <85 and MCH <28	MCH <27	MCV <80	MCV <80 and MCH <27	Normal
α-thalassemia, N	-α$^{3.7}$/αα	7	7	7	6	5	5	8
	-α$^{4.2}$/αα	9	9	9	6	3	3	9
	--^{SEA}/αα	634	635	634	634	631	631	636
	--^{THAI}/αα	2	3	2	3	2	2	3
	ααα$^{anti-3.7}$/αα	3	3	3	2	1	0	3
	α$^{init\ CD\ [T>C]}$α/αα	1	1	1	1	1	1	1
	α^{QS}α/αα	3	3	3	3	3	3	3
	α^{CS}α/αα	3	3	2	3	3	2	4
	-α$^{3.7}$/-α$^{4.2}$	1	1	1	1	1	1	1
	--^{SEA}/-α$^{3.7}$	8	8	8	8	8	8	8
	--^{SEA}/-α$^{4.2}$	3	3	3	3	3	3	3
	--^{SEA}/α^{CS}α	5	5	5	5	4	4	5
	--^{THAI}/α^{CS}α	1	1	1	1	1	1	1
	--^{SEA}/ααα$^{anti-3.7}$	4	4	4	4	4	4	4
	Total	684	686	683	680	670	668	689
	Others	275	266	264	248	252	243	
Total, N		959	952	947	928	922	911	
α-thalassemia (%)		71.3	72.1	72.1	73.3	72.7	73.3	
Other types of mutations (%)		28.7	27.9	27.9	26.7	27.3	26.7	

MCH=mean corpuscular hemoglobin; MCV=mean corpuscular volume

medical checkups, thalassemia screening tests, and preventive measures.

This study identified nine different α-thalassemia mutations, creating 15 different genotypes in pregnant women and their husbands in Vietnam. Among 21 mutations screened, $--^{SEA}$ deletion was the most prevalent mutation (91.9%), as also shown in another study in Southeast Asia.[11] Those carrying the $--^{SEA}$ deletion in heterozygous form are conventionally named α-thalassemia trait and typically have mild or no obvious clinical symptoms.[12] Couples, both of whom carry this mutation, have a 25% chance of having a fetus with Hb Bart's hydrops fetalis syndrome, which accounts for 44.8% of the total causes of placental edema, based on a study in Vietnam.[13] Evaluation of the α-globin gene mutation pattern showed that the $--^{SEA}$ mutation had the highest rate (51.4%), followed by the $^{3.7}$, $^{4.2}$, and α^{CS} mutations. The $--^{SEA}$ deletion was also found to be the most common α-globin gene defect in studies conducted in other Southeast Asian countries and China.[14–19] In contrast, surveys in Malaysia showed that α-thalassemia 2 with the 3.7 kb deletion was the most common α-globin gene defect, indicating ethnic heterogeneity of the α-thalassemia gene.[20]

MCV and MCH are two parameters recommended by the TIF for prenatal diagnosis of thalassemia[21] and are commonly used in Vietnam. Microcytosis with MCV <80 fL and hypochromia with an MCH <27 pg are laboratory features of α-thalassemia 1 carriers, homozygous α-thalassemia 1 carriers, and α-thalassemia disease (HbH disease in particular). Table 2 showed that the MCV <85 fL threshold was more effective in detecting mutations than the MCV <80 fL threshold because there were fewer missed mutations (five mutations compared to 19 mutations), indicating that it could detect more α-thalassemia carriers. However, MCV <85 fL still missed five cases of α-thalassemia carriers, with three cases having large deletion mutations. Similarly, MCH <28 pg could better detect thalassemia mutation than MCH <27 pg but still missed some carriers, including the $--^{SEA}$ mutation. We found a similar trend with both the indices indicating that despite boosting their thresholds to the highest level (MCV <85 fL and MCH <28 pg), α-thalassemia cases were still missed, including α-thalassemia trait mutation in 2/4 α genes, as shown in Tables 2 and 3, which still left a potential risk of having a baby with HbH disease and fetal edema. This suggests that attempting to raise the threshold as much as possible may not be an optimal strategy for α-thalassemia screening.

MCH <28 pg showed the highest sensitivity, whereas the combination of MCV <80 fL and MCH <27 pg showed the lowest sensitivity in screening for α-thalassemia (Table 4). However, different genotypes may have different optimal detection thresholds, and further research is required to determine the most effective threshold for each genotype. Different combinations of index thresholds also represent different screening efficiencies, with the positive predictive value (PPV) varying among populations. In this study, the PPV was limited to participants attending antenatal care and prenatal screening. Despite having the lowest sensitivity, the combination of MCV <80 fL and MCH <27 pg had the highest predictability of α-thalassemia among all thresholds. Using this combination for screening could detect fewer α-thalassemia carriers but higher possibility of α-thalassemia mutation in high-risk population.

This study did not evaluate the sensitivity and specificity of the tests, as the participants had been screened for MCH and MCV indices before genetic testing. Instead, we evaluated the minimum missed rate to compare different screening thresholds. The detection rate of α-thalassemia mutation in one (silent carriers) and two genes (α-thalassemia traits) varied among the different thresholds, while that in three gene mutations was similar in all testing methods. The severity of the disease forms may cause this difference. Silent carriers of α-thalassemia can be mostly normal or mildly anemic.[22,23] α-thalassemia traits are mostly microcytic or mild hypochromic anemia, whereas HbH disease had moderate to severe hypochromic microcytic anemia.

In addition, this study showed significant differences in the current use of common screening thresholds in Vietnam, providing reliable evidence to improve screening for α-thalassemia using clinical units. Among all the indices examined, MCH <28 pg had the lowest missing rate of α-thalassemia and is recommended for α-thalassemia screening in pregnant women and their husbands.

Countries with a high prevalence of thalassemia should consider implementing prenatal screening programs using the optimal screening thresholds identified in this study. Continuous monitoring and updating screening methods are needed to ensure the best possible detection rates for thalassemia. The

results of this study may also guide future research on α-thalassemia genotypes and screening methods in other populations.

In conclusion, the prevalence of the α-thalassemia gene is high among pregnant women and their husbands in Vietnam, with the $--^{SEA}$ mutation being the most common mutation. This study evaluated the effectiveness of various thresholds for MCV and MCH in prenatal screening for α-thalassemia and found that MCV <85 fL and MCH <28 pg had a higher detection rate but still missed a small number of cases. This study highlighted the need to optimize screening methods for α-thalassemia in Vietnam.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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Clinical Research

Comparative efficacy of intravenous levetiracetam and phenytoin in status epilepticus: a systematic review and meta-analysis of randomized controlled trials

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ABSTRACT

BACKGROUND Status epilepticus (SE) is a neurological emergency, with the current guidelines for second-line anticonvulsants may include phenytoin, levetiracetam, valproic acid, and phenobarbital. However, some studies suggest that levetiracetam may be better at stopping seizures in SE. This study aimed to compare the efficacy of intravenous (IV) levetiracetam and phenytoin in SE.

METHODS We searched PubMed, ScienceDirect, Cochrane, and Google Scholar for randomized controlled trials (RCTs) on administering IV levetiracetam or phenytoin in patients with SE. RCTs were screened using eligibility criteria, and their quality was assessed using the Cochrane risk of bias tool. Heterogeneity was assessed using the I^2 test, and publication bias was evaluated using Egger's test. All analyses were performed using Review Manager version 5.4 (The Cochrane Collaboration, UK) and Stata 17 (StataCorp LLC, USA).

RESULTS 12 RCTs involving 2,137 patients (1,099 receiving levetiracetam) met the inclusion criteria. Pooled analysis showed that levetiracetam therapy had a significantly higher rate of seizure cessation than phenytoin (RR: 1.10, 95% CI = 1.05–1.14, $p = 0.02$, $I^2 = 51\%$). Less adverse events were observed in the levetiracetam group (9.34%) than in the phenytoin group (11.62%; RR: 0.82, 95% CI = 0.66–1.02, $p = 0.07$). However, there was no significant difference regarding IV levetiracetam or phenytoin administration with the incidence of admission to critical care (RR: 1.01; 95% CI = 0.93–1.10, $p = 0.80$) and mortality (RR: 1.08; 95% CI = 0.54–2.15; $p = 0.82$).

CONCLUSIONS IV levetiracetam was significantly better in the cessation of seizures in SE patients than phenytoin.

KEYWORDS levetiracetam, phenytoin, status epilepticus

Status epilepticus (SE) is a neurological emergency characterized by prolonged or recurrent seizures, with an unconscious phase between seizures. Associated morbidity and mortality are high,[1] with an annual incidence of 6–41 per 100,000 individuals. Incidence in the elderly is approximately 3 to 10 times higher than that in young adults.[2] The mortality rate of SE ranges from 9% to 21%, increases with age, and depends on seizure duration, age at seizure onset, and etiology.[3]

SE requires immediate targeted treatment to reduce morbidity and mortality. Various clinical studies on SE treatments have been conducted to stop seizures early and prevent recurrence. Clinical practice guidelines recommend benzodiazepines as first-line treatment for patients with SE. If SE is refractory to benzodiazepines, the current guidelines for second-line anticonvulsants include phenytoin, levetiracetam, valproic acid, and phenobarbital. Each

drug has its advantages and disadvantages depending on the patient's clinical condition. However, there is no conclusive evidence that one choice is superior.[3,4]

Phenytoin is a hydantoin derivative anticonvulsant that inhibits nonspecific sodium channels in synapses. Intravenous (IV) phenytoin is widely used because of its wide availability. However, IV administration of phenytoin can cause adverse events such as prolonged QT interval, arrhythmias, and hypotension. Therefore, cardiac monitoring is required periodically following IV administration of phenytoin.

Levetiracetam, a drug in the pyrrolidine class and a newer anticonvulsant, is an alternative second-line SE treatment. The exact antiepileptic mechanism of levetiracetam is still unclear. However, some studies suggested that levetiracetam acts on synaptic vesicle glycoprotein 2A (SV2A), high-voltage activation calcium channels, and the gamma-aminobutyric acid (GABAergic) system. In several observational studies, levetiracetam is superior to phenytoin.[4] This study aimed to compare the effectiveness of levetiracetam and phenytoin as the second-line treatment for SE.

METHODS

Study protocol

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 guidelines[5] and registered in PROSPERO (CRD42022363772).

Study selection

A literature search was conducted using the following databases and search engines: PubMed, ScienceDirect, Cochrane, and Google Scholar from January 1 to October 1, 2022. A combination of Medical Subject Headings was used for the search strategy as the following "levetiracetam" AND "phenytoin" AND "status epilepticus." The inclusion criteria for the population, intervention, outcomes, and study design were as follows: (1) population: patients in all age groups with convulsive SE (focal or generalized) despite first-line antiepileptic drug (e.g., benzodiazepine) administration; (2) intervention: IV levetiracetam, phenytoin, or fosphenytoin; and (3) outcome: the primary outcome was clinical seizure cessation. The secondary outcomes were adverse events, admission to critical care, and all-cause mortality; (4) study design: randomized controlled trials (RCTs).

The included RCTs were required to include the primary outcome but did not require all secondary outcomes. Studies that had not been translated into English or provided the full text were eliminated. Studies using combination therapy with anticoagulants and/or antiplatelet agents were excluded.

Selection of studies

All reference lists from the electronic databases were stored and processed using the Rayyan Systematic Review Tool (Rayyan System Inc., USA). Two authors (GAT and NID) independently assessed the reference lists and screened the titles and abstracts. Duplicate and ineligible studies were also excluded. Two authors (GAT and NID) independently obtained full-text articles from relevant studies for further screening. Any disagreements during this process were resolved by discussion with a third author (FHFR or VKR), as needed.

Data extraction

GAT and NID extracted data on study characteristics, interventions, and outcome details. The baseline characteristics included the main study characteristics (first author, year of publication, study design, country of study, number of included patients, age, sex, and history of previous seizures) and quantitative outcomes (clinical seizure termination, adverse events, critical care admission, and death). GAT, NID, and FHFR developed a data extraction form (Microsoft Excel; Microsoft, USA) for data collection.

Data synthesis

Statistical analyses were performed using the Review Manager 5.4 (The Cochrane Collaboration, UK) and Stata 17 (StataCorp LLC, USA). The risk ratio (RR) and 95% confidence interval (CI) were analyzed using dichotomous outcomes and calculated using a fixed-effects model. Heterogeneity was estimated using the I^2 test, with I^2>50% indicating substantial heterogeneity. A two-sided test was performed for all analyses, with a *p*-value of <0.05 considered statistically significant.

Risk of bias assessment

GAT and NID independently assessed the eligibility criteria and study quality using the Cochrane risk of bias tool (Center for Evidence-Based Medicine Odense and Cochrane Denmark, Denmark) under five domains

(sequence generation, allocation concealment, blinding, detection bias, and attrition bias). Each domain was assessed as low, unclear, or high risk. Any disagreements during this process were resolved by discussion with a third author (FHFR). Egger's test was performed to statistically analyze possible publication bias.

RESULTS

Study selection result

A total of 467 records were obtained during the initial search. After removing duplicates, the titles and abstracts of 376 records were screened. Eighteen articles were assessed for feasibility, and only 12

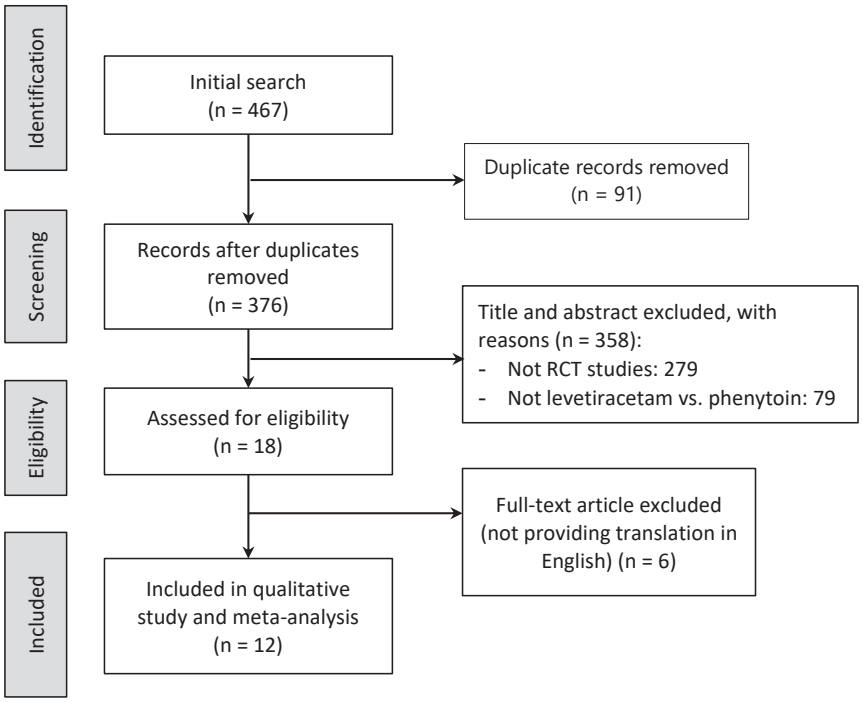

Figure 1. Searching strategy

Table 1. Summary of the studies

First author, year	Country	Ratio M:F (n)	Drug dosage (mg/kg)		Age (years), mean (SD)		History of previous seizure (n)		SE treatment (n)		Seizure cessation (n)	
			LEV	FEN	LEV	FEN	LEV	FEN	LEV	FEN	LEV	FEN
Appleton,[6] 2020	UK	147:139	40	20	3.15 (0.87)	3.15 (0.75)	83	85	152	134	106	86
Chakravarthi,[7] 2015	India	27:17	20	20	39.00 (18.40)	31.82 (12.68)	17	14	22	22	13	15
Chamberlain,[8] 2020	USA	180:137	60	20	28.6 (25.9)	28.2 (25.3)	112	97	175	142	82	66
Dalziel,[9] 2019	Australia and New Zealand	112:121	40	20	3.8 (1.25)	4.0 (1.5)	54	55	119	114	61	62
Gujjar,[10] 2017	Oman	34:18	30	20	38 (19)	37 (19)	NA	NA	22	30	18	22
Mundlamuri,[11] 2015	India	60:40	25	29	34.78 (13.64)	33.24 (13.39)	33	25	50	50	39	34
Nakamura,[12] 2023	Japan	118:58	1–3*	22.5	67 (16)	65 (19)	NA	NA	93	80	83	67
Nalisetty,[13] 2020	India	32:29	40	20	2.74 (2.6)†	2.74 (3.1)†	9	6	32	29	30	20
Nazir,[14] 2020	India	71:29	25	20	4.98 (4.14)	5.17 (3.71)	NA	NA	50	50	39	44
Noureen,[15] 2019	Pakistan	406:194	40	20	3.52 (0.24)	3.46 (0.22)	NA	NA	300	300	278	250
Vignesh,[16] 2020	India	37:30	20	20	58 (50)†	44 (43)†	NA	NA	32	35	30	31
Wani,[17] 2019	India	66:38	40	20	3.39 (3.32)	4.80 (4.11)	8	10	52	52	50	31

F=female; FEN=phenytoin; LEV=leviteracetam; M=male; NA=not available; SD=standard deviation; SE=status epilepticus
*In grams (single dose); †in months

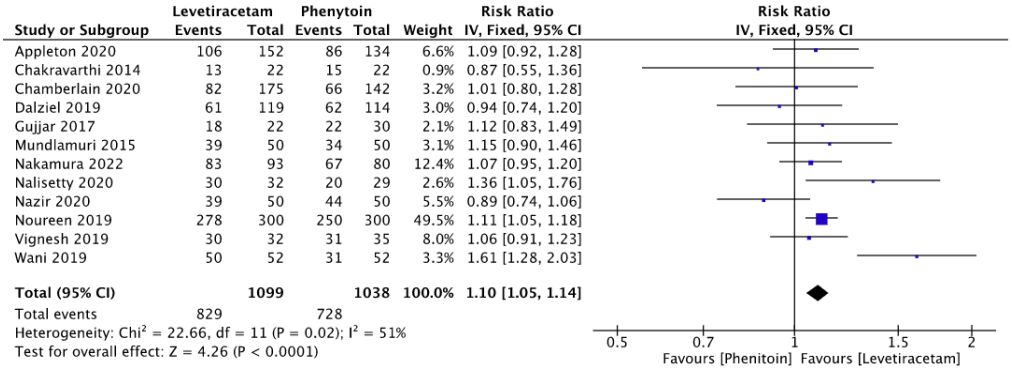

Figure 2. Forest plot of seizure cessation after levetiracetam or phenytoin treatment

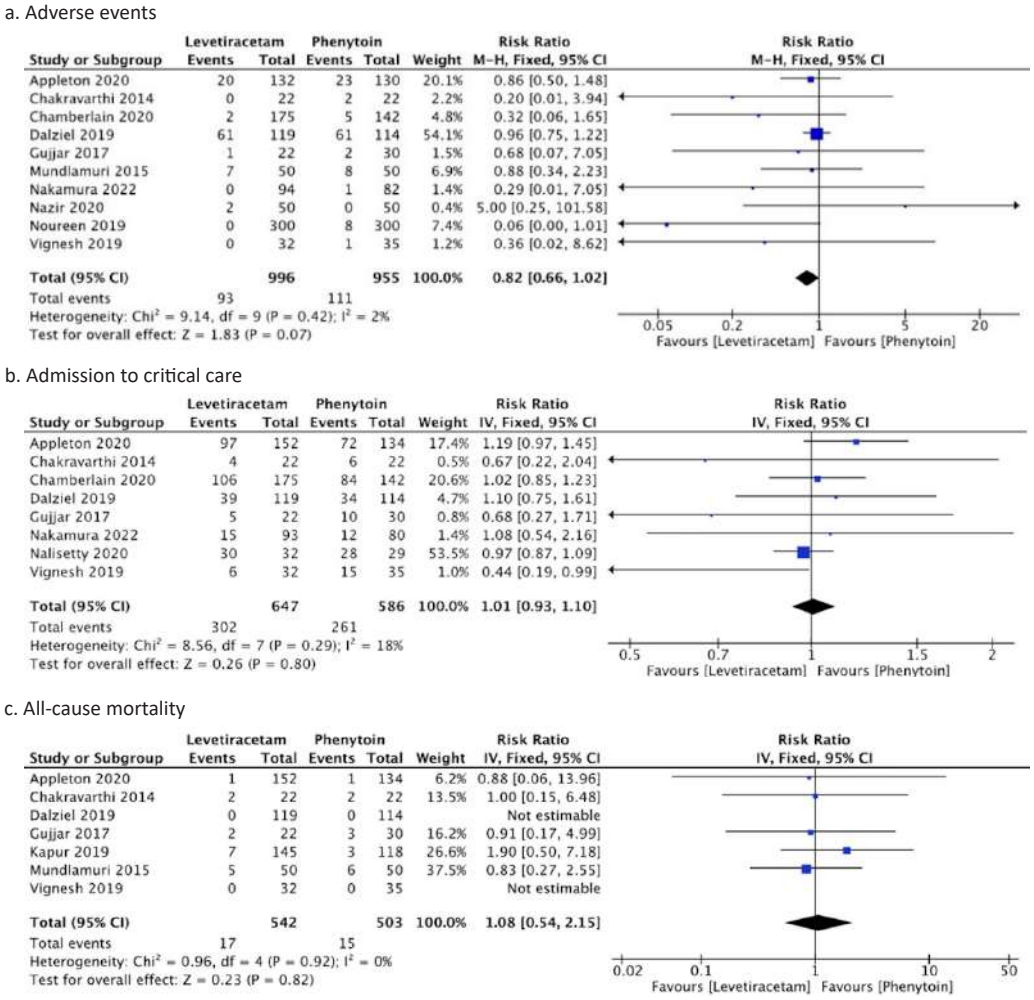

Figure 3. Forest plot of secondary outcomes in status epilepticus after levetiracetam and phenytoin treatment

studies[6–17] that met the criteria were included in the quantitative synthesis (Figure 1).

Clinical characteristics and demography of the included studies

The main characteristics of the studies are summarized in Table 1. In this study, most patients were males (59.1%). Patients' ages ranged from 2.74 (2.6) months to 67 (16) years for the levetiracetam group and 2.74 (3.1) months to 65 (19) years for the phenytoin group. In 12 clinical studies, 2,137 patients with SE were randomized to receive IV levetiracetam (1,099 patients) or phenytoin (1,038 patients). A total of 608 (53.1%) patients had a history of seizures.

Primary outcome

Of the 1,099 patients who received IV levetiracetam, seizures stopped in 829 (75.4%) patients. Seizure cessation was observed in 727 (70.0%) patients who received IV phenytoin. Pooled analysis showed a significant effect of levetiracetam therapy administration on seizure cessation, compared with phenytoin, in patients with SE (RR: 1.10, 95% CI = 1.05–1.14, and $p = 0.02$ with Z-score = 4.26) (Figure 2).

Secondary outcomes

The secondary outcomes evaluated were adverse events, the need for critical care, and all-cause death in patients with SE after administration of levetiracetam or phenytoin treatment. Approximately 9.34% of the adverse events occurred in the levetiracetam group, which was lower than that in the phenytoin group (11.62%) (Figure 3a). The administration of levetiracetam or phenytoin therapy did not significantly affect critical care needs (Figure 3b) or deaths (Figure 3c).

Risk of bias

Details of the risk of bias assessment are shown in Supplementary Figure 1. Most studies are sufficient to generate random sequences and selective reporting and are at a low risk of bias for incomplete outcome data. Approximately three-quarters of the included studies lacked blinding and were at high risk of performance and detection bias. Half of the studies had low risk of allocation concealment and other possible biases. Quantitative analysis using Egger's test in these 12 studies showed a *p*-value of 0.12, indicating no evidence of publication bias.

DISCUSSION

The efficacy of levetiracetam and phenytoin as secondary treatments for SE remains controversial. In line with Shi et al[18] and Xue et al,[19] this meta-analysis showed better efficacy of levetiracetam for seizure cessation than phenytoin (RR: 1.12 and 1.14, respectively). In contrast, meta-analyses conducted by Li et al[20] and Feng et al[21] showed that levetiracetam and phenytoin are as effective in SE seizure cessation. In addition, there was no significant difference in critical care admissions between the levetiracetam and phenytoin groups. Li et al[20] only included seven RCTs and did not include the RCT by Chamberlain et al[8] which was a multicenter study with a large sample size. The most recent RCT by Nakamura et al[12] in 2022 was not included from the meta-analysis by Feng et al[21]. Furthermore, the number of studies included in this review was greater than that in previous studies (1,028 *versus* 2,137).[20] Additional RCT will increase the sample size of this study, thus making it possible to obtain more accurate and convincing results.

There was no significant difference in the incidence of critical care admission and mortality between patients receiving IV levetiracetam and phenytoin. Several factors can affect admission to critical care and mortality, in addition to the adverse effects of drugs. SE can have long-term consequences, including nerve death, nerve injury, and changes in the nerve tissue, depending on the type and duration of the seizure. In addition, the presence of metabolic diseases, infections, and prolonged seizures can affect mortality in SE.[22]

Most of the included studies used the same dosage of IV phenytoin, while the dosage of IV levetiracetam varied from 20 to 60 mg/kg. This difference was due to the different guidelines for IV levetiracetam dosing in each country.[23] The present study showed a higher percentage of adverse events in the phenytoin group. Although phenytoin has been the antiepileptic drug of choice for several decades, it has several side effects including hypotension, cardiac arrhythmias, gingival hyperplasia, megaloblastic anemia, sedation, and hirsutism. Phenytoin acts on the sodium channels of the synapses by reducing the influx of sodium across nerve membranes and limiting the release of action potentials and overexcitation, which can lead to seizures. It is metabolized in the liver by cytochrome P450 (CYP450) enzymes, resulting in interactions with other drugs metabolized by CYP450, such as warfarin.[4]

The mechanism of action of levetiracetam is still not clearly understood; however, a recent study suggested that it inhibits neurotransmission by binding to SV2A in all synapses and decreases excitatory transmission by blocking the high-voltage-activated calcium channels (N-type, L-type, and P/Q-type calcium channels) of hippocampal pyramidal neurons.[4,24] It also works on the GABAergic system in the central nervous system. However, the results are still conflicting.[24] Although levetiracetam has no known serious adverse events in the organ system, the most common side effects are drowsiness, dizziness, headache, fever, dry mouth, asthenia, and behavioral changes. Acute thrombocytopenia and eosinophilia

are hematological adverse effects of levetiracetam, which often occur in SE patients.[24–26] No relevant pharmacokinetic interactions were found between levetiracetam and other antiepileptic drugs. In addition, levetiracetam does not interact with digoxin, warfarin, or low-dose contraceptive pills; however, an adverse pharmacodynamic interaction was found between levetiracetam, carbamazepine, and topiramate.[27]

This study provides high-quality evidence supporting the use of IV levetiracetam over phenytoin for treating SE. However, this meta-analysis had several limitations. Although all the studies were RCTs, most were open trials. In addition, different doses and infusion times of therapy across the studies might have caused potential bias, affecting the results. IV levetiracetam is not widely used in Indonesia, both in studies and clinical practice for managing SE, as the Indonesian Neurologist Association (PERDOSSI) guidelines recommend oral levetiracetam. Therefore, further studies are needed to compare the route of administration (oral and IV) and to determine the optimal dosage of levetiracetam for the treatment of SE.

In conclusion, IV levetiracetam was superior to IV phenytoin for seizure cessation. Moreover, a lower incidence of adverse events was observed with IV levetiracetam. No significant difference was found between IV levetiracetam or phenytoin administration with respect to the incidence of admission to critical care and all-cause deaths.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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Clinical Research

Association of perceived male sexual dysfunction and sexually transmitted disease to female sexual function among Indonesian women

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ABSTRACT

BACKGROUND Male sexual dysfunction (MSD)'s impact on female partners is challenging to understand. Male erectile dysfunction (ED) and ejaculation disorder likely affect female sexual function. This study aimed to examine the prevalence of female sexual dysfunction and disorder as well as the relationship between perceived MSD and female sexual function using the validated Indonesian short version of the 6-item Female Sexual Function Index (FSFI-6).

METHODS This cross-sectional study was conducted at Cipto Mangunkusumo Hospital, Jakarta, Indonesia, from February 2018 to February 2019. About 702 Indonesian married women, including patients, visitors, and medical and nonmedical staff, provided the sociodemographic, FSFI-6, quality of life, and sexual function (ED, ejaculation disorder, and desire problems), and sexually transmitted disease (STD) data. The association between categorical variables was evaluated using Fisher's test. Logistic regression was used for multivariate analysis, and a *p*-value of 0.05 was considered statistically significant.

RESULTS Among 702 women, about 242 had sexual dysfunction (34.5%), 20 had sexual disorder (2.8%), 172 had low desire (24.5%), 72 had low arousal (10.3%), 253 had orgasmic function (36.0%), and 575 had sexual pain (81.9%). The respondents reported their partners' STD, desire problems, ED, and ejaculation disorder. Female sexual disorder and low desire were associated with perceived ED. Female sexual disorder was associated with STD (Wald = 10.3, $p = 0.001$) and desire problems (Wald = 6.89, $p = 0.008$). No other MSD was associated with female sexual function.

CONCLUSIONS Perceived STD and male desire problems affected female sexual disorder.

KEYWORDS sexual arousal, sexual behavior, sexual health

According to the World Health Organization (WHO), sexual health encompasses sexual well-being and respect for human rights, development, and maturation.[1] Decreased sexual function is intertwined with decreased quality of life (QoL) and welfare. Female sexual dysfunction (FSD) includes an unpleasant situation disrupting a woman's ability to enjoy a satisfying sexual life.[2] The following three classifications are commonly used in defining FSD: a) sexual complaints as sexual function-related discomfort expressions; b) sexual dysfunction as an interruption of at least one phase in the sexual response cycle or painful sexual activity; and c) sexual disorder as a combination of sexual dysfunction and personal distress related to sexual dysfunction.[3]

Desire, arousal, orgasmic function, and pain-related issues are the four components of FSD.[4,5] Sexual dysfunction is more prevalent among older women,

whereas sexual complaints are more frequent in young women. Sex-related distress is also more common in premenopausal women than that in menopausal women.[3,6] Satyawan et al[7] examined FSD among medical and nonmedical professionals in Indonesia and disclosed that 19/206 participants (9.2%) had sexual dysfunction. Conversely, Taher et al[8] reported a 15.2% prevalence of FSD in Jakarta. In Western countries, the prevalence is substantial, with rates of 43–88% in the USA and 22% in Europe.[9] Based on population-based epidemiological surveys of FSD involving 6,000 women in Beijing, the prevalence of adults with FSD was 63.3%, and 30.3% did not seek care.[10] These results were quite similar to sexual dysfunction in women originating from biological (post-accident conditions or postoperative injuries, blood flow disorders, hormonal disorders, the presence of diseases in the reproductive organs, and side effects of certain drugs), psychological (the existence of certain psychological problems in the past or present, such as depression, anxiety disorders, problems with body image and self-esteem, and stress), and interpersonal factors (incompatibility or conflict with a partner).[3,6] The sexual condition of a partner is also a factor that may affect FSD. According to Nelson et al,[11] male sexual dysfunction (MSD) can impact partners in various ways, including reducing general happiness, generating feelings of unattractiveness, causing feelings of rejection and guilt, increasing anxiety, and impairing intimacy.

The impact of MSD on female partners remains unclear. Presumably, erectile and ejaculation disorders play a notable role in FSD.[9,10] To the best of our knowledge, this is the first study on MSD and its relationship with female sexual function in Indonesia, which we believe differs in sociocultural and perception aspects. Using a validated Indonesian short version of the 6-item Female Sexual Function Index (FSFI-6), this study aimed to assess how perceived MSD (including erectile dysfunction [ED], ejaculation disorder, and desire problems) and sexually transmitted diseases (STDs) affect female sexual function.

METHODS

This cross-sectional study was conducted at Cipto Mangunkusumo Hospital, Jakarta, Indonesia, from February 2018 to February 2019. Data were obtained from 702 Indonesian married women, including patients, visitors, and medical and nonmedical staff, using a consecutive sampling method, with a minimum of 422 participants. This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (No. 1023/UN2.F1/ETIK/2017). All respondents were adults aged >18 years; sexually active; able to read, write, and communicate well; and agreed to participate in this study. Women with psychiatric problems and no sexual activity and those who did not fill out all questionnaire items were excluded. All the respondents provided written informed consent.

FSFI-6

The collected data consisted of sociodemographic profiles, the FSFI-6, and participants' perceptions of male sexual function. FSFI-6 comprised of six categories, namely desire, arousal, lubrication, orgasm, satisfaction, and pain, in addition to QoL. Inquiries into any distress in their sexual lives were intended to assess their QoL.[12] Participants perceived their spouse's sexual function, whereas the Indonesian version of the FSFI-6 assessed their own sexual function. Sesari et al[12] performed a linguistic validation of the employed questionnaire, with a Cronbach's alpha of 0.664 and a standardized Cronbach's alpha of 0.714.

Perceived STDs and male sexual function include ED, ejaculation disorder, and problems in initiating desire. The assessed clinical symptoms of male STD included vesicular lesions over the affected areas, pruritus, dysuria, rash, lower urinary tract symptoms, and purulent discharge, as well as accompanying fever or malaise. Perceived male sexual function was assessed by guiding participants to complete the questionnaire. Participants were allowed to ask questions if they did not understand the terminology in the questionnaire, such as ED or libido disorder.

The results were categorized as follows: having sexual disorder (the participant had an FSFI-6 score of ≤19 and answered "mostly dissatisfied," "unhappy," or "terrible" regarding QoL); sexual dysfunction (participants with an FSFI-6 score of ≤19 or one or more interrupted sexual phases as outlined in FSFI-6, desire [item no. 1], arousal [item no. 2], orgasm [item no. 4], and pain [item no. 6]); low desire (participants who answered question no. 1 with "low," "very low," or "not at all"); low arousal (participants who answered question no. 2 with "low," "very low," or "not at all"), low orgasmic function (participants who answered question no. 4 with "sometimes," "a few times," or "almost never or never"); and sexual pain (participants

who answered question no. 6 with "sometimes," "most times," or "almost always or always").[4]

Data collection

We collected demographic data on age, marital status, educational level, body mass index (BMI), smoking and alcohol consumption habits, history of sexual abuse, menstrual and obstetric status, history of pelvic surgery, and urinary/gynecological problems. The BMI categorization was based on the WHO classification for the Asia-Pacific region: <18.5 kg/m^2, underweight; 18.5–22.9 kg/m^2, normal weight; ≥23–24.9 kg/m^2, overweight; ≥25–29.9 kg/m^2, obese I; and ≥30 kg/m^2, obese II.[13]

Meanwhile, the QoL of the respondents was measured using the QoL question of the International Prostate Symptom Score (IPSS). The QoL question utilizes a 7-point Likert scale, with responses ranked from best to worst as "delighted," "pleased," "mostly satisfied," "mixed," "mostly dissatisfied," "unhappy," and "terrible."[14] The respondents were asked to indicate their perception of living with their current sexual life condition using the Likert scale, in accordance with the QoL evaluation in IPSS.

Statistical analysis

The distribution of the numerical data was evaluated using the Kolmogorov–Smirnov test. The unpaired *t*-test or Mann–Whitney *U* test, depending on whether the data were normally distributed, was used to determine the mean differences between categorical and numerical variables. Alternatively, Fisher's exact test or the chi-square test was used to determine the association between categorical variables. Logistic regression was used in multivariate analysis, with a cut-off *p*-value of 0.25 for either continuous or categorical data. Adjusted odds ratios (AORs) were calculated by adjusting for significant variables in the multivariate analysis. Statistical analysis was performed using SPSS software for Macintosh version 20.0 (IBM Corp., USA). Statistical significance was set at $p<0.05$.

RESULTS

Participant characteristics are presented in Table 1. Among 702 women, 242 (34.5%) had sexual dysfunction, 20 (2.8%) had sexual disorder, 172 (24.5%) had low desire, 72 (10.3%) had low arousal, 253 (36.0%) had orgasmic dysfunction, and 575 (81.9 %) experienced

Table 1. Characteristics of the participants

Characteristics	n (%), N = 702
Age (years), median (IQR)	37 (20–60)
Marital age (years), median (IQR)	25 (16–50)
BMI (kg/m^2)	
Underweight	24 (3.4)
Normal	207 (29.5)
Overweight	119 (17.0)
Pre-obese	254 (36.2)
Obese	98 (14.0)
Educational background	
Elementary school	4 (0.6)
Junior high school	15 (2.1)
High school	110 (15.7)
Diploma	88 (12.5)
Bachelor	443 (63.1)
Master/Doctor of Philosophy	42 (6.0)
Current smoking habit	19 (2.7)
Current alcohol consumption habit	17 (2.4)
Urinary diseases	8 (1.1)
Urinary infections	45 (6.4)
Gynecologic diseases	14 (2.0)
Gynecologic infections	56 (8.0)
Pelvic surgery	137 (19.5)
Menstrual and obstetric history	
Dysmenorrhea	124 (17.7)
Menopause	59 (8.4)
History of sexual abuse	1 (0.1)
FSFI total score, median (IQR)	21 (3–29)
Sexual dysfunction	242 (34.5)
Sexual disorder	20 (2.8)
Low desire	172 (24.5)
Low arousal	72 (10.3)
Low orgasmic function	253 (36.0)
Sexual pain	575 (81.9)
Partner with STD perceived from female participants	5 (0.7)
Partner's sexual function perceived from female participants	
Desire problems	10 (1.4)
ED	8 (1.1)
Ejaculation disorder	17 (2.4)

BMI=body mass index; ED=erectile dysfunction; FSFI=female sexual function index; IQR=interquartile range; STD=sexually transmitted disease

Table 2. Bivariate analysis between FSD and MSD

MSD perceived by female partner	FSD (N = 702)											
	Sexual disorder	*p**	Sexual dysfunction	*p**	Low desire	*p**	Low arousal	*p**	Low orgasmic function	*p**	Sexual pain	*p**
STD	25.15 (3.96–159.84)	**0.007**	7.71 (0.86–69.40)	0.051	4.69 (0.78–28.28)	0.098	5.97 (0.98–36.35)	0.085	7.20 (0.80–64.72)	0.059	0.33 (0.05–1.98)	0.224
Desire problems	9.36 (1.85–47.24)	**0.030**	2.90 (0.81–10.37)	0.101	3.14 (0.90–10.99)	0.071	0.97 (0.12–7.78)	1.000	1.79 (0.51–6.24)	0.508	0.51 (0.13–2.00)	0.399
ED	8.82 (1.76–44.24)	**0.033**	1.14 (0.27–4.82)	0.594	4.69 (1.31–16.80)	**0.018**	1.25 (0.15–10.33)	0.581	0.59 (0.12–2.94)	0.718	1.55 (0.19–12.73)	1.000
Ejaculation disorder	4.94 (1.05–23.23)	0.081	1.34 (0.50–3.57)	0.068	1.29 (0.45–3.72)	0.579	0.54 (0.07–4.14)	1.000	1.25 (0475–3.32)	0.799	0.71 (0.23–2.22)	0.527

ED=erectile dysfunction; FSD=female sexual dysfunction; MSD=male sexual dysfunction; STD=sexually transmitted disease
Data are presented as odds ratios (OR) (95% confidence interval [CI]). *Fisher's test, a *p*-value of 0.05 was considered statistically significant

sexual pain. The participants also stated their partners' conditions (Table 1).

Further analysis revealed a significant proportional difference in sexual disorder among females whose partners had STDs and desire problems. Perceived ED was found to be associated with female sexual disorder and low desire. No associations were identified between other types of MSD and FSD, low desire, low arousal, low orgasmic function, or sexual pain (Table 2).

The multivariate analysis showed that women whose partners contracted an STD (AOR = 21.68 [95% confidence interval (CI) = 2.58–182.25], p = 0.007) or experienced desire problems (AOR = 18.00 [95% CI = 2.11–153.43], p = 0.008) had a higher risk for having female sexual disorder.

DISCUSSION

The prevalence of FSD in the current study was higher than that reported in a study conducted in 2014. The previous study involved 103 medical and 103 nonmedical professionals and showed a 9.2% prevalence of FSD in Indonesia. Moreover, it demonstrated at least one interrupted phase in the sexual response cycle.[7] Individuals with sexual dysfunction may not always experience personal distress. The clinical significance lies in the fact that out of approximately 242 participants who experienced sexual dysfunction, only 20 reported consequent personal distress. However, only a small percentage of the participants believed that their conditions caused personal distress and reduced their QoL.[7] Indonesian society considers sex-related talk culturally taboo; thus, people, especially women, usually try to avoid the topic. Morton et al[15] asserted that East Asian societies have historically viewed sex as primarily reproductive in nature. Asian women in the United States reported much lower levels of sexual desire and arousal and higher degrees of sexual pain than Euro-American women and were far less likely to report sex as vital.[15]

Among 702 women who participated in this study, 5 (0.7%) reported having a partner with an STD, 10 (1.4%) reported their partner's desire problems, 8 (1.1%) reported ED, and 17 (2.4%) reported an ejaculation disorder. A study by Jiann et al[16] in Taiwan, which involved 779 males (only 632 fully participated), reported 95 (15%) cases of ED based on the International Index of Erectile Function. Among these cases,

27.7% consisted of self-reported ED, and only 10.0% were reported by the partners. In 59% of cases, the perceptions of the severity of the male partners' ED by men and women were similar and highly substantially linked, with a chi-square *p*-value of $p<0.001$.[17]

In the present study, sexual dysfunction, low desire, low arousal, low orgasmic function, and sexual function were not associated with impaired perceived male sexual function. Female sexual disorder was found in women whose partners had STDs or desire problems. Furthermore, the multivariate analysis showed STDs and desire problems as contributing factors to developing sexual disorders. The odds of developing sexual disorders in women were 21.68 times higher in women whose partners had STDs and 18.00 times higher in those whose partners had desire problems. Since only female partners perceived the STDs, there might be a varying degree of perception, from curable to non-curable STDs. STDs usually impair the anatomy of the male genitalia and cause negative stigma in society (e.g., HIV/AIDS). These factors are believed to impair not only female sexual function but also QoL.

In Iran, the most frequent sexual dysfunction was the sexual desire problem (49.2%), while sexual pain disorder occurred in approximately 35.2% of all participants.[18] These results differed from those of Rizvi et al,[19] whose findings suggested libido and lubrication disorders as the most impaired domains. In contrast, the present study found that most females of a relatively young age experienced sexual pain. Presumably, the sociocultural background of Indonesian women shaped their perspectives on sexual discussion (e.g., talking about sexual fantasy or discomfort) as taboo, thus preventing them from disclosing the matter to their male partners and causing discomfort during sexual activity.

The lack of sex education in schools leads to a limited understanding of sexual anatomy and function. Jiann et al[16] showed that females lacked understanding of their partners' sexual functions. Elterman et al[17] also concluded that communication is essential for the uniformity of men's perceptions of sexual dysfunction. This issue may incite ignorance, potentially leading to lower participation in the partners' sexual health. In contrast, when males encounter sexual problems, they tend to avoid intimacy or sexual activities, resulting in infrequent sexual activity and impaired sexual relations.[20]

According to Nelson et al[11] MSD could affect partners by decreasing general happiness; generating feelings of unattractiveness, rejection, and guilt; causing anxiety, and impairing intimacy. These factors could impair not only one phase but also several phases of female function, which would affect the male partner's sexual dysfunction and cause a vicious cycle. Even in mild to moderate severity cases, ED was associated with sexual difficulty in female partners, especially in the desire and lubrication domains.[21] This finding was similar to that of the present study, which revealed that ED was associated with female sexual disorder and low desire. Cabral et al[22] observed less sexual activity among healthy women whose partners had ED. In this cohort, ED was the most frequent reason for reduced sexual activity in women aged <45 years, indicating that couples share sexual dysfunction. Martín-Morales et al[23] also showed an improvement in the sexual QoL of female partners following vardenafil treatment in men with ED. Furthermore, significant improvements in sexual arousal, lubrication, orgasm, satisfaction, and pain were found after their partners underwent ED treatment.[23] Since perceived MSD in the present study was lower than the actual prevalence reported in another study, females should understand sexual health information regarding MSD to improve sexual function and QoL in both parties. Both males and females contribute to FSD, either through organic or psychogenic factors. Although most of the participants were bachelors who surmised that they understood their partners' sexual health, self-administered questionnaires by their partners could provide more objective data.

Little is known about whether FSD affects MSD. Zhang et al[24] found that only the physical pain disorder of the wife was associated with the husband's ED; however, other domains, such as loss of interest in sex, finding sex unpleasant, lubricating problems, failure to achieve orgasm, delayed orgasm, and lack of lubrication, were not associated with ED in husbands. These findings, however, were thought to conflict with those of earlier research. Another study conducted in Taiwan with 632 sexually active couples found a minor to moderate link between the total female sexual function and its domains, and the erectile function of men.[16] Grondhuis Palacios et al[25] reported significant weak to strong relationships between female sexual function and their male partners' erectile function following radical prostatectomy for prostate cancer.

This study had several limitations. Using a validated questionnaire would have yielded more reliable results for MSDs. Additionally, comparing the actual results of MSD with perceived dysfunction would better assess the discrepancies. The cross-sectional design made establishing a causal relationship between MSD and FSD difficult. Therefore, prospective or retrospective cohort studies are warranted to establish causality between females and MSD. The included subgroups were too small for statistical analysis, which resulted in insignificant findings.

In conclusion, the perception of STDs and desire problems in males by females affected FSD. Raising awareness of sexual health information regarding MSD among female partners could lead to improved sexual function and QoL in both partners.

Conflict of Interest

Harrina Erlianti Rahardjo is the editorial board member but was not involved in the review or decision making process of the article.

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Basic Medical Research

Effect of lycopene and metformin combination on phagocytosis, glycemic control, and oxidative stress in rats with type 2 diabetes

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ABSTRACT

BACKGROUND Hyperglycemia and oxidative stress cause phagocytosis dysfunction in patients with diabetes. A combination of lycopene and metformin can reduce oxidative stress and blood glucose. This study aimed to determine the effect of combined lycopene and metformin on phagocytosis function, glycated hemoglobin A1c (HbA1c), nitric oxide (NO), reactive oxygen species (ROS), and advanced glycation end products (AGEs).

METHODS A randomized controlled study was conducted in rats at the Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta, Indonesia, from August to September 2022. 30 rats were divided into control (n = 5) and type 2 diabetes mellitus (T2DM) (n = 25) groups. Rats in the T2DM group were induced by a high-fat diet combined with streptozotocin-nicotinamide. The 25 rats were then divided into five subgroups: 1 ml coconut oil (DM), 250 mg/kg metformin in 1 ml coconut oil (DMet), 250 mg/kg metformin + 10 mg/kg lycopene in 1 ml coconut oil (DML-10), 250 mg/kg metformin + 20 mg/kg lycopene in 1 ml coconut oil (DML-20), and 250 mg/kg metformin + 40 mg/kg lycopene in 1 ml coconut oil (DML-40). Treatments were administered daily for 4 weeks. The macrophage phagocytosis index (PI), HbA1c levels, ROS, NO, and AGEs serum were evaluated.

RESULTS There was a significant difference in the PI, HbA1c, NO, ROS, and AGEs between the groups ($p<0.001$). The DML-20 and DML-40 groups had significantly increased PI and decreased NO, ROS, and AGEs levels than metformin alone ($p<0.05$).

CONCLUSIONS Lycopene combined with metformin could improve phagocytosis function, glycemic control, and oxidative stress.

KEYWORDS glycemic control, lycopene, metformin, oxidative stress, phagocytosis

A decreased phagocytic function is commonly experienced by patients with type 2 diabetes mellitus (T2DM).[1–5] It has previously been shown that phagocytic activity is significantly reduced by 14.53% in patients with T2DM compared with individuals without T2DM.[5] This decrease in phagocytosis causes an increased risk of infections, which occur at an incidence of 36.33 per 1,000 people per year.[6] Poor glycemic control results in oxidative stress, which in turn causes decreased phagocytosis.[1,3–5,7] However, due to metabolic memory, improving glycemic control does not prevent oxidative stress, which continues to affect phagocytic activity.[8] Patients with T2DM have higher levels of nitric oxide (NO), reactive oxygen species (ROS), and advanced glycation end products (AGEs) than individuals without T2DM.[9–11]

Currently, there is no standardized method of improving phagocytic function. Metformin is typically

used as the first-line treatment for patients with T2DM because of its antidiabetic and antioxidant properties.[12,13] It has been shown to reduce NO and AGE levels by 33.33%.[14,15] However, as the effect of metformin on oxidative stress is <50%, patients with T2DM remain susceptible to infection. Therefore, additional antioxidants and immunomodulators are needed to maximize the reduction in oxidative stress and improve phagocytic function in patients with T2DM.

Figueiredo et al[15] recommended additional antioxidant therapy to increase the effectiveness of metformin treatment. It has previously been shown that carotenoid lycopene can reduce oxidative stress and improve phagocytic function.[16,17] Lycopene alone can reduce NO and ROS levels in lipopolysaccharide-stimulated macrophages by 30–90% and AGE levels by 33.3%.[15,18] It can also improve glycemic status by lowering fasting blood glucose (FBG) and glycated hemoglobin A1c (HbA1c) levels.[19]

A combination of lycopene and metformin has been shown to decrease FBG levels and reduce ROS and AGE levels to a greater degree than either metformin or lycopene alone.[15,20] This study aimed to evaluate the effect of combined lycopene and metformin treatment on the phagocytosis index (PI), and levels of HbA1c, NO, ROS, and AGEs in a rat model of T2DM.

METHODS

This randomized controlled study was conducted in rats at the Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta, Indonesia, from August to September 2022.

Animals and ethics

Healthy male albino Wistar rats weighing 160–200 g were purchased from the Animals Laboratory of the Center for Food and Nutrition Studies and housed in an animal room with individual stainless steel cages, a 12-hour light–dark cycle, a temperature of 24°C ± 2°C, and a relative humidity of 50.00–60.00%. All the rats had *ad libitum* access to water and food. The experimental procedures were approved by the Health Research Ethics Committee of the Faculty of Medicine of Universitas Diponegoro (No. 28/EC/H/FK-UNDIP/IV/2022). All animals were cared according to the Animal Laboratory Guidelines of the Center for Food and Nutrition Studies.

Experimental design

Thirty Wistar rats were randomly divided into control (n = 5) and T2DM (n = 25) groups. Rats in the T2DM group were fed a high-fat diet containing Comfeed Par S (Japfa Comfeed Indonesia, Indonesia; 60.00%), flour (27.80%), cholesterol (2.00%), folic acid (0.20%), and lard (10.00%). After 2 weeks, rats in the T2DM group were intraperitoneally injected with 45 mg/kg streptozotocin (Sigma-Aldrich, USA) and 110 mg/kg nicotinamide (Nacalai Tesque, Inc., Japan) in citrate buffer (pH 4.6). T2DM was diagnosed after 72 hours of FBG levels of 200 mg/dl.[21] The 25 rats in the T2DM group were divided into five subgroups, which received the following treatment: 1 ml coconut oil (DM), 250 mg/kg metformin in 1 ml coconut oil (DMet), 250 mg/kg metformin + 10 mg/kg lycopene in 1 ml coconut oil (DML-10), 250 mg/kg metformin + 20 mg/kg lycopene in 1 ml coconut oil (DML-20), and 250 mg/kg metformin + 40 mg/kg lycopene in 1 ml coconut oil (DML-40).

Metformin hydrochloride (99.60%) (PT Phapros Tbk, Indonesia) and tomato extract powder containing 98% lycopene (Sigma-Aldrich) were used in this study. Treatment was administered daily for 4 weeks via oral gavage. The doses of lycopene and metformin were chosen according to previous studies by Eze et al[22] and Figueiredo et al,[15] respectively.

Biochemical assays

Four weeks following the last intervention and after an overnight fast, all rats were sacrificed under ketamine anesthesia. Blood samples were collected immediately from the retro-orbital plexus using a capillary glass tube. Blood was allowed to clot, and serum was separated by centrifugation at 3,500 rpm for 10 min. Serum HbA1c, NO, and AGE levels were quantified using rat HbA1c, NO, and AGE FineTest enzyme-linked immunosorbent assay kits (Wuhan Fine Biotech Co., Ltd., China) according to the manufacturer's instructions. Serum ROS levels were quantified following the procedure described by Tang et al[23] with thiobarbituric acid reactive substances (Animals Laboratory of the Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta, Indonesia) using the colorimetric method and a spectrophotometer set at a wavelength of 532 nm.

Isolation and culture of peritoneal macrophages

The abdominal skin of each rat was disinfected with 70% alcohol and cut open. Next, 10 ml cold Roswell

Park Memorial Institute (RPMI) medium was injected into the peritoneal cavity, and the same syringe was used to aspirate the peritoneal fluid. The fluid was centrifuged at 1,200 rpm for 10 min, following which the supernatant was discarded, and the cells were resuspended in 3 ml RPMI medium. The cells were counted using a hemocytometer and adjusted to obtain a cell suspension with 2.5 × 10^6/ml density. The cell suspension was cultured in a microplate with round coverslips, with 200 µl (5 × 10^5 cells) per coverslip. The cells were incubated in a 5.00% carbon dioxide (CO_2) incubator at 37°C for 24 hours.[24]

PI testing

Following the culture for 24 hours, the medium was removed using a pipette, and the cells were washed twice with RPMI. Latex beads resuspended in RPMI were added to each well in volumes up to 200 µl, and the cells were further incubated in 5% CO_2 at 37°C for 1 hour, after which cells were washed three times with phosphate-buffered saline, dried 20–25°C, and fixed with methanol for 30 min. The methanol was then removed, and the coverslip was left to dry and then stained with Giemsa 20.00% (v/v) for 20 min, washed with distilled water, removed from the culture wells, and dried at 20–25°C. The number of macrophages that had phagocytosed latex beads and the number of latex beads within macrophages was calculated under a light microscope at 400× magnification following a previously described procedure.[24]

Statistical analysis

Data are expressed as mean (standard error of the mean). Differences between groups were analyzed by one-way analysis of variance followed by the least significant difference test. Statistical analyses were performed using SPSS software version 23 (IBM Corp., USA). Differences between mean values were considered significant at $p<0.05$.

RESULTS

Glycemic control

Figure 1a shows the serum HbA1c levels after 4 weeks of treatment. There was a significant decrease ($p<0.001$) in serum HbA1c levels, both in the rats with diabetes treated with metformin alone and in those treated with metformin combined with lycopene, compared with the diabetic control group.

The combination of metformin and lycopene exerted a significant effect at all doses compared with the DM group. The effect was dose-dependent (Table 1),

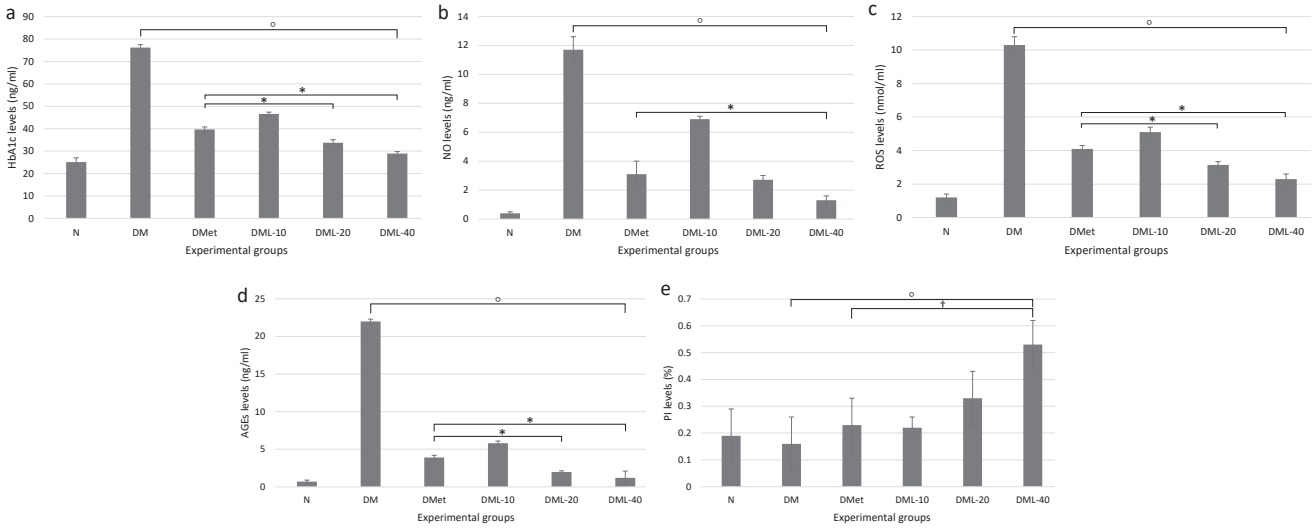

Figure 1. Effect of 4 weeks of lycopene and metformin combination on HbA1c (a), NO (b), ROS (c), AGEs (d), and PI (e) in rats with T2DM. Values are expressed in terms of mean (SEM). All variables showed significantly different results ($p<0.05$) between the DM group and those treated with metformin alone and those with metformin combined with lycopene (shown in circle). Differences in HbA1c, NO, ROS, and AGEs levels between groups were analyzed using one-way ANOVA followed by the LSD test: *$p<0.05$; PI levels using Kruskal-Wallis test followed by the Mann–Whitney test: †$p<0.05$. AGEs=advanced glycation end products; ANOVA=analysis of variance; DM=rats with T2DM treated with coconut oil; DMet=250 mg/kg metformin in coconut oil; DML-10=250 mg/kg metformin + 10 mg/kg lycopene in coconut oil; DML-20=250 mg/kg metformin + 20 mg/kg lycopene in coconut oil; DML-40=250 mg/kg metformin + 40 mg/kg lycopene in coconut oil; HbA1c=glycated hemoglobin A1c; LSD=least significant difference; N=normal rats treated with coconut oil; NO=nitric oxide; PI=phagocytosis index; ROS=reactive oxygen species; SEM=standard error of the mean; T2DM=type 2 diabetes mellitus

Table 1. Inferential analysis on HbA1c, NO, ROS, AGEs, and PI levels following lycopene and metformin administration in rats with T2DM

Item	HbA1c* (ng/ml)	NO* (ng/ml)	ROS* (mmol/ml)	AGEs* (ng/ml)	PI‡ (%)
N vs. DM	−50.68 (0.818)	−11.13 (0.269)	−9.04 (0.183)	−21.19 (0.151)	4.1 (5.54)
DMet vs. DML-10	−8.91 (0.818)	−3.14 (0.269)	−1.08 (0.183)	−1.93 (0.151)	−0.5 (5.54)
DMet vs. DML-20	5.25 (0.818)	0.46 (0.269)†	0.99 (0.183)	1.89 (0.151)	−7.7 (5.54)
DMet vs. DML-40	10.55 (0.818)	1.74 (0.269)	1.77 (0.183)	2.63 (0.151)	−14.5 (5.54)§
DML-10 vs. DML-20	12.16 (0.818)	3.60 (0.269)	2.07 (0.183)	3.81 (0.151)	−7.2 (5.54)
DML-10 vs. DML-40	17.46 (0.818)	4.89 (0.269)	2.85 (0.183)	4.55 (0.151)	−14 (5.54)¶
DML-20 vs. DML-40	5.3 (0.818)	1.29 (0.269)	0.78 (0.183)	0.74 (0.151)	−6.8 (5.54)

AGEs=advanced glycation end products; ANOVA=analysis of variance; DM=rats with T2DM treated with coconut oil; DMet=metformin 250 mg/kg in coconut oil; DML-10=250 mg/kg metformin + 10 mg/kg lycopene in coconut oil; DML-20=250 mg/kg metformin + 20 mg/kg lycopene in coconut oil; DML-40=250 mg/kg metformin + 40 mg/kg lycopene in coconut oil; HbA1c=glycated hemoglobin A1c; LSD=least significant difference; N=normal rats treated with coconut oil; NO=nitric oxide; PI=phagocytosis index; ROS=reactive oxygen species; SEM=standard error of the mean; T2DM=type 2 diabetes mellitus

Values are expressed in terms of mean (SEM), n = 5

Differences between groups were analyzed using one-way ANOVA followed by the LSD test, *$p<0.001$, †$p = 0.101$; ‡Kruskal-Wallis test followed by the Mann–Whitney test: §$p<0.01$, ¶$p<0.05$

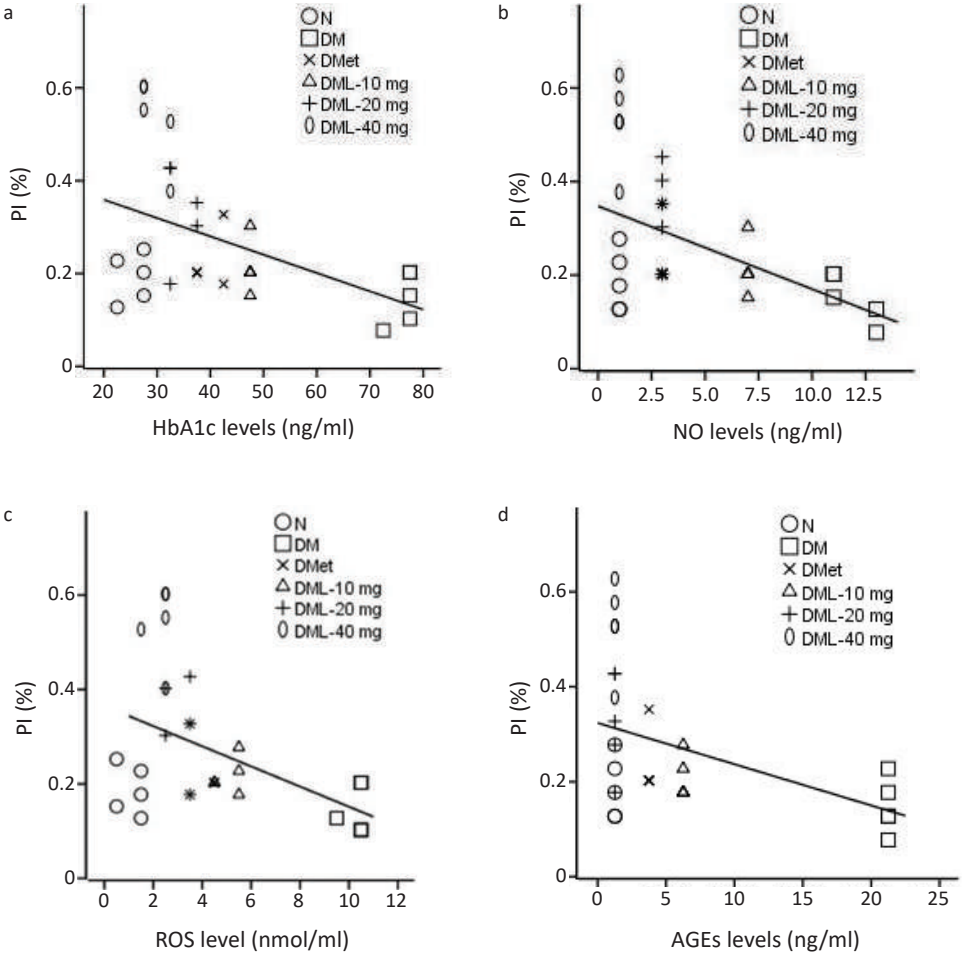

Figure 2. Linear regression of HbA1c levels (a), NO (b), ROS (c), and AGEs (d) with PI in rats with T2DM. AGEs=advanced glycation end products; DM=rats with T2DM treated with coconut oil; DMet=metformin 250 mg/kg in coconut oil; DML-10=250 mg/kg metformin + 10 mg/kg lycopene in coconut oil; DML-20=250 mg/kg metformin + 20 mg/kg lycopene in coconut oil; DML-40=250 mg/kg metformin + 40 mg/kg lycopene in coconut oil; HbA1c=glycated hemoglobin A1c; N=normal rats treated with coconut oil; NO=nitric oxide; PI=phagocytosis index; ROS=reactive oxygen species; T2DM=type 2 diabetes mellitus

and the greatest decrease was observed in the DML-40 group. HbA1c levels in the DML-20 and DML-40 groups were significantly lower than those in the DMet group, with mean differences of 5.25 and 10.55 ng/ml, respectively.

Oxidative stress

The levels of NO, ROS, and AGEs after 4 weeks of treatment are shown in Figure 1b–d. All treatment groups showed significant differences in oxidative stress ($p<0.001$). The DMet group showed a significant decrease in NO, ROS, and AGE levels compared with the DM group ($p<0.001$). The combination of metformin and lycopene treatment had a significant effect at all doses compared with the DM group. The effect was dose-dependent (Table 1), and the greatest decrease was observed in the DML-40 group.

PI

The PI of the macrophages after 4 weeks of treatment is shown in Figure 1e. The PI was significantly different in all experimental groups ($p = 0.01$). Metformin administration was associated with a slightly increased PI compared with the DM group. However, rats treated with metformin and 20 or 40 mg/kg lycopene showed significantly increased PI in macrophages ($p<0.05$). The effect of lycopene was dose-dependent, and the DML-40 group showed the greatest improvement in phagocytic function compared with the DMet group (Table 1).

Rats with lower HbA1c levels had a higher macrophage PI ($r = -0.456$) (Figure 2a). A combination of metformin and lycopene improved phagocytosis by lowering blood glucose levels in T2DM rats. Rats with lower oxidative stress levels had a higher macrophage PI, with r values for ROS, NO, and AGE levels of −0.423, −0.446, and −0.440, respectively (Figure 2b–d). Therefore, combining metformin and lycopene may improve phagocytosis by reducing oxidative stress in rats with T2DM.

DISCUSSION

This study found higher HbA1c, NO, ROS, and AGE levels and a lower PI in rats with diabetes compared with the control. In terms of improving phagocytic function, treatment with a combination of 20 or 40 mg/kg lycopene and metformin was superior to metformin alone. Lycopene can act as an immunomodulator at the cellular level and increase phagocytosis. The results of the present study are in accordance with those obtained by Durairajanayagam et al,[25] who showed that the administration of the antioxidant lycopene increased phagocytosis by reducing oxidative stress and through non-oxidative pathways by effects on the immune system.

Metformin is a hypoglycemic therapy as a first-line treatment option for patients with T2DM.[12] In line with previous studies,[13–15,20] the present study found lower HbA1c, NO, ROS, and AGE levels in diabetic rats treated with metformin, compared with the DM group. Moreover, the decrease in these levels was greater in rats treated with a combination of metformin and 20 or 40 mg/kg lycopene than in those treated with metformin alone, indicating that this combination was effective in improving HbA1c levels and reducing oxidative stress. However, a previous study revealed that a lower lycopene dosage effectively decreased AGE levels compared with the present study. Following the recommended daily dosage of 20 mg lycopene,[26] this study found that a combination of metformin and lycopene at 20 mg/kg was the minimum dose required to reduce oxidative stress and improve phagocytic function; treatment with 40 mg/kg lycopene further improved these results.

This study was limited by the fact that it was conducted only in rats. Additionally, the experiments were carried out only at the end of the intervention period (post-test only); thus, changes could not be observed in real-time. Therefore, further studies with a pre-post-test design are required to confirm the effect of the metformin and lycopene combination in humans.

In conclusion, the combination of lycopene and metformin treatment improved phagocytic function and decreased HbA1c, NO, ROS, and AGE levels in rats with diabetes. The addition of lycopene to metformin treatment regimens might increase the effectiveness of metformin in improving glycemic control, oxidative stress, and phagocytosis.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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Case Report/Series

Split cornea transplantation in anterior lamellar keratoplasty for limbal dermoid surgery: a case report

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ABSTRACT
Limbal dermoid is a rare congenital lesion that can impair vision and raise aesthetic concerns. Surgery is frequently required to reduce discomfort and enhance visual outcomes. A 20-year-old woman presented with a limbal dermoid measuring 4.5 mm in diameter and half the depth of the stroma. Excision was performed with anterior lamellar keratoplasty (ALK) using a post-Descemet's membrane endothelial keratoplasty graft, which resulted in signs of graft failure. Re-surgery was then performed with post-Descemet's stripping endothelial keratoplasty graft. It yielded a clear graft with good visual acuity. The first corneal graft utilized 95% of the graft thickness to cover 55% of the defect, leading to poor host-donor apposition. The second graft employed 55–65% to cover the same portion of the defect. The proportional thickness of the graft is crucial for a successful ALK. Split cornea transplantation produces respectable results; however, the corneal thickness must be carefully considered.

KEYWORDS cornea transplantation, dermoid cyst, host-graft reaction, keratoplasty, lamellar

Limbal dermoid tumors are benign congenital tumors of the neurological tissues, connective tissues, skin, fat, sweat glands, and lacrimal glands. It is most frequently observed in the inferotemporal region, partially across the cornea and sclera. The estimated incidence of limbal dermoid worldwide is 1 to 3 per 10,000.[1] A retrospective study in China in 2017 showed that the prevalence of limbal dermoid was about 0.177 per million people within 10 years.[2]

Anatomically, limbal dermoid is divided into three grades based on size and depth of involvement.[3] Grade I limbal dermoid is a superficial lesion on the limbus less than 5 mm in size, while grade II limbal dermoid is a larger lesion that involves most of the cornea and extends deep into the stroma without affecting Descemet's membrane. Larger lesions covering the entire cornea and extending through the structures between the anterior surface of the eyeball and the pigmented epithelium of the iris are classified as grade III limbal dermoid.[4] Deciding which technique to apply should involve an imaging study to measure lesion depth. Simple lesion excision, lamellar keratoplasty, penetrating keratoplasty, lamellar

keratoplasty with a full-thickness corneal graft, excision with a pericardial patch graft, and fibrin glue-assisted multilayered amniotic membrane transplantation, with or without a limbal allograft, are among the surgical techniques available.[5–8]

Anterior lamellar keratoplasty (ALK) can treat limbal dermoid with a thickness greater than 100 µm stroma depth and/or a diameter greater than 5 mm.[9] Previous studies have shown good results when treating limbal dermoid using the ALK technique with a partial- or full-thickness corneal graft, with minimal complications.[10,11] Shimmura et al[12] in 2004 were the first to propose component surgery for the cornea. This newly discovered surgical procedure involves replacing the affected region of the recipient's cornea with the donor cornea's lamellar buttons. This method, in which single cornea donor can be transplanted to multiple recipients, aimed to alleviate the corneal tissue shortage crisis during transplantation. Therefore, this study aimed to evaluate the surgical outcomes of limbal dermoid excision using a partial-thickness corneal graft based on split cornea transplantation in patients with ALK. This concept was the first to be applied in our setting, thus becoming a learning point for other limbal dermoid cases.

CASE REPORT

A 20-year-old woman complained of a white lump in her left eye since birth, which grew slowly, was painless, and was without visual disturbances. An elevated, well-defined, painless, and vascularized mass was found in the inferotemporal limbus measuring 4.5 mm in diameter (Figure 1a). Anterior segment optical coherence tomography (AS-OCT) revealed a mass in the corneal-limbal-conjunctiva with half the depth of the stroma (Figure 2a). ALK tumor excision was performed using a post-Descemet's membrane endothelial keratoplasty (post-DMEK) corneal graft. The remnant corneal donor after DMEK, previously split by Descemet removal, was used, leaving approximately 95% of the anterior lamellar button. A conjunctival peritomy was performed at the inferotemporal site. The lesion was dissected in a lamellar fashion using a crescent blade and blunt scissors dissection. The remnant corneal graft was then trephined to have the same diameter as the defect without adjusting the graft thickness. The graft was placed on the recipient bed and radially fixed using 12 interrupted 10-0 nylon sutures. The conjunctiva was attached to the limbus and covered half of the graft

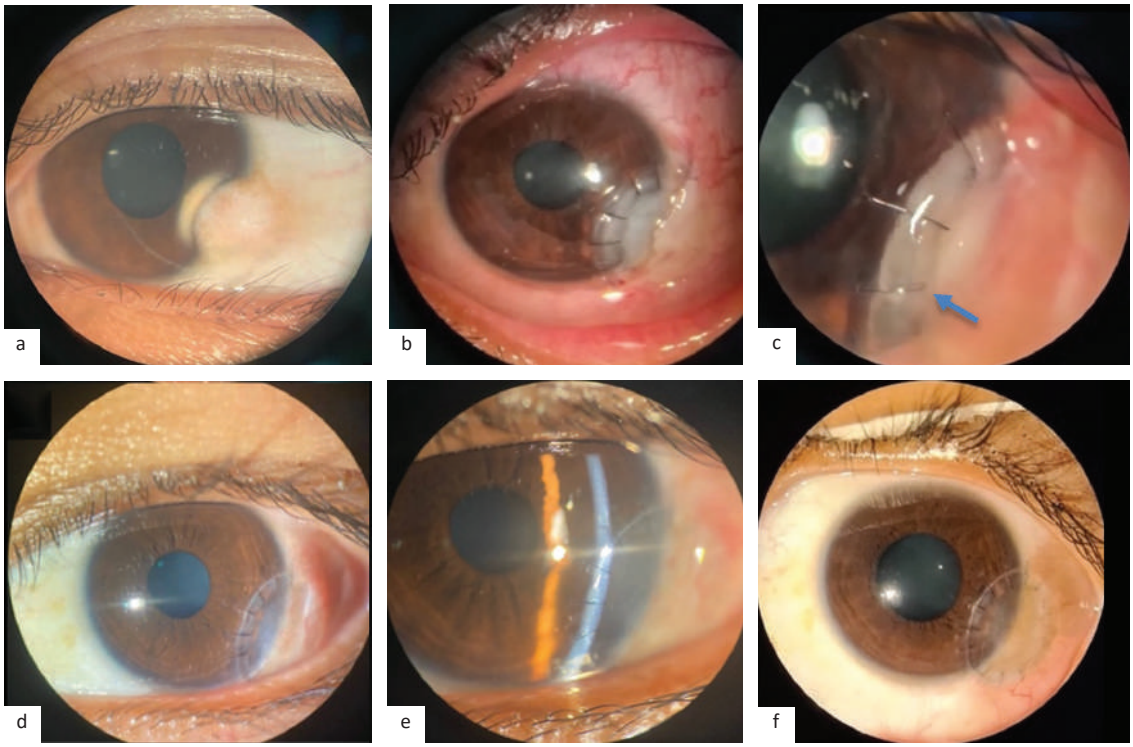

Figure 1. Slit-lamp examination of the patient's left eye. (a) Limbal dermoid before surgery; (b and c) 5 weeks after the first surgery, blue arrow showing loose suture; (d and e) 4 weeks after re-surgery, well-apposed host-graft junction; (f) 3 months after re-surgery

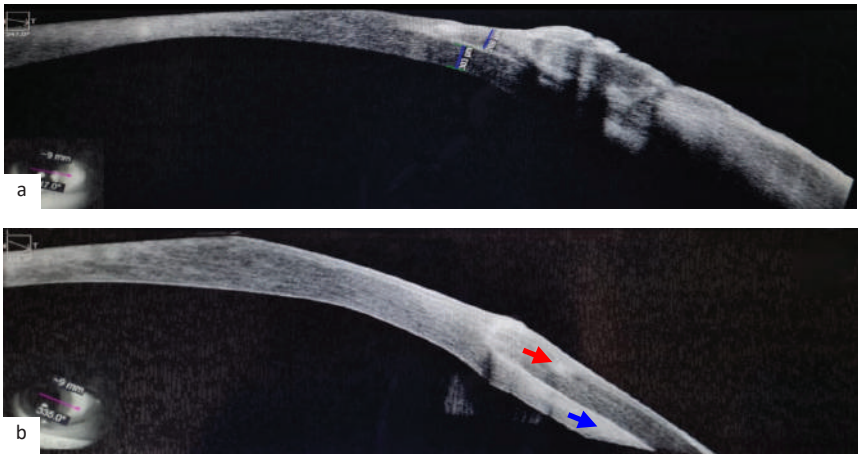

Figure 2. Anterior segment optical coherence tomography (AS-OCT) examination before surgery. (a) The thickness of extending tumor on the cornea measuring 269 µm; (b) 3 months after release and regraft showing good donor-host apposition (red arrow: donor; blue arrow: host)

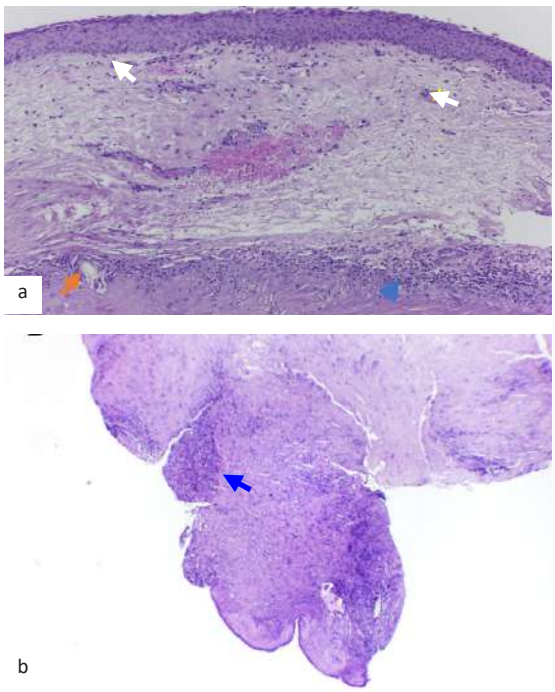

Figure 3. Histopathological examination of the first corneal graft. (a) Corneal grafts lined by hyperplastic squamous epithelial layer and subepithelial consisting of loose and fibrotic tissue, with neovascularization (white arrows), multinucleated foreign-body giant cells (orange arrow), and acute and chronic inflammatory cells (blue arrow) (H&E, 100× magnification); (b) area with dense infiltration of acute inflammatory cells (blue arrow) (H&E, 40× magnification). H&E=hematoxylin and eosin

area. Prednisolone acetate and levofloxacin eye drops were given every 3 hours postoperatively.

After 5 weeks, the graft showed overlapping signs of failure and infection (Figure 1, b and c). The patient complained of redness and foreign-body sensation in the left eye. Edema of the corneal graft, loosening of the suture with infiltration, and graft melting were noted on slit-lamp examination. An immediate plan for release and regrafting using the remnant cornea from post-Descemet's stripping endothelial keratoplasty (post-DSEK) was performed for re-treatment. The corneal tissue surrounding the host corneal defect was dissected using a crescent blade and blunt corneal scissors. The corneal donors were trephined to a defect size of 6.5 mm in diameter. The graft was placed on the recipient bed and secured using a 10-0 nylon suture for 16 radial sutures. The conjunctiva was sutured using vicryl 8-0 around it. The released graft was sent to the Department of Pathological Anatomy for histopathological examination (Figure 3).

Four weeks after re-surgery, good apposition of the host-graft junction with minimal conjunctival hyperemia was observed (Figure 1, d and e). The result at 3 months follow-up was a clear graft and well-apposed graft-host junction on slit-lamp examination (Figure 1f) and in AS-OCT (Figure 2b). Good visual acuity was observed: 0.00 logMAR with −0.50 cylindrical lens correction.

DISCUSSION

Surgery is usually recommended for grade II and III limbal dermoids because they frequently cause refractive or occlusive amblyopia. Surgical techniques include simple excision to the lamellar, penetrating keratoplasty with a corneal relaxing incision, a corneal-limbal-scleral graft transplant, and surgical

excision followed by sutureless multilayered amniotic membrane transplantation or pericardial patch graft, depending on the depth, size, and placement of the lesion.[4–8] The limbal dermoid, in this case, was categorized as deeper-Grade I since it was 4.5 mm in diameter (<5 mm) and extended half the depth of the stroma (>100 µm).[9] We performed partial thickness corneal splitting, followed by lamellar delineation and partial thickness corneoscleral graft implantation in accordance with the recommendation from Pirouzian et al's[9] study on pediatric corneal limbal dermoid management. Lamellar excision of large limbal dermoid with matching partial thickness corneoscleral grafts is safe and provides good cosmetic and tectonic stability without postoperative astigmatism.[10] However, simple keratectomy with or without tattooing may be sufficient if the dermoid is relatively superficial. However, in this case, the dermoid was lodged in the cornea, and simple keratectomy was insufficient. Penetrating keratoplasty is indicated when the dermoid involves the anterior chamber.

Deep anterior lamellar keratoplasty (DALK) has several theoretical advantages over penetrating keratoplasty, including retention of the recipient's healthy endothelium, maintenance of Descemet's membrane integrity, and reduce intraocular surgical complications, which minimizes allograft endothelial rejection and reduces the need for immunosuppression. Lamellar keratoplasty of the limbal dermoid also decreases the incidence of postoperative sequelae, such as corneal opacity, neovascularization, and pseudopterygium. However, DALK is a more time-consuming and technically challenging procedure that might cause interface irregularity and scarring, resulting in lower best-corrected visual acuity and a higher risk of early postoperative interface haze and inflammation.[13,14]

In the present case, during observation from the first day after surgery until 3 weeks follow-up, the graft was clear without edema. However, at 5 weeks follow-up, it showed signs of graft failure, possibly due to graft infection or rejection. According to the literature, the process of graft rejection starts at 3 weeks (10 days) in a successful clear graft. During these periods, follow-ups every week or 2 weeks are advisable to detect signs of failure earlier. Because the corneal endothelium is not transplanted in the ALK technique, endothelial rejection cannot occur and usually responds to corticosteroid therapy.[15] However, in this case report, the etiology of failure was doubtful, stroma rejection showed significant haze, neovascularization occurred, and the graft was released.

Ideally, a histological examination should be performed for every tissue removed from the human body for a definitive diagnosis.[16] Histopathological examination of the first graft should be performed to determine whether the failure was caused by infection or an immunological reaction. The full-thickness donor tissue was lined with hyperplastic squamous epithelium and sub-epithelium consisting of loose and fibrotic tissue. The graft tissue is also infiltrated with mild-to-moderate acute and chronic inflammatory cells, along with foreign-body giant cells and blood vessel proliferation. These findings correlated with possible graft rejection. Graft rejection after ALK/DALK is likely caused by infection (herpetic keratitis, bacterial infection, or fungal infection) or immunological reactions.[13,16,17] Microscopic investigation of corneal transplant failure demonstrates loss of the epithelial basement membrane with invasion of monocytes, lymphocytes, plasma cells, and fibroblasts into the stroma. Alterations in stromal keratocytes may occur when they come in contact with lymphocytes. New capillary development was observed in the anterior and middle layers of the stroma, with immunoblast-like cells in the rejection region. Lymphocytes and plasma cells are predominantly found outside of the endothelial capillary wall.[15] The presence of multinucleated giant cells in the graft can result from persistent inflammation caused by foreign material presence or ongoing infections.[18,19] The donor corneal cells do not appear to be involved in corneal graft healing; instead, healing appears to be a function of the recipient corneal cells. Impairment of this delicate equilibrium during corneal wound healing can result in pathological corneal vascularization.[20–22] Lymphatic vessels form near the corneal limbus under both normal and pathological conditions. Lymphatic vessels can emerge abruptly in the corneal stroma and are not related to the lymphatic vessels in the limbus. Thus, graft failure is associated with interfacial vascularization and stromal opacification.[23]

Previous case reports have successfully demonstrated that a single-donor cornea can be used for multiple recipients in various types of corneal transplantations, including ALK, posterior lamellar keratoplasty, and limbal stem cell transplantation.

The techniques for splitting the cornea vary among studies.[24–27] Sati et al[28] used a manual dissection technique from a single-donor corneal button for three recipients, while Sharma et al[25] dissected the corneal donor with a microkeratome. Manual dissection is considered inferior to microkeratome dissection, but both corneal splitting techniques yield successful visual acuity and cosmesis outcomes.[25,28] Shen et al[11] used full-thickness central corneal grafts in 10 patients with limbal dermoids undergoing lamellar keratoscleroplasty. The results showed good cosmetic outcomes with minimal postoperative complications. All patients had relatively deep lesions, and lamellar dissection was performed until a clear cornea was reached, accounting for approximately 70–80% of the corneal thickness. Owing to the depth of the excised hole, lamellar dissection of the central corneal graft was not performed, and no tissue overriding occurred after surgery.[11]

In the present study, the first corneal graft was obtained from a post-DMEK graft and dissected manually. It left approximately 95% of the corneal thickness to fill 55% of the recipient's corneal defect, resulting in poor host-donor apposition. Regrafting a post-DSEK graft of 55–65% thickness yielded smooth host-donor apposition, good cosmesis, and minimal postoperative astigmatism. Complications after the first surgery possibly occurred because of the thickness difference between the donor graft and the host cornea, which hampered the healing process. Disturbance of the corneal epithelium and anterior stromal integrity during subsequent wound healing promoted the formation of new vessels inside the donor stroma and hastened transplant rejection. It is essential to determine the depth of corneal penetration by the tumor before deciding on the surgical approach. If lamellar keratoplasty is performed, the donor cornea must be trimmed to match the remainder of the patient's cornea after tumor removal.

To date, no case reports have described the use of split-corneal donors in Indonesia. This case report will be useful for other ophthalmologists applying this method. Subsequently, this may reduce the use of donor corneas, which is helpful as most donor corneas in our setting are donated overseas. Split cornea transplantation is a promising strategy for alleviating the corneal tissue shortage crisis in Indonesia. Future studies with longer follow-up durations are needed to evaluate the stability of corneal grafts.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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Basic Medical Research

DNA quality from buccal swabs in neonates: comparison of different storage time

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ABSTRACT

BACKGROUND Genomic medicine has great potential for diagnoses, disease prediction, and targeted treatment. Buccal swabs are a suitable non-invasive method for neonates to obtain DNA samples. Due to Indonesia's geographical conditions, samples require a prolonged time to reach the genetic laboratory. This study aimed to compare the DNA quality of buccal swabs in neonates between immediate and after-storage extraction.

METHODS This study was part of a study about the profile of human milk oligosaccharide and *FUT2* genotype in Indonesian mother-infant dyads consisting of 20 neonates. 1 swab stick for each participant was taken using a standardized buccal swabbing protocol and divided into 2 isovolume aliquots, which were grouped into the immediate (extraction was performed within 3 days after sampling) and storage groups (extraction was performed on the 14th day after storage in 4°C). DNA yield and purity $A_{260/280}$ ratio were measured by spectrophotometry. The PCR amplification and Sanger sequencing were performed to validate the DNA isolate quality for downstream application.

RESULTS The DNA yield for the immediate group was similar compared with the storage group (9.50 [4.89] *versus* 9.10 [5.05] µg), $p = 0.659$, as well as DNA purity $A_{260/280}$ (1.58 [0.24] *versus* 1.56 [0.28]), $p = 0.785$. PCR and sequencing of FUT2 results also showed similar quality between both groups.

CONCLUSIONS The similar DNA quality and sequencing results between immediate and storage extraction confirmed that buccal swabs could be stored for 2 weeks, allowing ample time for sample shipping from remote areas to the laboratory.

KEYWORDS buccal, DNA, isolation, quality, swabs

Understanding the genetic basis of disease has substantial healthcare benefits. Exploring single-nucleotide variations to explain human variations in metabolism, physiology, and disease risk factors is another cutting-edge development in the genomic medicine era. Obtaining genetic material for analysis is thus essential and has broad implications for understanding disease pathogenesis, establishing a diagnosis for complicated cases, and designing individualized therapies.[1]

Buccal swabs are a suitable non-invasive method for obtaining DNA samples from neonates. However, the literature regarding DNA quality from buccal swabs has yielded various results. Siswanto et al[2] reported

much lower DNA concentration in buccal swab than in the whole blood of premature infants. In contrast, Said et al[3] obtained a significantly higher DNA yield from buccal epithelial cells than that from the whole blood of premature infants. Moreover, buccal swabs pose additional concerns regarding storage time and temperature. As Indonesia has numerous islands, DNA samples often require a prolonged time to reach the genetic laboratory. Various studies have recommended different storage duration and temperature for buccal swabs. Navarro et al[4] mentioned that buccal swabs could be stored for up to 2 weeks at 4°C before processing without a noticeable loss in DNA yield or quality. Rogers et al[5] kept buccal swabs at –80°C before extraction but did not compare the storage time. Grujičić et al[6] compared three groups of different storage time from buccal swabs kept at room temperature. Therefore, this study aimed to compare the DNA quality between immediate and after-storage extractions and to validate the DNA isolate with Sanger sequencing between the immediate extraction (within 3 days) and 2-week-storage extraction groups. This result will help justify the storage of buccal swabs from remote areas before shipment to a genetic laboratory.

METHODS

This research was a part of the main study aiming to learn the profile of human milk oligosaccharides and *FUT2* genotype in Indonesian mother-infant dyads. The primary study recruited 120 mother-newborn dyads from Bunda Mother and Child Hospital, Jakarta, Indonesia. However, the participants of this study were a subset of the last 20 participants. All participants provided informed consent. The DNA extraction, polymerase chain reaction (PCR), and sequencing were conducted at the Human Genetic Research Center, Indonesian Medical Education and Research Institute (IMERI), Faculty of Medicine, Universitas Indonesia, from December 2021 to August 2022. This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (No: KET-838/UN2.F1/ETIK/PPM.00.02/2021).

Sample collection and storage

Samples were collected by rubbing the inside of the right and left cheeks 10 times each without touching the tongue or lips, as described in the kit manual. The ORAgene® OraCollect OCR-100 (DNA Genotek, USA)[7] used in this study is designed for DNA collection in pediatric patients and is painless because of its soft sponge-tip properties. The swab was directly placed into the 500 µl preservation reagent in the kit and shaken vigorously 10 times.[7] One swab stick was taken for each participant. The swabbing was performed by a trained physician who collected numerous samples for newborns in the main study. The 500 µl-raw samples were divided into two aliquots (250 µl each) for each participant. One aliquot was assigned to the immediate group (extracted within 3 days after sampling) and the other to the storage group (extracted on the 14th day after sampling) which was kept in 4°C refrigerator.

DNA extraction from buccal swabs

Raw samples (250 µl) were put into a 1.5 ml microtube. As much as 20 µl of PrepIT.L2P reagent (DNA Genotek) was added to the sample. The microtubes were vortexed for 10 sec to ensure that the reagent and raw samples were homogenized. The samples were incubated for 10 min in grated ice cubes and then centrifuged (Eppendorf 5415R, Germany) for 5 min with 10,000 × *g* (room temperature); the supernatant was transferred to a fresh, sterilized microcentrifuge tube. As much as 400 µl absolute ethanol (Merck, Germany) was added and mixed thoroughly by inverting the tubes 10 times to precipitate the DNA. The samples were incubated at room temperature (24–26°C) for 10 min and then centrifuged (Eppendorf 5415R) at 10,000 × *g* (room temperature) again for 2 min. The upper aqueous layer was discarded, and the pellet was stored in microtubes. After decanting the supernatant, 250 µl of 70% ethanol (Merck) was added carefully, and the sample was incubated for 1 min. After 1 min of incubation, the solution was removed. Note that no solution should remain in the microtubes. The pellet was then resuspended in 80 µl 10 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid (EDTA) (TE) buffer, and frozen at –20°C or –80°C for further use.

Concentration and purity determination

DNA quality was determined by DNA yield (concentration) and purity. A quantitative spectrophotometric assay of DNA was performed using a Varioskan microplate reader (Thermo Fisher Scientific, USA) to measure the DNA yield and purity.[8] Absorbance was measured at wavelengths of 260 and 280 (A_{260} and A_{280}, respectively) nm. The absorbance quotient ratio (OD_{260}/OD_{280}) was used to express DNA

purity. An absorbance quotient ratio between 1.8 and 2.0 was considered good for purified DNA. A ratio of <1.8 indicated protein contamination, whereas a ratio of >2.0 indicated RNA contamination.[9]

PCR and Sanger sequencing

PCR for the *FUT2* gene was performed using a primer pair from Lefebvre et al[10] and its sequences as follows: forward 5'ACACACCCACACTATGCCTG'3 and reverse 5'AAGAGAGATGGGTCCTGCTC'3. MyTaq HS Redmix (Meridian Bioscience, USA)[11] was used as a PCR mix. The PCR program consisted of pre-denaturation at 95°C for 1 min, denaturation at 95°C for 15 sec, annealing at 65°C for 15 sec, extension at 72°C for 10 sec, repeated for 35× cycles, and completed by a final extension at 72°C for 10 min.[11] Agarose gel electrophoresis was performed to verify the quality of the PCR products. The PCR products were separated on a 0.8% agarose gel at 100 volts for 45 min. The gels were visualized using a gel documentation system (Accuris Instruments, USA).[12]

RESULTS

DNA was successfully extracted from all samples. The DNA yield for the immediate group was similar from that of the storage group (9.50 [4.89] *versus* 9.10 [5.05] µg, respectively; $p = 0.659$). The DNA purity $A_{260/280}$ ratio was also similar between the immediate and storage groups (1.58 [0.24] *versus* 1.56 [0.28], respectively; $p = 0.785$). A comparison between several studies comparing DNA yield at different storage time and temperature is detailed in Table 1. DNA purity showed no difference between the groups (Table 2).

To evaluate the impact of slight differences in the DNA yield and purity between the immediate and storage groups on the downstream process, PCR and Sanger sequencing results were compared to capture the coding region of the *FUT2* gene. Visualization of the PCR products indicated the successful amplification of specific fragments with a size of 1,238 bp, as shown in Figure 1. In addition to successful target amplification, we did not observe the significant appearance of multiple bands, dimers, smears, and off-target fragments. This suggests that even at a purity lower than the recommended value, the presence of chemical contaminants did not significantly interrupt fragment amplification.

Sanger sequencing was performed on each participant to validate whether the DNA quality was sufficient for downstream applications. Sample

Table 1. Comparison of DNA yield from buccal swabs based on storage time and temperature

References	Population (n)	Storage temperature	DNA yield (ng/µl)		
			Immediate extraction	Storage group	
			Day 0–3	Day 7	Day 14
This study, mean (SD)	Neonates (20)	4°C	9.50 (4.89)	NA	9.10 (5.05)
Mulot et al,[13] mean (range)	Adult (20)	−20°C	3.4 (0.4–8.5)	3.5 (0.9–9.0)	NA
Grujičić et al,[6] mean (range)	Adult (7)	4°C or −20°C	3.91 (1.54–8.3)	3.98 (1.25–8.1)	4.29 (0.87–7.9)
Ghatak et al,[9] mean (SD)	Adult (5)	4°C or −20°C	3.55 (0.60)	2.53 (0.31)	NA

NA=not available; SD=standard deviation

Table 2. Comparison of DNA purity ($A_{260/280}$) from buccal swabs based on storage time

References	Population (n)	DNA purity ($A_{260/280}$), mean (SD)		
		Immediate extraction	Storage group	
		Day 0–3	Day 7	Day 14
This study	Neonates (20)	1.58 (0.24)	NA	1.56 (0.28)
Mulot et al[13]	Adult (20)	NA	1.6 (0.2)	NA
Grujičić et al,[6] mean (SD), range	Adult (7)	1.68 (0.15), 1.45–1.88	1.64 (0.16), 1.37–1.88	1.67 (0.19), 1.32–1.88
Ghatak et al[9]	Adult (5)	1.60 (0.05)	1.70 (0.09)	NA

NA=not available; SD=standard deviation

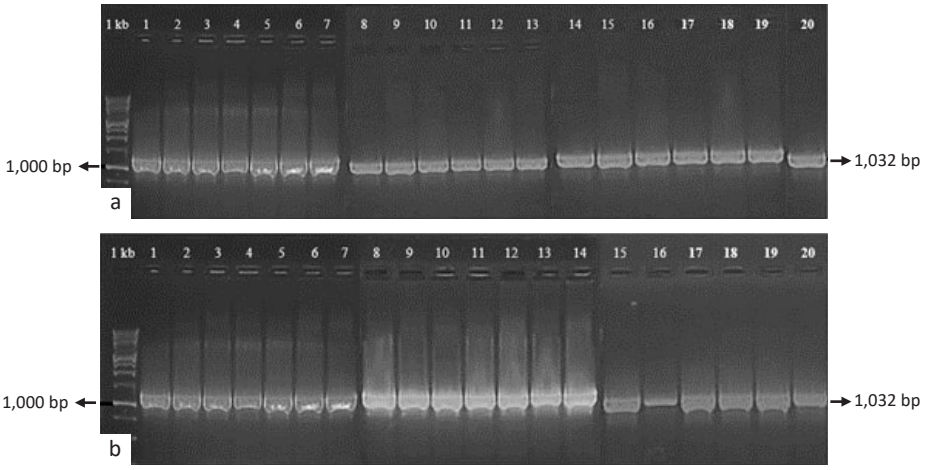

Figure 1. Comparison of polymerase chain reaction (PCR) products extracted (a) within 3 days and (b) on day 14 after sampling

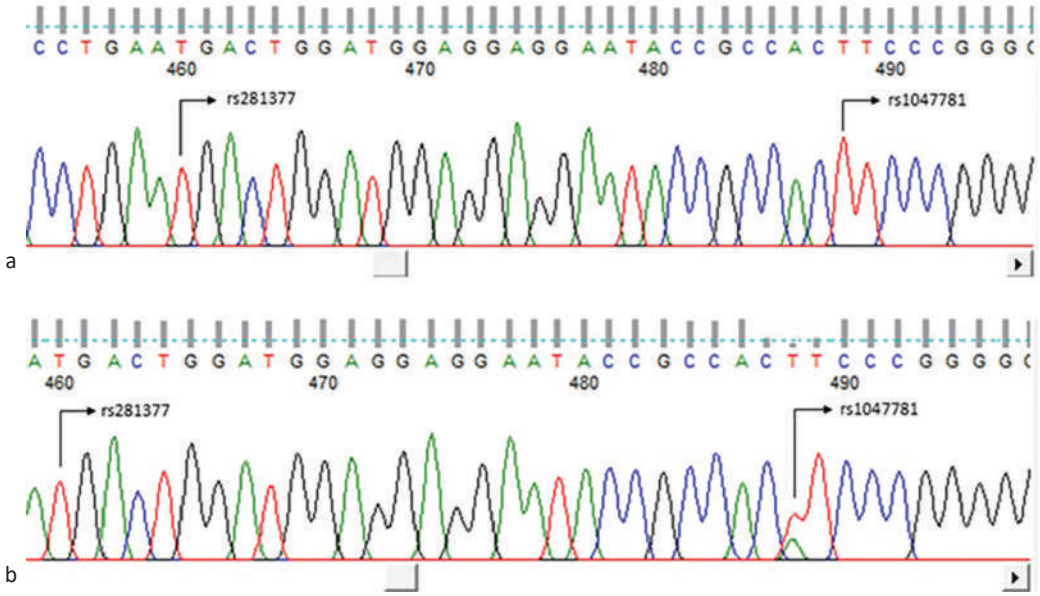

Figure 2. Sanger sequencing results for (a) participant 2: DNA extraction within 3 days and (b) participant 5: DNA extraction after 2 weeks in a 4°C refrigerator

aliquot were randomly assigned to each participant for sequencing. Sequencing was performed using both forward and reverse primers to increase confidence in base calling. High-quality sequencing results were obtained for both groups (Figure 2). No significant noise was observed in the baseline sequence. The average quality value was >20 for a single base and >10 for a mixed base.

DISCUSSION

In this study, the DNA yield and purity $A_{260/280}$ were similar between the immediate and storage groups. This result aligns with three previous studies that compared the impact of different storage time and temperature on DNA quality from buccal swabs. Grujičić et al[6] showed no significant differences in the yield and purity of isolated DNA between three different storage times (day 0, day 7, and day 14). Mulot et al[13] also obtained insignificant results for the DNA yield of cytobrush buccal samples stored at room temperature for 2, 5, and 7 days. Ghatak et al[9] also found no difference in the DNA yield between immediate processing and 1-week storage at 4°C and –20°C. However, these studies were performed in adults, whereas the present study was the first to be conducted in neonates.

Differences in swab procedures, swab kits, and raw sample volumes might explain the variation

in the DNA yield. Livy et al[14] obtained a higher DNA yield of 21.03 (17.1), compared with the yield in this study, primarily due to a larger raw sample volume of 400 µl, whereas this study used 250 µl. Ghatak et al[9] used one cotton swab and put the sample in 500 µl of self-prepared fluid consisting of 10 mM Tris (pH 8.0) + 10 mM EDTA + 2% sodium dodecyl sulfate. Despite using a larger raw sample volume, Ghatak et al[9] achieved a lower DNA yield than the present study. This difference could be attributed to the swabbing technique used. In contrast to the swabbing technique used by Ghatak et al[9] in which the participants swabbed themselves, swabbing in this study was conducted by a trained physician following a standardized protocol, as described in the kit manual. In the storage time aspect, immediate extraction in Ghatak et al's[9] study resulted in a higher average yield of 3.55 (0.60) µg than samples that went to storage before processing (2.53 [0.31] µg). This was probably due to DNA degradation by the bacteria and nucleases in the buccal epithelial cell samples.[4] Another study by Said et al[3] in 85 premature infants implied that the average DNA yield from buccal swabs was 25.5 µg (range 8.95 to 42.1 µg), much higher than the present study that obtained a DNA yield of 9.50 µg for immediate extraction and 9.10 µg for after-storage extraction. This was probably because Said et al[3] used two cotton swab sticks (cytology brushes) with 900 µl cell lysis fluid, whereas this study used one cotton swab with 500 µl preservation reagent, as included in the ORAgene® OraCollect OCR-100 (DNA Genotek) swab kit. Interestingly, significantly higher DNA yields were obtained from buccal swabs than from blood.

All studies revealed DNA purity below the recommended value, between 1.8 and 2.0. Livy et al,[14] who did not compare storage time, also obtained low $A_{260/280}$ purity (1.33 [0.32]) from adult buccal swabs. Although the present study showed lower DNA purity, the sequencing results were of high quality (Figure 2). Good sequencing results despite lower than recommended DNA purity was also demonstrated by Grujičić et al.[6]

According to previous studies, the recommended $A_{260/280}$ nucleic acid purity is in the 1.8–2.0 ratio. A lower $A_{260/280}$ purity ratio may indicate the presence of protein contaminants that may inhibit downstream applications.[15] The difference in DNA $A_{260/280}$ purity result might be due to the DNA extraction method. We used conventional methods to extract DNA, whereas Livy et al[14] used QIAamp DNA Blood Kits (Qiagen, Netherlands). The reagents used in this study consisted of PrepIT.L2P DNA reagent (DNA Genotek) for sample preparation, ethanol 100% for precipitation, ethanol 70% as a wash buffer, and TE solution as a DNA elution buffer. The QIAamp DNA Blood Kit (Qiagen) included a lysis buffer, wash buffers 1 and 2, and an elution buffer. Using a column during DNA extraction might increase the DNA purity since the column captures DNA better and leaves the impurities in the flow-through.[16] In addition to that, adding the wash buffer twice also improves the DNA $A_{260/280}$ purity.[17] To prevent food contamination to purity, the participants were asked to rinse their mouths thoroughly and refrain from eating for around 45 min. In this study, infants were required not to be breastfed for at least 45 min before swabbing; therefore, the possibility of breast milk contamination was considered very low. The PCR product from a buccal swab was sequenced to validate whether the DNA isolate could be used for genetic analysis. The sequencing quality was at a high confidence level for variant analysis, represented by two common single-nucleotide variants of rs281377 and rs1047781 in the *FUT2* gene, which were superimposed on both DNA sample groups.[18,19]

In conclusion, buccal swabs could be stored for a certain period without affecting DNA quality, which benefits health services and neonatology research. Buccal swabs have great potential as a non-invasive sampling method for genetic analyses in neonates.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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