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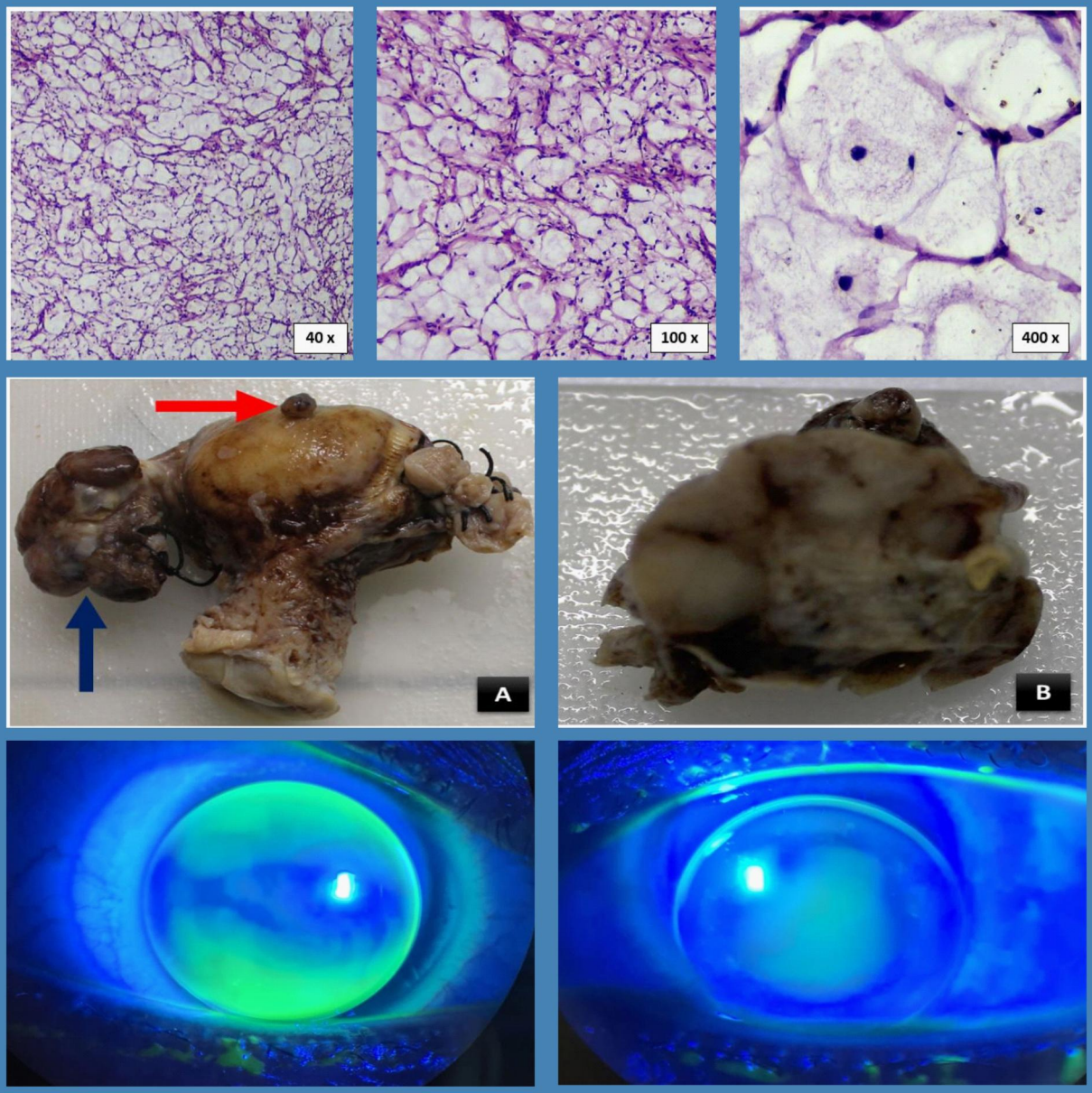

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Development of a SYBR Green real-time PCR-based assay system for detection of *Neisseria gonorrhoeae*

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ABSTRACT

Diagnosis of *Neisseria gonorrhoeae* infection is needed for patient therapy and for reducing this bacterial transmission in the population. The culture method is a gold standard method for *N. gonorrhoeae* detection, however, it has low sensitivity. Among molecular methods with high sensitivity and specificity, SYBR Green real-time PCR is the potential method for *N. gonorrhoeae* detection. In this study, we developed an SYBR Green real-time PCR-based system assay for *N. gonorrhoeae* detection. Several PCR conditions were optimized and analyzed including primer annealing temperature, DNA template volume, the limit of detection (LoD), cross-reaction with others (bacteria, viruses, fungus, and protozoa), and quality assurance. The results showed that the annealing temperature and DNA template volume were 60°C and 5 µL, respectively. The LoD was 29 DNA copies corresponding to 3 bacterial cells per reaction. No cross-reaction was detected for other bacteria, viruses, fungus, and protozoa. The external quality assurances enrolled in 2019 and 2021 showed 100% concordance. The preliminary testing for clinical samples was also 100% concordance. In conclusion, the SYBR Green real-time PCR-based system assay developed in this study is promising for application in clinical laboratories.

ABSTRAK

Diagnosis infeksi *Neisseria gonorrhoeae* diperlukan untuk pengobatan pasien dan untuk menurunkan peneyebarannya dalam populasi. Metode kultur merupakan standar emas untuk deteksi *N. gonorrhoeae,* namun sensitivitasnya rendah. Di antara metode molekular dengan sensitivitas dan spesifisitas tinggi, *SYBR Green real-time PCR* adalah metode yang potensial untuk deteksi *N. gonorrhoeae*. Dalam penelitian ini sebuah metode pengujian berbasis *SYBR Green real-time PCR* telah dikembangkan. Beberapa kondisi PCR dioptimasi dan dianalisis termasuk suhu annealing primer, volume templat DNA, limit deteksi (LoD), reaksi silang dengan mikroorganism lain seperti bakteri, virus, jamur, dan protozoa serta uji jaminan kualitasnya. Hasil penelitian menunjukkan suhu *primer annealing* dan volume DNA templat berturut-turut adalah 60 °C dan 5 µL. Batas deteksi (LoD) DNA adalah 29 salinan atau sekitar 3 sel bakteri per reaksi. Tidak ada reksi silang dengan bakteri lain, virus, jamur, dan protozoa. Kesesuaian pemantapan mutu eksternal yang diikutkan pada tahaun 2019 dan 2021 mencapai 100%. Pengujian tahap awal untuk sampel klinis juga menunjukkan hasil 100%. Dapat disimpulkan, pengembangan metode pengujian berbasis *SYBR Green real-time PCR* dalam penelitian ini menjanjikan untuk diaplikasikan dalam laboratorium klinis.

Keywords:
Neisseria gonorrhoeae;
PCR;
SYBR Green;
sensitivity;
specificity

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INTRODUCTION

Gonorrhea is a sexually transmitted disease (STD) caused by *Neisseria gonorrhoeae*.[1] In the male, the disease is usually symptomatic such as urethral discharge, dysuria, and testicular pain.[2] In females, gonorrhea is often asymptomatic and the individuals may not be aware of it.[2,3] However, some individuals are symptomatic such as vaginal discharge, dysuria, lower abdominal and/or rectal pain, dyspareunia, and abnormal uterine bleeding.[2] Untreated gonorrhea in females can cause pelvic inflammatory disease that will lead to chronic pelvic pain and cause infertility and ectopic pregnancy.[2,4]

In 2016, WHO reported 87 million new cases occurred among adolescents and adults aged 15-49 years worldwide, with a global rate of 20 per 1000 women and 26 per 1000 men.[5] The highest prevalence of gonorrhea in the African region was 1.9% (women) and 1.6% (men), followed by other regions, namely America was 0.9% (women) and 0.8% (men), Western Pacific was 0.9% (women) and 0.7% (men), and Europe was 0.3% in women and men.[6] In Europe, ECDC reported 100.673 confirmed gonorrhea cases in 2018. The highest rates (>30/100000 population) were reported in the United Kingdom, Ireland, Denmark, and Norway. Meanwhile, the lowest rates (<1 per 100 000) were reported in Bulgaria, Croatia, Cyprus, Poland, and Romania.[7] In the United States, there were 616.392 gonorrhea cases in 2019, which prevalence in men is higher than in women.[8] In South-East Asia, gonorrhea had decreased by two-thirds, from 118 million in 1990 to 39 million in 2012.[9,10] In Indonesia, the prevalence of gonorrhea is still high in asymptomatic cases in men (56.2%), women (33.0%), and trans-women (10.8%).[11]

To prevent and reduce gonorrhea transmission, screening, and testing of risk populations should be addressed.[12] Early diagnosis is important to perform for asymptomatic and symptomatic individuals.[13] There are some microbiological methods to support the diagnosis of gonorrhea, such as culture (gold standard), microscopy, biochemical test, chromogenic enzyme substrate test, immunoassay, and nucleic acid method.[12,14] The ideal diagnostic test for screening *N. gonorrhoeae* should be sensitive, specific, easy to use, rapid, and affordable.[12]

Among those alternatives, nucleic acid amplification technologies (NAATs) based on the real-time Polymerase Chain Reaction (qPCR) method are the best solution. It is a rapid, sensitive, and specific method to identify the *N. gonorrhoeae* infection.[12,14] There are two types of qPCR assays, SYBR Green and Taqman probe. The Taqman probe assay is based on double (fluorescence and quencher) labeled oligonucleotide, whereas the SYBR Green assay is based on the binding of the florescent dye to dsDNA.[15] Both methods are sensitive and specific to detect a specific gene target.[16] However, SYBR Green is simpler and more economical than the Taqman probe assay.[17] Therefore, in this study we developed an assay system based on SYBR Green qPCR for the detection of *N. gonorrhea*.

MATERIALS AND METHODS

Clinical specimen

Twenty cervical swab samples (Collection of Clinical Microbiology Laboratory, Faculty of Medicine Universitas Indonesia) were used for a preliminary trial of the optimized assay system developed in this study. The 10 samples have been confirmed as *N. gonorrhoeae* positive and the other 10 samples have been confirmed as *N. gonorrhoeae* negative. The confirmation

test was conducted by VITEX 2 NH (BioMerieux). This study was approved by the Ethics Committee, Faculty of Medicine, Universitas Indonesia (KET-667/UN2.F1/ ETIK/PPM.00.02/2020).

Positive control

Genomic DNA of *N. gonorrhoeae* ATCC 43069 was used as a positive control for optimization of SYBR Green qPCR assay developed in this study.

DNA extraction

DNA extractions from cervical swab samples and isolate *N. gonorrhoeae* ATCC 43069 were performed by using QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instruction, with 40 μL final elution. The extracted DNA samples were stored at -35°C.

Primers

Forward (5'-GTT GAA ACA CCG CCC GG-3') and reverse (5'-CGG TTT GAC CGG TTA AAA AAA GAT-3') primers were used in qPCR assay as reported by Geraarts-Peters *et al.*[13]

SYBR Green qPCR assay

The qPCR Assay was performed by the following formulation (20 μL of reaction volume): 1x SensiFAST SYBR No-ROX Mix (Bioline, Cat. no: BIO-98005), 0.3 μM each of primer, and 4 μl of DNA template. The thermal cycles were performed by the following condition (LC96, Roche): 95°C for 3 min; 45 cycles at 95°C for 10 sec, 60°C for 30 sec, and 72°C for 30 sec; and temperature melting (Tm) analysis following standard machine setting (LC96, Roche).

Optimization of primer annealing temperature and DNA template volume

To obtain the optimal primer annealing temperature, a PCR gradient was performed using a range of annealing temperatures (54, 55.5, 58, 60.7, 62.9, and 64°C). A comparison of DNA template volume was performed to know the possible PCR inhibitor contained in elute solution of the DNA extraction result. The PCR inhibitor analysis was performed by comparing the DNA template volumes of 3, 4, and 5 μL.

Limit of detection (LoD)

LoD of the qPCR was determined by serial dilution of DNA concentration. Minimal DNA detection was defined as the lowest DNA concentration which can be detected by the qPCR. To obtain the DNA copy number as LoD, Avogadro's calculator was used (cels.uri.edu/gsc/cndna.html).

Cross-reaction over other microorganisms

The potential cross-reaction of the qPCR assay was evaluated against bacteria, fungi, viruses, and protozoa.

Internal and external quality assurance

Internal quality assurance of qPCR assay was conducted by using negative and positive controls of *N. gonorrhoeae* ATCC 43069 DNA genomes. External quality assurance was assessed by QCMD EQA Management System (Scotland, The United Kingdom) in 2019 and 2021.

RESULTS

SYBR Green qPCR assay

To obtain the optimal reaction formulation and condition, several parameters were optimized, including annealing temperature, DNA template volume, LoD, and potential cross-reaction with other microbes. Based on the annealing temperature analysis

result, the melting peaks resulting from temperatures 55.5 (E), 58 (C), and 60.7°C (D) showed the overlapped peaks (FIGURE 1A). Considering the specificity of primers, we set the annealing temperature at 60°C. For DNA template volume, the melting peak from the DNA template volume of 5 μL was slightly above the melting peak from that of 4 μL (FIGURE 1B); therefore, we set the DNA template volume at 5 μL.

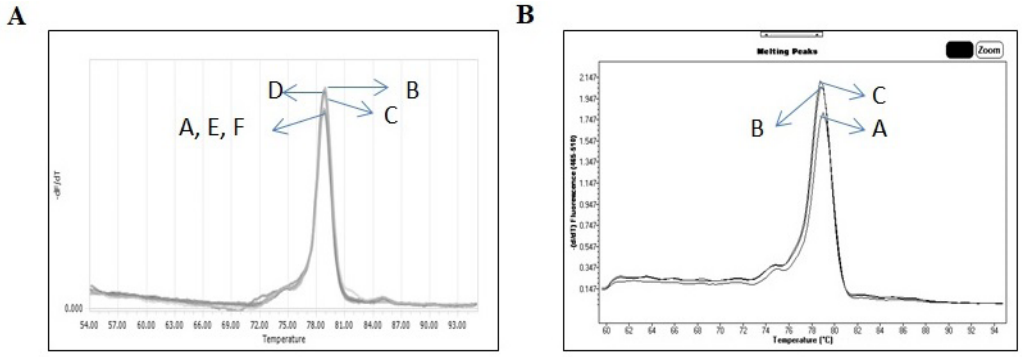

FIGURE 1. The qPCR results. **A)** the gradient annealing temperatures of 54 (A), 55.5(B), 58 (C), 60.7 (D), 62.9 (E), and 64°C (F). **B)** the DNA template volumes of 3 (A), 4 (B), and 5 μl (C).

The specificity of the primers used in the qPCR was *in silico* analyzed on Primer-BLAST GenBank. The results showed that the primers only bind with the *N. gonorrhoeae* (data not shown) target gene. Consistently, the qPCR assay was specifically detected *N. gonorrhoeae* with no cross-reaction with other organisms such as bacteria (*N. meningitidis, Mycobacterium tuberculosis, Chlamydia trachomatis, M. leprae, Bordetella pertussis, C. pneumonia, Helicobacter phylori, Legionella pneumophilae, Leptospira, Mycoplasma pneumonia, Salmonella enterica, Streptococcus pneumonia*), fungi (*Pneumocystis jirovecii*), viruses (Cytomegalovirus, Herpes Simplex Virus, Epstein-Barr Virus, Varicella Zoster Virus, *Influenza A & B, Rubella* virus), and protozoa (*Toxoplasma*) (FIGURE 2).

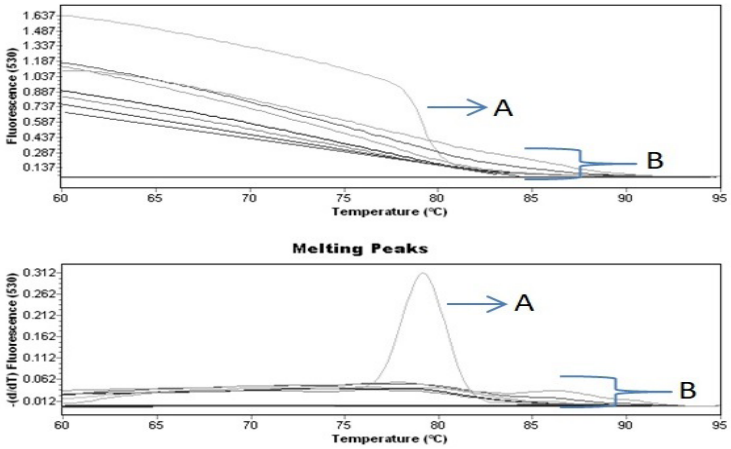

FIGURE 2. The qPCR Results (top [melting curve] and bottom [melting dynamic] images) for bacteria, viruses, fungi, and protozoa. A: The qPCR Result for the positive control (DNA genome of *N. gonorrhoeae* ATCC 49226). B: The qPCR Results for microbes (bacteria, viruses, and fungi) and protozoa.

The LoD of qPCR showed a highly sensitive assay, namely 25 x 10^{-10} ng DNA (FIGURE 3). Estimation using Avogadro's formulation, the 25 x 10^{-10} ng DNA yielded 29 DNA copies. Based on the number of *opa* gene alleles (11 copies) in *N. gonorrhoeae* (18), the LoD was thus corresponding to 3 cell/reaction.

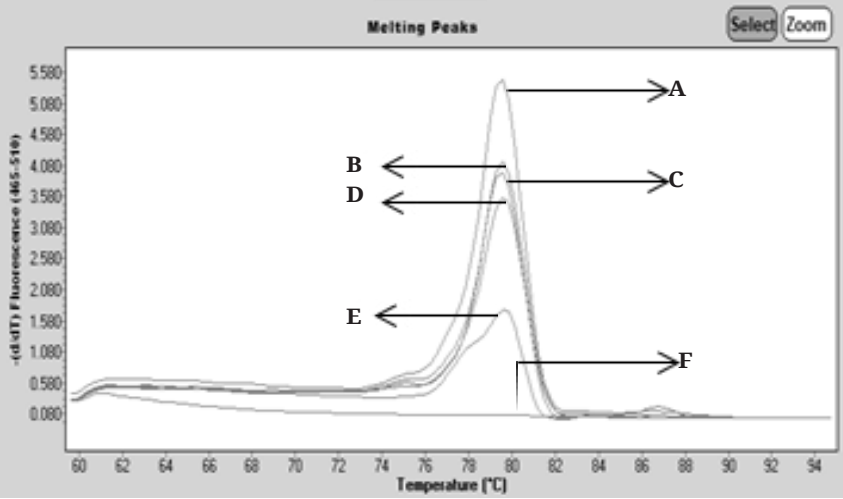

FIGURE 3. The qPCR result for determining the limit of detection (LoD). This assay was still able to detect the DNA at 25x10^{-10} ng. A-F: 25x10^{-6}, 25x10^{-7}, 25x10^{-8}, 25x10^{-9}, 25x10^{-10}, and 25x10^{-11} ng DNA respectively.

External quality assurance

To validate the qPCR optimized in this study, we enrolled the external quality assurance in 2019 and 2021 by QCMD EQA Management System (Scotland, The United Kingdom). The results showed score 0, meaning the testing agreement of 100% (FIGURE 4).

Preliminary trial for the clinical specimen

For preliminary testing, 20 cervical swab samples were used. The results showed 100% concordance with 10 positive- and 10 negative-confirmed samples (FIGURE 5).

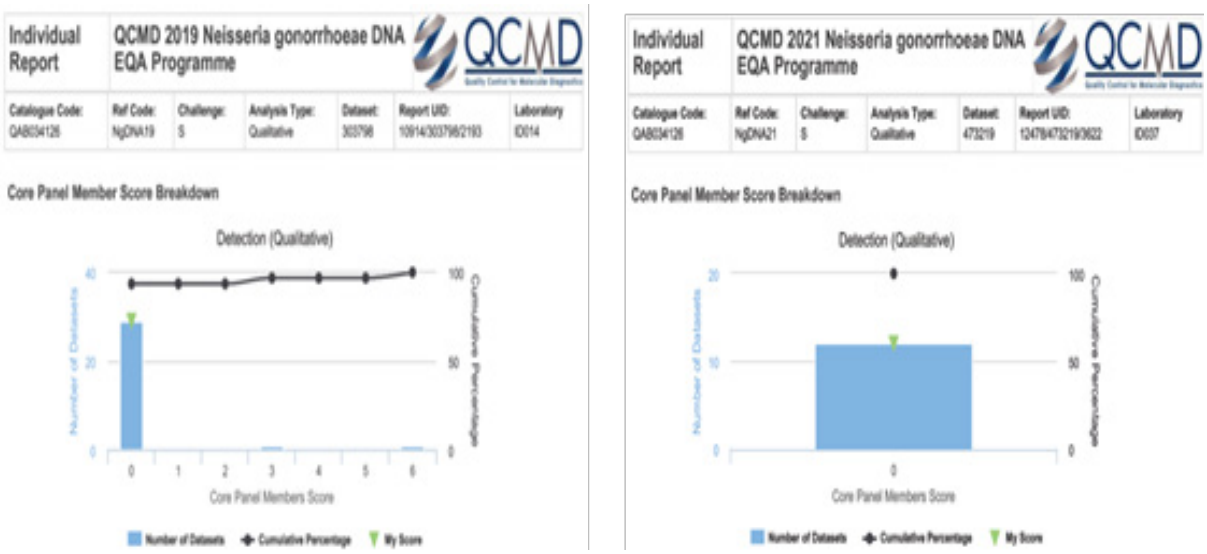

FIGURE 4. External quality assurance reports enrolled in 2019 and 2021. The score 0 is 100% concordance (The higher score, the higher discordance).

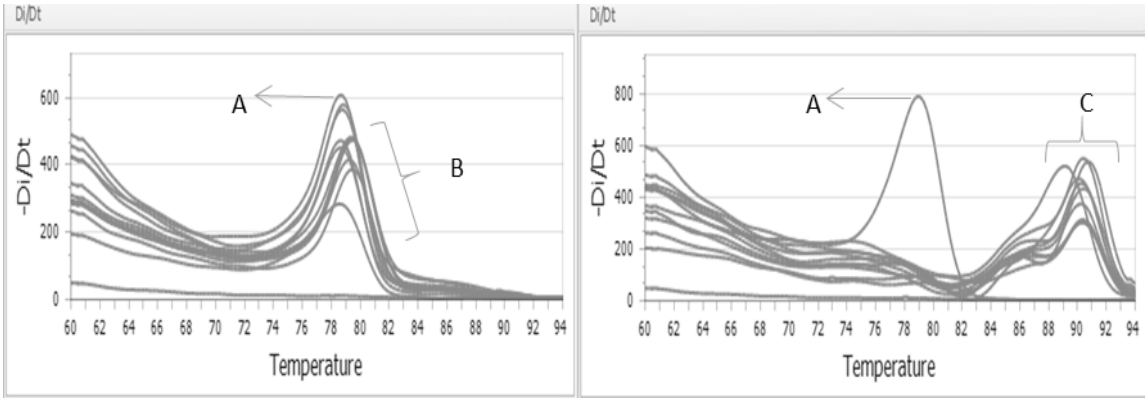

FIGURE 5. Preliminary test results for 10 positive (left)- and 10 negative (right)-confirmed samples. A: Positive control of *N. gonorrhoeae* ATCC 43069. B: Melting dynamic for 10 *N. gonorrhoeae* positive samples. C: Melting dynamic for 10 *N. gonorrhoeae* negative samples.

DISCUSSION

For the development and establishment of PCR assay on the laboratory scale, many factors affect the sensitivity and specificity of the PCR assay, including primer design, PCR composition, annealing temperature, and PCR inhibitor.[19–21] In this study, we optimized annealing temperature and DNA template volume. The optimal annealing temperature is the temperature in which the primers attach to the DNA sequence targets completely, leading to high DNA amplification efficiency.[19] Low annealing temperature could amplify non-specific DNA fragments, whereas too high annealing temperature could reduce the PCR product purity due to poor annealing of primers.[19]

The Optimal DNA template volume is important to reduce the PCR inhibitor available in the DNA solution of DNA extraction. The inhibitor influences the efficiency of *Taq* DNA polymerase in incorporating deoxynucleoside triphosphate when the DNA strand is synthesized. The inhibitor analysis is highly important, particularly for clinical samples, such as respiratory, feces, sputum, and other body fluids (cerebrospinal fluid, blood, urine, saliva, etc.).[22] Various extraction DNA methods have a different performances to diminish the possible inhibitor in clinical samples; thus each new method has to be optimized.[23]

Another important factor that affected the PCR performance, particularly PCR specificity is primer design. In this study, we used primers specific to the *opa* gene that have been reported by Geraats-Peters *et al.*[13] Based on *in silico* and *in vitro* analysis, the primers were only reacted with the *N. gonorrhoeae* target gene, and no cross-reaction with bacteria, viruses, fungi, and protozoa that might cause the possible false-positive results. It indicates that the qPCR assay system developed in this study has high specificity.

Besides specificity, the LoD also affects the PCR performance. An important factor affecting the PCR LoD is the target gene.[24] There are several genes used for *N. gonorrhea* PCR, including *opa*, *16S rRNA*, *porA*, *cppB*, and *CMT* genes.[24] The *opa* gene is the conserved region and encodes proteins with physiological function.[13] Moreover, the *N. gonorrhoea* genome has multiple copies (about 11 alleles) of *opa* gene,[18] thus leading the PCR to be more sensitive than other genes with fewer copies. Geraats-Peters reported that the *opa* gene-based PCR is more sensitive than

16S rRNA.[13] Other study also report that *opa* gene has higher sensitivity than *porA* pseudogene.[24] The *cppB* gene and *CMT* gene have wide range sensitivity because of lacking the genes in certain *N. gonorrhea* strains.[25,26] The *CMT* gene is less specific because can cross-react with commensal *Neisseria* species.[26] Thus, the *cppB* and *CMT* genes are not suitable for *N. gonorrhoeae* detection.

The LoD of qPCR developed in this study was 29 DNA copies (25x 10^{-7} pg) corresponding to 3 bacterial cells per reaction. This LoD is more sensitive than what has been reported by Verma *et al*, namely 0.4 pg (*16S rRNA* and *opa* genes) and 4 pg (*porA* pseudogene) DNA of *N. gonorrhoeae.*[24] For internal quality control, this study used *N. gonorrhoeae* ATCC 43069 as positive control and nuclease-free water as the negative control. *N. gonorrhoeae* ATCC 43069 is often used as a reference strain in the diagnosis of *N. gonorrhoea* infection.[27,28] For external quality assurance (QCMD, Scotland, United Kingdom), the assay developed in this study had score 0 (100% concordance) in 2019 and 2021. Based on the preliminary trial for 20 clinical samples, the assay showed 100% concordance. Overall, the data indicate that the SYBR Green qPCR-based assay system developed in this study is highly promising for clinical laboratories application.

CONCLUSION

We conclude that an SYBR Green qPCR-based assay system that was developed in this study has high sensitivity and specificity for *N. gonorrhoeae* detection. This assay also can be applied to cervical swab samples. However, larger cervical swab samples are required for further analysis. Also, this qPCR is needed to evaluate some types of samples such as urine, urethral swab, and other clinical samples.

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An increase in mean platelet volume (MPV) as a predictor of mortality in children with sepsis

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ABSTRACT

Sepsis is associated with increased morbidity and mortality in children worldwide, mainly in developing countries. This fatal risk emphasizes the importance of finding accessible and inexpensive parameters to be used as predictors for mortality in children with sepsis. The aim of this study was to determine the role of increased mean platelet volume (MPV) as a predictor for mortality in children with sepsis. A case control study was applied using medical records of all in-patients aged 1 mo -18 y diagnosed with sepsis at Dr. Sardjito General Hospital, Yogyakarta from January 2015-December 2016. Bivariate and multivariate analyses by Chi-square and logistic regression to evaluate the correlations between increased MPV within the first 24-72 h (ΔMPV>0) and mortality were applied. Eighty-one eligible subjects met the inclusion/exclusion criteria with the mortality was 52%. Chi-square analysis showed significant correlations between increased MPV and mortality (p=0.005). Multivariate analysis showed increased MPV within the first 24-72 h after sepsis diagnosis as a predictor for mortality after controlling for sex and AKI (adjusted OR 3.851; 95% CI:1.354-10.948; p= 0.011). In conclusion, an increase in MPV within the first 24-72 h after diagnosed is an independent predictor for mortality in children with sepsis.

ABSTRACT

Kejadian sepsis berhubungan dengan peningkatan morbiditas dan mortalitas anak-anak di seluruh dunia, khususnya di negara berkembang. Risiko fatal ini menekankan pentingnya menemukan parameter yang murah dan mudah diakses untuk digunakan sebagai prediktor kematian pada anak dengan sepsis. Penelitian bertujuan mengkaji peran peningkatan rerata *platelet volume* (MPV) sebagai prediktor mortalitas pada anak dengan sepsis. Penelitian ini merupakan penelitian kasus kontrol menggunakan rekam medis seluruh pasien rawat inap anak umur 1 bulan-18 tahun di RSUP Dr. Sardjito, Yogyakarta yang didiagnosis sepsis dari Januari 2015-Desember 2016. Analisis bivariat dan multivariat dengan uji *Chi-square* dan regresi logistik dilakukan untuk menentukan korelasi antara peningkatan MPV dalam 24-72 jam pertama (ΔMPV>0) dengan mortalitas. Sebanyak delapan puluh satu subjek memenuhi kriteria inklusi/eksklusi dengan kematian sebesar 52%. Uji *Chi-square* menunjukkan korelasi signifikan antara peningkatan MPV dengan mortalitas (p=0,005). Analisis multivariat menunjukkan peningkatan MPV dalam 24-72 jam pertama sejak diagnosis sepsis ditegakkan sebagai prediktor mortalitas setelah mengontrol variabel jenis kelamin dan *acute kidney injury*/AKI (*adjusted* OR 3,851; 95%CI: 1,354-10,948; p= 0,011). Dapat disimpulkan bahwa peningkatan MPV dalam 24-72 jam pertama setelah penegakkan diagnosis merupakan prediktor independen untuk mortalitas anak dengan sepsis.

Keywords:
children;
mean platetet volume;
mortality;
sepsis

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INTRODUCTION

Sepsis is a life-threatening organ dysfunction caused by a dysregulated immune system due to infection.[1] Sepsis affects millions of people worldwide each year, and is associated with high morbidity, mortality, and health-maintenance costs. Sepsis is also a major cause of infant and child mortality worldwide. The incidence of sepsis increased in recent years, especially in developing countries.[2] Based on epidemiological studies in the United States, the incidence of severe sepsis involved 0.56 cases per 1000 population per year, with a mortality reaching 10.3% (more than 4383 deaths per year).[3] The incidence of sepsis in the Pediatric Intensive Care Unit (PICU) Dr. Cipto Mangunkusumo Hospital (RSCM), Jakarta was 19.3% with a mortality rate 10%.[4] High mortality rate encourages applied research to discover easily accessible and inexpensive methods that can be widely used to know the severity of sepsis when diagnosed so an optimal management approach can be developed to reduce mortality.

Mean platelet volume (MPV) is a simple and accurate marker of functional status and size of the platelets. Some research hypothesized that cytokines such as interleukin 3 (IL-3) and IL-6 in sepsis patients will affect megakaryocytes ploidy that leads to the production of more reactive and larger platelets, so MPV will increase.[5,6] In healthy populations, MPV has an inverse correlation with the number of platelets, but biological effects, clinical significance, and its association with sepsis is still not clearly understood.

Previous study explains that the measurement of the MPV can be used as predictors of mortality in patients with sepsis and critical illness.[7-12] Study on the role of MPV as a predictor of mortality in children with sepsis is still rarely conducted. The procedure of MPV measurement is very simple and does not require high cost. This study aimed to determine the MPV role as a predictor of mortality in children with sepsis.

MATERIALS AND METHODS

Design of study

We conducted a prognostic study with matched case control design at Dr. Sardjito General Hospital, Yogyakarta by reviewing medical records. Subjects were patients aged 1 mo-18 y who were diagnosed with sepsis based on Indonesian Pediatric Society 2016 criteria[1] and were hospitalized in the Pediatric Intensive Care Unit (PICU) since January 2015 to December 2016, with performed blood cultures and MPV measurement.

Protocol of study

Mean platelet volume value came as part of complete blood count measurement using a quantitative automated hematology analyzer which analyzed at the clinical pathology laboratory of Dr. Sardjito General Hospital. Mean platelet volume measurement was taken at diagnosis and within 24-72 h thereafter. Mean platelet volume values were recorded from the medical record. Subjects were excluded if there was comorbidity such as hematologic abnormalities (leukemia, immune thrombocytopenic purpura, essential thrombocytosis, abnormal platelet

function, aplastic anaemia), asplenia, chronic diseases, organ transplantation, malignancies, congenital abnormalities, immunosuppressive drugs or discharge against medical advice.

The case group was children based on inclusion and exclusion criteria who died during hospitalization, and the control group was children who were survived, match by age. The data taken including age, gender, nutritional status, number of leukocytes, MPV value at the time diagnosed as sepsis (MPV_1), MPV values within 24-72 h after diagnosed (MPV_2), changes in the value MPV within 24-72 h after diagnosed (ΔMPV), the number of platelets, acute kidney injury (AKI),[13] disseminated intravascular coagulation (DIC),[14] septic shock[15] and the outcomes (dead or alive). Mean platelet volume values taken were the result of blood sample analysis in the clinical pathology laboratory that were written in medical records or tracked from clinical laboratory installation. If there were more than one examination within the time range (24-72 h), the MPV value that was recorded is the latest MPV. Mean platelet volume is considered high when MPV value > 10.4 fl, and increased when ΔMPV>0 (MPV_2-MPV_1>0). Other data were taken from examination performed within 72 h after sepsis diagnosis.

Statistical analysis

Descriptive analysis was conducted to describe the basic characteristics of the subjects and the data was displayed in the form of proportions. Bivariate analysis by Chi-square test was conducted to determine any correlation between an increase in MPV (ΔMPV> 0), thrombocytopenia, leukopenia, age, gender, AKI, DIC and septic shock with mortality. Multivariate analysis by logistic regression was performed for an increase in MPV (ΔMPV> 0) and confounders with p value < 0.25 to determine the adjusted OR. This study was approved from the Medical Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/ Dr. Sardjito General Hospital, Yogyakarta (ref. number KE/FK/0546/EC/2017).

RESULTS

Within 2 years (January 1st, 2015 - December 31th, 2016) there were 81 subjects who met the study criteria. Subjects' characteristics are shown in Table 1. Median of age was 25-month (1-205-month), with higher proportion of patients younger than 5 years old (66,7% *versus* 33,3%). Diseases related to the occurrence of sepsis in this study include pneumonia (28.4%), dengue shock syndrome (24.7%), intracranial infection (19.8%), surgery (9.9%), gastrointestinal infections (4.9%), skin infections (3.7%), and typhoid fever (1.2%). Pneumonia is most prevalent in the case group (43.9%), while dengue shock syndrome is most prevalent in the control group (30.0%). Positive culture was found in 65.4% of the subjects. The most common pathogens are *Streptococcus viridans* (17.3%), *Klebsiella pneumoniae* (11.8%), *Candida sp.* (10.9%), *Acinetobacter baumanii* (9.1%), *E. coli* (9.1%), *Enterobacter cloacae* (5.5%), and *Pseudomonas aeruginosa* (5.5%).

TABLE 1. Basic characteristics of subjects

Characteristics	Died (n = 41) n (%)	Alive (n = 40) n (%)	Total (n = 81) n (%)
Age			
• <5 years	27 (65.9)	27 (67.5)	54 (66.7)
• ≥ 5 years	14 (34.1)	13 (32.5)	27 (33.3)
Gender			
• Boys	24 (58.5)	34 (85.0)	58 (71.6)
• Girls	17 (41.5)	6 (15.0)	23 (28.4)
Underlying diseases			
• Surgical cases	1 (2.4)	7 (17.5)	8 (9.9)
• Non-surgical cases	40 (97.6)	33 (82.5)	73 (90.1)
• Pneumonia	18 (43.9)	5 (12.5)	23 (28.4)
• Dengue shock syndrome	8 (19.5)	12 (30.0)	20 (24.7)
• Intracranial infection	8 (19.5)	8 (20.0)	16 (19.8)
• Gastrointestinal infections	1 (2.4)	3 (7.5)	4 (4.9)
• Skin infections (burns, cellulitis, abscesses)	1 (2.4)	2 (5.0)	3 (3.7)
• Typhoid fever	0 (0.0)	1 (2.5)	1 (1.2)
• > 1 source of infection	4 (9.8)	2 (5.0)	6 (7.4)
Wasted or severely wasted	13 (31.7)	9 (22.5)	22 (27.2)
Positive culture	20 (48.8)	33 (82.5)	53 (65.4)
• Blood	0 (0.0)	5 (12.5)	5 (6.2)
• Feces	1 (2.4)	0 (0.0	1 (1.2)
• Respiratory tract	7 (17.1)	10 (25.0)	17 (21.0)
• Others	1 (2.4)	1 (2.5)	2 (2.5)
• > One kind of sample	11 (26.8)	17 (42.5)	28 (34.6)

This study showed that MPV_1 and MPV_2 values had no effect on mortality, while an increase in MPV (ΔMPV> 0) did have a significant effect on mortality (p = 0.005) (TABLE 2). The results of the bivariate analysis of other variables also shown in TABLE 2. Thereafter, multivariate analysis by logistic regression was performed for an increase in MPV (ΔMPV> 0) and confounders with p value < 0.25 to determine the adjusted OR. Confounders which included was female gender, leukopenia, AKI, DIC, and septic shock. We obtained adjusted OR of ΔMPV>0 based on reference model (model 1) was 4.144 (95% CI 1.328 to 12.933; p = 0.014) (Table 3). After analyzed for the best model that would be used based on its validity and precision, we obtained final adjusted OR of ΔMPV>0 was 3.851 (95% CI 1.354 to 10.948; p = 0.011) (TABLE 3).

TABLE 2. Result of bivariate analysis on the factors that affect mortality in sepsis

Variables	Died (n = 41) n (%)	Alive (n = 40) n (%)	p*
MPV_1			
>10.4 fl	10 (24.4)	15 (37.5)	0.202
≤10.4 fl	31 (75.6)	25 (62.5)	
MPV_2			
>10.4 fl	19 (46.3)	11 (27.5)	0.079
≤10.4 fl	22 (53.7)	29 (72.5)	0.005
ΔMPV increased	28 (68.3)	15 (37.5)	0.875
Aged <5 years	27 (65.8)	27 (67.5)	0.008
Female gender	17 (41.5)	6 (15.0)	0.118
Leukopenia	4 (9.7)	9 (22.5)	0.745
Thrombocytopenia	18 (43.9)	19 (47.5)	0.352
Wasted or severely wasted	13 (31.7)	9 (22.5)	0.000
Acute kidney injury (AKI)	21 (51.2)	5 (12.5)	0.002
Disseminated intravascular coagulation (DIC)	24 (58.5)	10 (25.0)	0.005
Septic shock	28 (68.3)	18 (45.0)	0.034

TABLE 3. Validity and precision of potential models based on multivariate analysis by logistic regression

Model	OR	95%CI	OR changes from reference model (%)	Precision	p
Model 1 or reference model (controlling for gender, leucopenia, AKI, DIC and septic shock)	4.144	1.328-12.933	-	11.605	0.014
Model 2 (controlling for gender, AKI, DIC and septic shock)	3.926	1.275-12.089	5.3	10.814	0.017
Model 3 (controlling for AKI, DIC and septic shock)	3.609	1.220-10.677	12.9	9.457	0.020
Model 4 (controlling for gender, AKI and septic shock)	4.413	1.477-13.190	6.5	11.713	0.008
Model 5 or final model (controlling for gender and AKI)	3.851	1.354-10.948	7.1	9.594	0.011
Model 6 (controlling for gender)	3.859	1.458-10.211	6.9	8.753	0.007
Model 7 (Unadjusted)	3.590	1.434-8.988	13.4	7.554	0.006

DISCUSSION

In the present study, we have evaluated the MPV role as a predictor of mortality in children with sepsis. The bivariate analysis showed there was no correlation between MPV_1, MPV_2 and mortality in children with sepsis (p = 0.202 and p = 0.079 respectively), while an increase in MPV within 24-72 h after diagnosed as sepsis (ΔMPV > 0) is associated with higher mortality (p = 0.005). After conducting multivariate analysis, we obtained adjusted OR for ΔMPV>0 was 3.851 (95% CI 1.354 to 10.948; p = 0.011).

Sepsis causes immune dysregulation resulting in the release of various pro-inflammatory cytokines that affect megakaryocytes. Sepsis also

leads to increase growth factors such as thrombopoietin which regulates megakaryocytes proliferation and differentiation.[16] As the result, the young platelets which are larger and more active are released.[17,18] These large platelets are characterized by an increase in MPV. Furthermore, an increase in MPV also indicates that the platelets are more active functionally, metabolically and enzymatically than the small ones. This difference is due to an increase in the content of alpha granules and dense granules, thromboxane A2 intracellular, and pro-coagulation surface proteins such as P-selectin and glycoprotein IIa/IIIa.[10]

The results of this study are similar to several previous study, which showed that MPV increased significantly within 72 h after admission in the group of deceased patients caused by sepsis (p = 0.001).[10] Similarly, the study by Erdogan concluded that there was no correlation between mortality and MPV value obtained at admission and 48 h later in critically ill children (p = 0.480 and p = 0.213 respectively), but an increase in MPV within 48 h after treatment in the pediatric intensive care unit is associated with higher mortality (p <0.001).[19] Therefore, serial MPV measurements may help to predict poor outcome in pediatric patients with sepsis to optimize the treatment and reduce mortality.

Another factor associated with mortality in children with sepsis is the presence of AKI (OR 6.129; 95%CI 1.844 to 20.369; p = 0.003). This is consistent with a previous study that concluded AKI as a risk factor of mortality in children with severe sepsis (OR 2.5; 95%CI 1.5 to 4.2; p = 0.001).[20] Septic patients with AKI are associated with poorer outcome due to organ dysfunction, so that the risk of death is greater. Meanwhile, the influence of gender on mortality was not statistically significant (OR 3.157; 95%CI 0.944 to 10.562; p = 0.062).

The mortality rate of sepsis based on this study is 50.6%. This result is consistent with a previous study that showed the mortality rate in children with sepsis who were hospitalized at PICU Dr. Sardjito General Hospital from July to November 2012 was 52%.[21] The diagnosis of sepsis is confirmed by PELOD-2 score with cut-off ≥11 which is associated with an increase in mortality ≥ 30.5%.[1]

Most subjects were aged <5 years (66.7%) with the youngest only 1 month old. Research determined the maturity of immune system depends on the age, so the younger patients had lower maturity to eliminate pathogens thereby increasing the risk of sepsis.[22] In this study, there was no age difference in the case group or control group. This finding is similar to Saraswati's study which reported that the ages <5 y.o. are not proved to be a risk factor for mortality in children with sepsis.[4] However, the proportion of children aged 1-12 mo in case group was higher than the control group, although the finding was not statistically significant (46.3% versus 35.0%, p = 0.299).

We found male predominance with ratio of 2.5:1. However, the proportion of female subjects in the case group was higher than the control group (41.5% vs. 15.0%). This finding is consistent with the results of Pietropaoli's study that concluded although the incidence of sepsis was greater in male patients, still females with severe sepsis/septic shock have higher mortality risk (OR 1.11; 95%CI 1.04 to 1.19; p = 0.002).[23] Positive culture found in this study was similar to a previous study that found etiological pathogens in as many as 40-60% cases.[24]

This study has several limitations. The retrospective design by reviewing medical records gave us difficulties to obtain complete information. Mean platelet volume data also were obtained retrospectively, so the timing of blood sampling was different in all subjects and we could not control the time interval

between blood sampling and laboratory processing. One previous study showed that the duration of blood sample in ethylenediaminetetraacetic acid (EDTA) tube can affect MPV results, so the measurement processing should be done within one h after blood sampling.[25]

CONCLUSION

Elevation MPV within 24-72 h after being diagnosed (ΔMPV>0) predict poor outcome in pediatric patients with sepsis.

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Association between the level of high-sensitivity troponin I (Hs-Trop I) and major adverse cardiovascular events in patients with acute myocardial infarction of segment elevation (STEMI) with primary percutaneous coronary intervention (PCI)

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ABSTRACT

ST-segment elevation myocardial infarction (STEMI) is a condition which increases the risk of developing major adverse cardiovascular events (MACEs). For patients with STEMI, an efficient method of risk stratification is necessary in order to evaluate the clinical outcome. Troponin has been commonly used in the diagnosis of both STEMI and NSTEMI. The use of high sensitivity assays of troponin has been extensively studied in order to measure the size of myocardial damage caused by STEMI. This study aimed to investigate the association between the level of high sensitivity troponin I (Hs-Trop I) and the incidence of MACEs in patients with primary percutaneous coronary intervention (PCI) in Dr. Sardjito General Hospital, Yogyakarta, Indonesia. It was a cross-sectional observational analytic study involving a total of 195 patients. Data were obtained from both the SCIENCE (Sardjito Cardiovascular Intensive Care) registry and the medical record of Dr. Sardjito General Hospital. Pearson's Chi square test to evaluate the association between variables was applied. To determine the effect of confounding variables, a multivariate analysis was used. A significant difference in the baseline characteristics between the supramedian and inframedian Hs-Trop I groups (cutoff value of 2063.8 ng/mL) in age, onset, total ischemic time, wire crossing time and the smoking history of both groups was observed. Bivariate analysis showed a significant associations between Hs-Trop I and MACEs (p = 0.033), acute heart failure (p = 0.009) as well as mortality (p = 0.024). Meanwhile, no significant association between Hs-Trop I and cardiogenic shock (p = 0.977) and malignant arrythmia (p = 0.551) was reported. Furthermore, multivariate analysis showed Hs-Trop I, age and wire crossing time were significantly associated with the incidence of MACEs (p = 0.045). In conclusion, there is a significant association between the Hs-Trop I levels and the MACEs events in STEMI patients with primary PCI in Dr. Sardjito General Hospital, Yogyakarta, Indonesia.

ABSTRAK

Infark miokardium akut dengan elevasi segmen ST (IMA-EST) adalah kondisi yang meningkatkan risiko kejadian kardiovaskular mayor (KKM). Metode stratifikasi risiko efisien diperlukan untuk menilai luaran klinis pasien IMA-EST. Salah satu biomarker yang banyak digunakan untuk mendiagnosis baik IMA-EST dan IMA-nonEST adalah troponin. Penggunaan metode penetapan troponin sensitifitas tinggi banyak diteliti sebagai salah satu biomarker yang akurat dalam menilai luas kerusakan miokardium akibat IMA-EST. Penelitian ini bertujuan untuk mengkaji hubungan antara kadar *Hs-Trop I* pasien IMA-EST yang dilakukan intervensi koroner perkutan (IKP) primer pada saat admisi

Keywords:
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ACEs;
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STEMI;
cardiogenic shock

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dengan insidensi dari KKM selama durasi perawatan di RSUP Dr. Sardjito Yogyakarta, Indonesia. Penelitian ini merupakan penelitian observational analitik dengan desain potong lintang. Sebanyak 195 pasien diikutsertakan dalam penelitian ini, data pasien diperoleh dari instalasi catatan medis RSUP Dr. Sardjito dan registry SCIENCE (*Sardjito Cardiovascular Intensive Care*) secara *consecutive sampling*. Analisis dilakukan dengan uji Chi-Square pada variabel *Hs-Trop I* dan insidensi KKM. Analisa multivariat dilakukan dengan membuat model multivariat regresi logistik. Perbedaan yang signifikan ditemukan pada karakteristik dasar subjek penelitian yang dibagi berdasarkan nilai *Hs-Trop I* menjadi kelompok supramedian dan inframedian (*cutoff* 2063.8). Ditemukan perbedaan usia, onset, *total ischemic time*, *wire crossing time*, dan status merokok pada kedua kelompok. Pada analisis bivariat, ditemukan hubungan signifikan antara *Hs-Trop I* dengan KKM (p = 0,033), gagal jantung akut (p = 0,009), dan mortalitas (p = 0,024), tidak ada hubungan yang signifikan antara *Hs-Trop I* dengan syok kardiogenik (p = 0,977) dan aritmia maligna (p = 0,551). Pada analisis multivariat regresi logistik, ditemukan variabel *Hs-trop I*, usia, dan *wire crossing time* memiliki hubungan yang signifikan terhadap insidensi KKM (p = 0,045). Penelitian ini menunjukkan adanya hubungan yang signifikan antara kadar *Hs-Trop I* admisi pasien dengan insidensi dari KKM pada pasien IMA-EST yang menjalani IKP Primer di RSUP Dr. Sardjito, Yogyakarta.

INTRODUCTION

Acute coronary syndrome (ACS) is a term used to describe a range of conditions associated with sudden, reduced blood flow to the heart. It is one of the main health problems due to its high mortality and morbidity, including its high numerical value and medical costs. Worldwide, ACS is the leading cause of death and loss of disability-adjusted life years (DALYs). Most of ACS happens in low-middle income countries, such as Indonesia and other South East Asian countries.[1]

Acute myocardial infarction with ST-segment elevation (STEMI) is one of the clinical conditions of ACS. This STEMI is characterized by an increase in the ST segment in two leads on electrocardiography (ECG) examination and an increase in biomarkers of cardiac muscle cell necrosis (CK-MB, troponin, and others).[2] Patients with STEMI are at a high risk of experiencing post-infarction major adverse cardiovascular events (MACEs) with an incidence of 4.2-5%.[3]

Troponins are biomarkers released by the heart muscle when there are damages and death to these cells. Since early 2000, these biomarkers have been used and recommended for evaluating the diagnosis of patients with acute myocardial infarction (AMI). In the recent years, a method of accurately measuring the troponin levels has been discovered, in the form of high sensitivity troponin I (Hs-Trop I) assays. It is capable of detecting elevation of cardiac troponin to the 99th percentile of the upper reference limit, which in turn will be able to detect signs of myocardial ischemia and/or infarction earlier in the progression of the disease when compared to conventional troponin assays that have a less sensitive detection of early ischemia and infarction. Other cardiac biomarkers such as CK-MB, lactate dehydrogenase, and myoglobin are not preferred due to their low sensitivity and low specificity towards the myocardium.[4]

Furthermore, the measurement of high-sensitivity troponin (Hs-Trop I) is considered as a standard clinical practice in the management of patients with ACS. Therefore, Hs-Trop I admission data are easy to discover and obtain. The risk assessment methods for post-MI sequelae such as the global registry of acute coronary events (GRACE) and the thrombolysis in myocardial infarction (TIMI) risk score require a lot of patient

data combination and increase in the delay to treatment time in STEMI patients. This is in accordance with this study which suggests that there is a need for a risk stratification method which is fast, simple, and easy to use in clinical settings. This study aimed to investigate the association between the Hs-Trop I levels taken at admission and the MACEs prevalence that occurred during the treatment of STEMI patients with primary PCI.

MATERIALS AND METHODS

Study design

This was an observational analytic study with a cross-sectional design using the data obtained from patients diagnosed with STEMI since August 2019 to December 2020 from the Sardjito Cardiovascular Intensive Care (SCIENCE) registry and the medical records installation at Dr. Sardjito General Hospital, Yogyakarta, Indonesia. The protocol of the study was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/Dr. Sardjito General Hospital, Yogyakarta (ref. KE/FK/0558/EC/2019).

Patients

A total of 195 patients met the inclusion and exclusion criteria needed to be involved in this study. The prevalence of MACEs including acute heart failure, cardiogenic shock, malignant arrhythmias, and patient mortality during the treatment duration at the hospital were considered as the dependent variables. Meanwhile, the independent variable was the patient's Hs-Trop I levels taken at admission. There were also several variables assessed for their effect in this study, namely age, onset, patient risk factors (smoking habits, diabetes mellitus, hypertension, stroke history) and length of treatment (wire crossing time, total ischemic time). The target population of this study were patients diagnosed with STEMI while the sample population were STEMI patients that underwent primary PCI reperfusion in Dr. Sardjito General Hospital, Yogyakarta.

The study subjects were the source population that met the criteria and data collection was carried out using consecutive sampling techniques until the sample size was met. Inclusion criteria for the subjects were: (1) STEMI patients with complaints of chest pain <12 h, (2) 30-75 y.o. and (3) with reperfusion therapy in the form of primary PCI with the results of TIMI Flow 3. Meanwhile, the exclusion criteria for the subjects were as follows: (1) history of chronic kidney failure stage >IV, defined as patients with GFR <15 (2) a history of chronic heart failure before a heart attack and (3) patients with malignancy. Samples were obtained using the consecutive sampling method until the minimum size of 142 was met.

Data collection

Hs-Trop I levels were determined using the VIDAS® Hs-trop I kit with a value range of 1.5-40,000 ng/L. The data obtained from 195 patients were analyzed and divided into two groups based on a median value of 2063.8 ng/L, namely the supramedian and inframedian groups. Furthermore, bivariate categorical analysis was used to assess the association between Hs-Trop I and MACEs.

Data analysis

Statistical analysis was performed using SPSS software while the association between the two variables was assessed through bivariate Pearson's Chi-square test for data with normal distribution or the Fisher exact test for data with an abnormal distribution. In addition, an odds ratio (OR) of each variable

was analyzed. Multivariate logistic regression analysis was used to examine the effect of confounding variables. Analyzed variables that fulfills statistical significance with a p of < 0.25 would be included in multivariate analysis to be further analyzed for variable independent factor.

RESULTS

Characteristics of patients

A total of 195 patients were involved in this study consisted of 171 male (87.7%) and 24 females (12.3%). The mean age of patients was 57.18 ± 8.29 y.o. The median value of the Hs-Trop I level was 2063.8 ng/L. Among the patients, 64 patients (32.8%) had type 2 diabetes melitus, 115 patients (59%) had hypertension, and 18 patients (9.2%) had a history of stroke attack. A total of 130 patients smoked cigarettes (66.7%), this study did not further differentiate whether the patient was an ex-smoker or an active smoker. The median wire-crossing time of patients was 145 min, with 124 patients (63.6%) classified as not have the ideal wire-crossing time.

TABLE 1. Characteristics of patients

Variables	Total (n=195)
Age (mean ± SD, y.o.)	57.18 ± 8.29
Gender [n (%)]	
• Male	171 (87.7)
• Female	24 (12.3)
Cardiovascular Profile	
• Hs-Troponin I [median (range)]	2063.8 (2.7-40000)
- Supramedian [n (%)]	98 (50.3)
- Inframedian [n (%)]	97 (49.7)
• Onset [median (range) min]	360 (60-660)
• Wire Crossing Time [median (range) min]	145 (45-1020)
- Non-Ideal (>120 min) [n (%)]	124 (63.6)
- Ideal (<120 min) [n(%)]	71 (36.4)
• Total Ischemic Time [median (range) min]	490 (212-1200)
Comorbidity	
• Diabetes Mellitus [n (%)]	64 (32.8)
• Hypertension [n (%)]	115 (59.0)
• Stroke History [n (%)]	18 (9.2)
• Smoking Habits [n (%)]	130 (66.7)
Clinical Outcomes	
• MACEs [n (%)]	79 (40.5)
- Acute heart failure [n (%)]	34 (17.4)
- Cardiogenic Shock [n (%)]	28 (14.4)
- Malignant arrhythmias [n (%)]	54 (27.7)
- Mortality [n (%)]	17 (8.7)

*MACEs = major adverse cardiovascular events

Character of study subjects based on Hs-Trop I

The results of univariate analysis showed a significantly different in onset, wire crossing time ($p < 0.001$) and total ischemic time ($p = 0.023$) between supramedian and inframedian were observed (TABLE 2). Furthermore, a significantly different between ideal/non-ideal categories of the wire crossing time was reported (p=0.022). However, the comorbidities such as diabetes mellitus, hypertension, stroke and smoking habits were not significantly different ($p> 0.05$). Furthermore, variables with p value < 0.25 i.e. age, onset, wire crossing time, total ischemic time and smoking habits were continued for bivariate analysis before being declared as confounding variables and included in the logistic regression multivariate analysis with the main variable (troponin status).

TABLE 2. Characteristics of study subjects based on Hs-Trop I levels

Variables	Supramedian (n=98)	Inframedian (n=97)	P value
Age (mean ± SD, y.o.)	58.06 ± 8.46	56.29 ± 8.06	0.136**
Gender [n (%)]			
• Male	88 (89.8)	83 (85.6)	0.369
• Female	10 (10.2)	14 (14.4)	
Cardiovascular profile			
• Onset [median (range) min]	420 (60-660)	270 (60-660)	0.000*
• Wire Crossing Time [median (range) min]	152.5 (45-703)	134 (48-1020)	0.023*
- Non-Ideal (>120 min) [n (%)]	70 (71.4)	54 (55.7)	0.022*
- Ideal (<120 min) [n(%)]	28 (28.6)	43 (44.3)	
• Total Ischemic Time [median (range) min]	566.5 (224-1123)	415 (212-1200)	0.000*
Comorbidities			
• Diabetes Mellitus [n (%)]	34 (34.7)	30 (30.9)	0.575
• Hypertension [n (%)]	58 (59.2)	57 (58.8)	0.952
• Stroke History [n (%)]	11 (11.2)	7 (7.2)	0.334
• Smoking Habits [n (%)]	70 (71.4)	60 (61.9)	0.156**

Nonparametric data analyzed with Mann-Whitney test. Categorical data was analyzed with Chi-square test; *significant at $p < 0.05$; ** significant at $p < 0.25$

Association between Hs-Trop I and MACEs

A significantly association between Hs-Trop I levels with the overall MACEs prevalence (OR= 1.872; 95%CI: 1.048 – 3.343; p = 0.033), the incidence of acute heart failure (OR= 2.822; 95%CI: 1.268 – 6.281; p = 0.009), and the mortality (OR=3.556; 95% CI: 1.116-11,327; p = 0.024]. Patients with supramedian level of Hs-Trop I are at 1.872 times risk of having MACEs, 2.822 times risk to experience an acute heart failure, and 3.556 times risk of a death compared to those with inframedian of Hs-Trop I (TABLE 3). However, no association between Hs-Trop I levels with the incidence of cardiogenic shock (OR=0.988; 95% CI: 0.440-2.200; p = 0.977], and the malignant arrythmias (OR=1.210; 95% CI: 0.646 - 2.270; p = 0.511].

TABLE 3. Chi-Square analysis between the level of Hs-Trop I with MACEs

Variables		MACEs		p	OR (95% CI)
		Yes [n (%)]	No [n (%)]		
Hs-Troponin I	Supramedian	47 (48)	51 (52)	0.033	1.872 [1.048 – 3.343]
	Inframedian	32 (33)	65 (67)		
		Acute Heart Failure			
	Supramedian	24 (24.5)	74 (74.5)	0.009	2.822 [1.268 – 6.281]
	Inframedian	10 (10.3)	87 (89.7)		
		Cardiogenic Shock			
	Supramedian	14 (14.3)	84 (85.7)	0.977	0.988 [0.44 – 2.2]
	Inframedian	14 (13.9)	83 (83.1)		
		Malignant Arrythmias			
	Supramedian	29 (29.6)	69 (70.4)	0.551	1.210 [0.646 – 2.27]
	Inframedian	25 (25.8)	72 (74.2)		
		Mortality			
	Supramedian	13 (13.3)	85 (86.7)	0.024	3.556 [1.116 – 11.327]
	Inframedian	4 (4.1)	93 (95.9)		

P value calculated using Pearson's Chi Square, significant on $p < 0.005$.

Multivariate analysis and logistic regression model

Bivariate analysis showed only the wire crossing time was associated to the MACEs incidence ($p < 0.05$), whereas the total ischemic time ($p = 0.373$), the onset ($p = 0.738$), the age ($p= 0.149$) and the smoking habits ($p = 0.180$) were not associated to the MACEs incidence (TABLE 4).

TABLE 4. Feasibility of multivariate analysis

Variables	P*
Age	0.149
Smoking habits	0.180
Wire crossing time	
▪ Dichotomous variable	0.04
▪ Continuous variable	0.028
Onset	0.738
Total ischemic time	0.373

Nonparametric variables were analyzed with Mann-Whitney U test, while categorical variables were analyzed with Pearson's chi square test. *included on multivariate analysis at $p \leq 0.25$

The unadjusted model in this study only tested the Hs-Trop I level and MACEs using logistic regression (TABLE 5). These results showed a significant association between Hs-Trop I and MACEs (OR= 1.872; 95%CI: 1.048-3.343; p=0.034). Model A involved the wire crossing time (dichotomous) and age variable, therefore, the effect of Hs-Trop I on MACEs was adjusted to the presence of these two variables (TABLE 5). These results showed that Hs-Trop I levels still had a significant association with the incidence of MACEs and had an effect on the variable of wire crossing time and patient age (OR=1.848; 95%CI: 1.015-3.366; p=0.045).

TABLE 5. Multivariate logistic regression model

	Risk of MACEs		
	OR	95% CI	p
Unadjusted (Hs-Trop I)	1.872	1.048 – 3.343	0.034
Model A (Hs-Trop I, wire crossing time, age)	1.848	1.015 – 3.366	0.045

*Significant on $p < 0.05$

DISCUSSION

The association between Hs-Trop I and MACEs

Among 195 patients involved in this study, 98 patient were categorized as supramedian Hs-Trop I group and 97 patients were categorized as inframedian Hs-Trop I group. A significant association between the Hs-Trop I and MACEs was observed (TABLE 3). In previous study, a positive association between troponin levels with infarct area in patients with STEMI was reported.[5] Furthermore, another study investigated the infarct area and the extent of damage to the myocardium by comparing the size of the infarct area with the total left ventricular myocardial mass (LVMM). They are the main predictors of the MACEs incidence in STEMI patients with primary PCI, with hazard ratio (HR) of 1.03 times [95% CI 1.01-1.06] ($p < 0.001$). In addition, it was discovered that an infarct size of ≥15% LVMM had a significant negative linear association ($r^2 = 0.66$; $p < 0.001$) with left ventricular ejection fraction (LVEF), which was discovered for every 5% increase in infarct size, followed by a 6.1% decrease in LVEF.[6,7] This decrease in left ventricular ejection fraction is believed to be the cause of the post-infarction sequelae, such as mortality and MACEs.[8,9]

In sub-analysis, a significant association was observed between the supramedian Hs-Trop I with mortality and the incidence of acute heart failure which is common in ischemic/infarction cases in the myocardium (TABLE 3). This causes a decrease in myocardium contractility and leads to hemodynamic instability.[10] Generally, the association between Hs-Trop I levels and mortality may be seen in the decline in cardiac function. It was discovered that a decrease in left ventricular ejection fraction had a significant association with patient mortality. Meanwhile, patients with higher troponin levels on admission had a higher mortality rate as well.[8,11] An insignificant association was also discovered between Hs-Trop I with the incidence of cardiogenic shock and malignant arrhythmias, a condition caused by a single point and not serial troponin measurements. As

described in the previous section, serial measurements of Hs-Trop I describe the progression of myocardial damage over time intervals.[12]

Association between Hs-Trop I, age, and wire crossing time on the incidence of MACEs

The association between the increase in baseline troponin levels (both troponin I and troponin T isoforms) and age has been widely discussed in previous studies. A positive association between troponin levels and age has been reported.[13-15] An increase in Hs-Trop I levels when measured in serial caused long wire crossing times. However, because the measurements in this study were carried out during admission, the low Hs-Trop I condition in detected admission may increase if the subject's wire crossing time was not ideal. Therefore, both of them may influence each other and also affect the outcome, namely the incidence of MACEs.[16] It was also discovered that delayed STEMI treatment performed by primary PCI had a significant association with large infarct size and decreased left ventricular function.[17] Serial measurements of Hs-Trop I show a stronger association between these three variables.[12]

Comparison of Hs-Trop I levels to established risk score

Thrombolysis in myocardial infarction (TIMI) risk score and global registry of acute cardiac events (GRACE) risk score are the main risk scoring system used as a risk stratification method to differentiate patients into low, intermediate, or high-risk cardiac complications. Although they provide a high degree of risk stratification of the patients, they were developed by enrolling patients from North America, South America, and Europe.[18] It is not entirely clear whether the two risk scoring systems provide a good insight to Asian, especially Indonesian patients.

Elevated Hs-Trop I levels was one of the criteria included in GRACE risk score to stratify the risk of the patient. However, the system does not include the troponin levels to the incidence of MACEs. This study may help close the gap by providing the quantitative range of Hs-Trop I that is associated with a higher prevalence of MACEs.

Study limitations

This study has several limitations, such as data obtained from a single-center, Hs-Trop I measurements carried out in a single point unable to show a causal association between the two variables investigated. This study could not examine the incidence of MACEs due to it being a cross-sectional in design. A cohort study is required to determine MACEs incidence and its relation to the level of Hs-Trop I in patients with STEMI.

CONCLUSION

In conclusion, the Hs-Trop I at the time of patient admission, has a significant association with the incidence of MACEs occurring during the hospitalization duration of STEMI patients with PCI at Dr. Sardjito General Hospital, Yogyakarta.

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The significance of glycated haemoglobin, randomized admission blood glucose, and fasting blood glucose on in-hospital adverse cardiac events in patients with ST-elevation acute myocardial infarction

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ABSTRACT

In an ST-segment elevation acute myocardial infarction (STEMI), glucose metabolism undergoes disturbance secondary to acute myocardial injury, which affects the clinical outcome during the acute phase. Glucose metabolic disturbance indices are glycated haemoglobin, admission random glucose, and fasting glucose in blood circulation during STEMI. This is a retrospective cohort study, aimed to investigate whether glycated haemoglobin, admission random blood glucose, and fasting blood glucose levels are the risk factors for developing in-hospital adverse cardiac events in STEMI. The result showed that among the three glucose metabolic disturbance indices, fasting glucose was an independent predictor (adjusted OR: 1.010 (95% CI: 1.001-1.018) and the most accurate factor (AUC 64.9 %) for adverse cardiac events. Other glucose metabolic indices, namely random blood glucose and glycated haemoglobin, were associated with increased odds to develop adverse cardiac events but they did not independently predict adverse cardiac events. Therefore, fasting blood glucose was an independent predictor and the most accurate factor for adverse cardiac events in the acute event of STEMI.

ABSTRAK

Pada infark miokard akut dengan elevasi segmen ST (IMA-EST), metabolisme glukosa mengalami gangguan sekunder akibat cedera miokard akut, yang berpengaruh pada luaran klinis selama fase akut. Indeks gangguan gluco metabolik yaitu hemoglobin terglikasi, glukosa acak saat admisi dan glukosa puasa dalam sirkulasi darah selama periode STEMI. Penelitian ini adalah studi kohort retrospektif, bertujuan untuk menyelidiki apakah hemoglobin terglikasi, glukosa darah acak saat admisi dan kadar glukosa darah puasa merupakan faktor risiko kejadian luaran klinis jantung yang merugikan di rumah sakit pada STEMI. Hasil penelitian menunjukkan bahwa diantara ketiga indeks gangguan gluco metabolik, glukosa darah puasa merupakan prediktor independen (adjusted OR: 1,010 (95% CI: 1,001-1,018) dan faktor yang paling akurat (AUC 64,9%) untuk kejadian klinis jantung yang merugikan. Glukosa darah acak saat admisi dan hemoglobin terglikasi, dikaitkan dengan peningkatan peluang untuk kejadian klinis jantung yang merugikan tetapi tidak secara independen memprediksi kejadian jantung yang merugikan. Oleh karena itu, glukosa darah puasa merupakan prediktor independen dan faktor paling akurat untuk kejadian klinis jantung yang merugikan pada STEMI.

Keywords:
STEMI;
blood glucose;
glycated haemoglobin;
fasting glucose level

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INTRODUCTION

Acute myocardial infarction with ST-segment elevation (STEMI) is a disease due to transmural injury of myocardial cells because of the thrombus occlusion in the coronary artery. Among acute myocardial infarctions, 25% to 45% are STEMI.[1] The complication and mortality in STEMI are still high especially in patients with high-risk profiles.[2] Diabetes mellitus (DM), a chronic state of increased blood glucose level, is one of the high-risk profiles associated with complications and mortality in STEMI.[3] Our previous study indicated the detrimental impact of increased blood glucose levels in STEMI, especially in DM.[4]

The glucose metabolism undergoes several adjustments which produce a stress hyperglycemic state STEMI, associated with increased catecholamine levels secondary to acute myocardial injury.[5] During the acute phase of STEMI, continuous glucose metabolic disturbance is reflected by glycated haemoglobin, admission random glucose, and fasting glucose measured in blood circulation.[6] These biomarkers are functional as early markers of long-standing glucose metabolic disturbance, as opposed to stress hyperglycemia which is an acute phase associated with current acute myocardial infarction.[6]

The poorly-controlled long-standing glucose metabolic control, as reflected by glycated haemoglobin level, is associated with a risk of cardiovascular diseases.[7] Studies showed the relationship between admission blood glucose and the mortality rate after acute myocardial infarction.[8] This study acknowledged the involvement of glycated haemoglobin in this prognostication and as an earlier marker of unrecognized DM during acute events[8,9] Previous study also showed that elevated admission random blood glucose was associated with in-hospital fatal events during STEMI.[10] The importance of acute or chronic gluco metabolic control in STEMI has been extensively studied with various conclusions.

The association between fasting blood glucose in the early phase of STEMI and adverse cardiac events have been established.[3,11,12] These studies lead to the clinical trial to overcome the acute hyperglycemia by lowering it into a basal fasting state with intensified-insulin treatment by 24 h insulin–glucose infusion protocol.[12,13] Within 24 h blood glucose control, the target fasting glucose level is an important predictor of adverse cardiac events.[13] In this study, we aimed to investigate the role of glycated haemoglobin, admission of random blood glucose, and fasting blood glucose levels on the risk of developing in-hospital adverse cardiac events in patients hospitalized with STEMI.

MATERIALS AND METHODS

Study design and Subjects

The study design was a retrospective cohort study. The subjects were patients with STEMI who were hospitalized in the Intensive Cardiac Care Unit of Dr. Sardjito General Hospital, Yogyakarta, Indonesia. The subjects were enrolled consecutively from 2014 to 2018. The subject's data were retrieved from the case report form of the previous study.[14,15] The inclusion criteria were: (1) diagnosis of STEMI, (2) age between 30 and 75 years, (3) onset of anginal pain less than 24 h, and (4) the data of glucometabolic, namely admission random glucose, fasting glucose and glycated haemoglobin parameters were available. The exclusion criteria were: (1) history of chronic kidney disease stage V, chronic heart failure NYHA ≥ II, hepatic cirrhosis, and malignancy, (2) the comorbidities during acute phase: acute systemic infection or/and sepsis, and (3) revascularization procedure before reaching our hospital. All subjects

were given consent information. The study had been approved by the ethics committee of the Faculty of Medicine, Public Health, and Nursing Universitas Gadjah Mada-Dr. Sardjito General Hospital, Yogyakarta, Indonesia (No: KE/FK/817/EC.).

Laboratory examination

Subjects were admitted and followed up during hospitalization in the intensive cardiac care unit. All procedures and treatments received by subjects were in accordance with the attending physicians. The blood sample was withdrawn on admission from antecubital veins before the revascularization procedure was performed if indicated. The random blood glucose was measured from this admission blood sampling. The fasting blood sampling, for 6-8 h fasting, was withdrawn in the morning within 24 h of admission, for measurement of fasting blood glucose and glycated haemoglobin. The central hospital laboratory performed these parameters by standard laboratory practice.

Outcomes

The characteristics of subjects were recorded during intensive hospitalization. The subject's observation was performed during intensive hospitalization to identify the outcome. The outcome of this research was adverse cardiac events occurring during intensive hospitalization, namely the composite of death, acute heart failure, cardiogenic shock, reinfarction, and ventricular arrhythmia requiring resuscitation. Death was determined as death from the cardiac cause. Acute heart failure was the symptom and sign of breathlessness, fatigue, and congestion with the use of intravenous diuretics. Cardiogenic shock was systolic blood pressure <90 mmHg, the signs of low perfusion, and the use of vasopressor drugs. Reinfarction was the recurrence of chest pain, ST-segment elevation and, elevation of creatine kinase-MB/troponin-I after subjects subsided clinically. Ventricular arrhythmia was ventricular tachycardia/fibrillation episodes requiring cardiopulmonary resuscitation.[14] The outcomes were assessed by the attending cardiologists and recorded in a report form. Subjects who discharge from intensive care without any aforementioned events were classified as subjects without adverse cardiac events.

Statistics analysis

The continuous data were tested for normality distribution with the Kolmogorov-Smirnov test after logarithmic transformation as a standard normalization procedure. The parametric and non-parametric tests were used accordingly. The comparison between subjects with adverse cardiac events and those without was performed with Chi-square or Fisher-exact test for categorical data, and for continuous data, the comparison was performed with the Student t-test or Mann-Whitney U test. The receiver operator characteristics (ROC) curve was constructed to compare the accuracy of random blood glucose, fasting blood glucose, and glycated haemoglobin for predicting adverse cardiac events. The univariate and multivariable analyses were performed to establish the independent predictors of adverse cardiac events. A logistic regression test was performed for multivariable analysis by including co-variables from univariate analysis with $p < 0.250$. For comparison, a $p < 0.05$ was determined as statistical significance.

RESULT

The subjects enrolled for this research were 269, mostly male (84.8%) and, a mean age of 56.6 years old.

During observation, 55 subjects (20.4 %) had adverse cardiac events. The subjects with adverse cardiac events were significantly older (p <0.001) and had greater creatinine levels (p =0.011) as compared to subjects with no adverse cardiac events. The proportion of diabetes mellitus, and other risk factors, were comparable between groups. The revascularization procedures did not differ significantly between the groups. Significant increases in random blood glucose, fasting blood glucose, and glycated haemoglobin levels occurred in subjects with adverse cardiac events. TABLE 1 and TABLE 2 show the characteristics of all subjects and their comparison between subjects with adverse cardiac events and those with no adverse cardiac events.

TABLE 1. The characteristics of all subjects with STEMI

Characteristics	All subjects (n=269)
Male sex [n (%)]	228 (84.8)
Age [(years), mean±SD]	56.6±9.1
Hypertension, [n (%)]	146 (54.3)
Diabetes mellitus, [n (%)]	64 (23.8)
Ischemic heart disease [n (%)]	34 (12.6)
Current smoker [n (%)]	110 (40.9)
Dyslipidemia [n (%)]	35 (13.0)
Obesity [n (%)]	86 (32.0)
Haemoglobin [(g/mL), mean±SD]	14.0±1.8
Leukocytes [(x103/mL), mean±SD]	13.3±3.7
Platelet [(x106/mL), mean±SD]	263.9±79.6
Creatinine [(g/dL), mean±SD]	1.2±0.5
Onset of STEMI [n (%)]	7.9±7.4
Anterior STEMI [n (%)]	140 (52.0)
Primary PCI [n (%)]	108 (40.1)
Fibrinolysis [n (%)]	102 (37.9)
Random glucose [(g/mL), mean±SD]	182.5±98.6
Fasting glucose [(g/mL), mean±SD]	134.4±58.3
Glycated haemoglobin [(%), mean±SD]	6.8±2.3

SD: standard deviation; STEMI: ST-elevation acute myocardial infarction; PCI: percutaneous coronary intervention.

TABLE 2. The comparison of subjects with adverse cardiac events and those with no adverse cardiac events

Characteristics	Adverse cardiac events(n=55)	No adverse cardiac events(n=214)	p
Male sex [n (%)]	47 (85.5)	181 (84.6)	0.872
Age [(years), mean±SD]	60.0±8.5	55.7±9.1	0.001
Hypertension, [n (%)]	34 (61.8)	112 (52.3)	0.208
Diabetes mellitus, [n (%)]	14 (25.5)	50 (23.4)	0.745
Ischemic heart disease [n (%)]	4 (7.3)	30 (14.0)	0.130*
Current smoker [n (%)]	25 (45.5)	85 (39.7)	0.440
Dyslipidemia [n (%)]	6 (10.9)	29 (13.6)	0.603
Obesity [n (%)]	34 (65.4)	66.3)	0.897
Haemoglobin [(g/mL), mean±SD]	13.7±1.8	14.1±1.7	0.168
Leukocytes [(x103/mL), mean±SD]	13.9±4.4	13.1±3.5	0.215
Platelet [(x106/mL), mean±SD]	246.2±66.4	268.4±82.2	0.065
Creatinine [(g/dL), mean±SD]	1.4±0.6	1.2±0.4	0.011
Onset of STEMI [n (%)]	7.9±5.8	7.9±7.7	0.930
Anterior STEMI [n (%)]	31 (56.4)	109 (50.9)	0.371
Primary PCI [n (%)]	24 (43.6)	84 (39.3)	0.554
Fibrinolysis [n (%)]	17 (30.9)	85 (39.7)	0.230
Random glucose [(g/mL), mean±SD]	165.5 (125.5-252.5)	140.0 (119.2-195.8)	0.027**
Fasting glucose [(g/mL), mean±SD]	129.0 (110.5-175.8)	114.0 (96.0-136.5)	<0.001**
Glycated haemoglobin [(%), mean±SD]	6.2 (5.6-8.9)	5.8 (5.3-7.1)	0.020**

SD: standard deviation; STEMI: ST-elevation acute myocardial infarction; PCI: percutaneous coronary intervention ;IQR: interquartile range; * Fischer exact test; ** Mann Whitney test

TABLE 3 shows the type of adverse cardiac events that occurred during intensive hospital care. Acute heart failure was the most common adverse cardiac event (58.2%), followed by ventricular arrhythmia (14.5%) and cardiogenic shock (9.1%). The death occurred in 10.9% of subjects. The random glucose level was highest in subjects with cardiogenic shock. The fasting glucose level was also highest in subjects with cardiogenic shock. In all subjects with all types of adverse cardiac events, the fasting glucose levels were higher than in subjects with no adverse cardiac events. There were no significant differences in glycated haemoglobin among subjects of all types and no adverse cardiac events.

TABLE 3. The comparison of random glucose, fasting glucose, and glycated haemoglobin levels based on the type of adverse cardiac events

Type of adverse cardiac events (n=55)	Random glucose	Fasting glucose	Glycated haemoglobin
Death (n=6, 10.9%)	131.0 (118.0-399.5)	128.0 (89.5-348.0)	6.1 (4.6-12.3)
Acute heart failure (n=32, 58.2%)	160.0 (119.0-227.0)	128.0 (113.0-162.0)	6.2 (5.6-8.9)
Cardiogenic shock (n=5, 9.1%)	373.0 (205.5-436.0)	135.0 (108.5-247.5)	7.9 (5.7-11.2)
Ventricular tachycardia/ fibrillation (n=8, 14.5%)	133.0 (127.0-228.0)	129.0 (97.0-197.0)	6.2 (5.7-7.5)
No adverse cardiac events(n=211)	142.0 (120.0-200.0)	115.0 (98.0-139.0)	5.8 (5.3-7.1)
p	0.006*	0.005**	0.315

*One-way ANOVA, with post-hoc test significant for: acute heart failure vs. cardiogenic shock, cardiogenic shock vs. ventricular tachycardia/ fibrillation, cardiogenic shock vs. no adverse cardiac events; **One-way ANOVA, with post-hoc significance for: death vs. no adverse cardiac events, acute heart failure vs. no adverse cardiac events

The ROC curve is shown in FIGURE 1. It indicates that the area under the curve (AUC) of fasting glucose level was the highest (64.9 %), followed by the AUC of glycated haemoglobin (61.3 %) and AUC of random glucose level (58.0 %).

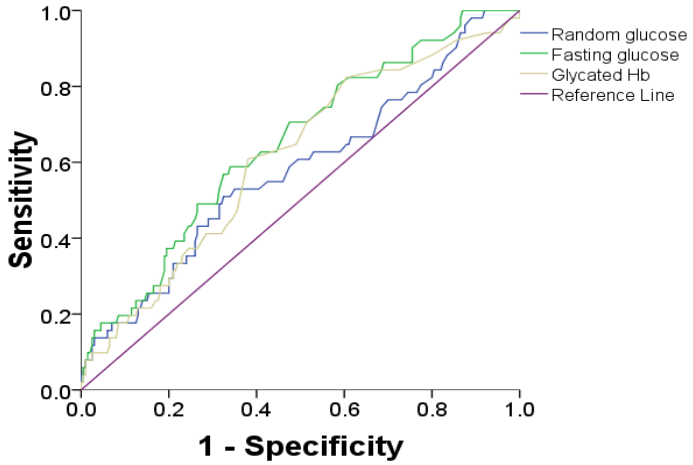

FIGURE 1. The receiver operator characteristics (ROC) curve to compare the area under the curve of random blood glucose, fasting blood glucose, and glycated haemoglobin in relation to adverse cardiac event

The univariate analysis shows that age, creatinine, random glucose, fasting glucose, and glycated haemoglobin levels were associated with increased odds to develop adverse cardiac events. The unadjusted OR of age was 1.058 (95% CI: 1.021-1.096), creatinine level was 2.501 (95% CI: 1.375-4.548), random glucose level was 1.003 (1.001-1.006), fasting glucose level was 1.008 (95% CI: 1.003-1.013) and glycated haemoglobin was 1.127 (95% CI: 1.001-1.270) (TABLE 4).

The multivariable analysis shows that age, creatinine level, and fasting glucose level were associated with increased odds to develop adverse cardiac events. The adjusted OR of age was 1.059 (95% CI: 1.019-1.100), creatinine level was 2.191 (95% CI: 1.188-4.041), and fasting glucose level was 1.010 (95% CI: 1.001-1.018) (TABLE 4).

TABLE 4. The univariate and multivariable analysis for parameters associated with adverse cardiac events

Co-variables	Univariate		Multivariable*	
	Unadjusted OR	95%CI	Adjusted OR**	95%CI
Age	1.058	1.021-1.096	1.059	1.019-1.100
Creatinine	2.501	1.375-4.548	2.191	1.188-4.041
Random glucose	1.003	1.001-1.006	1.000	0.995-1.006
Fasting glucose	1.008	1.003-1.013	1.010	1.001-1.018
Glycated haemoglobin	1.127	1.001-1.270	0.967	0.757-1.235

*Adjusted with age, hypertension, ischemic heart disease, haemoglobin level, leucocyte counts, platelet counts, creatinine level, random glucose level, fasting glucose level, and glycated haemoglobin; ** n = 251

TABLE 5. The changes and ratio of glucose level from admission to fasting state in all subjects

	All subjects (n=251)	Subjects with adverse cardiac events(n=51)	Subject with no adverse cardiac events(n=200)	p
Glucose reduction	30.0 (10.0-61.0)	27.0 (6.0-76.0)	31.0 (10.0-59.75)	0.789
Glucose ratio	1.26 (1.07-1.52)	1.22 (1.05-1.43)	1.28 (1.08-1.53)	0.318

The reduction of blood glucose level from admission to a fasting state was not significantly different between subjects with adverse cardiac events and those with no cardiac events. The glucose ratio, the value of random glucose:fasting glucose, also did not significantly differ (TABLE5).

DISCUSSION

Our current study inferred that among the three glucose metabolic disturbance indices, fasting glucose was an independent predictor and the most accurate factor for adverse cardiac events in the acute period of STEMI. Other glucose metabolic index measures, namely random blood glucose and glycated haemoglobin, were associated with increased odds to develop adverse cardiac events but they did not independently predict adverse cardiac events. Other independent predictors were age and creatinine level.

In the DIGAMI-2 trial, the intense hyperglycemia treatment initiated by insulin–glucose infusion throughout

the first 24 h with the target of normoglycemia after acute myocardial infarction was performed.[13] During the first 24 h, the insulin-glucose infusion was associated with a more reducing blood glucose level and increased incidence of hypoglycemia, while glycated haemoglobin level remained stable.[13] The insulin-initiated infusion was intended to rapidly achieve a normalized blood glucose level.[13] This study concluded that random blood glucose, along with glycated haemoglobin, was a significant and independent mortality predictor jointly with the other conventional risk factors, namely age, heart failure, and elevated serum creatinine.[13] This landmark study supports the suggestion that hyperglycemia is associated with increased adverse events, while normalization of glucose levels benefits patients.

Studies show that admission blood glucose and peak glycemia state are independent predictors for in-hospital mortality in STEMI patients.[16,17] The systematic review and meta-analysis demonstrated that on admission hyperglycemia had an increased risk of reperfusion failure in STEMI patients undergoing primary percutaneous coronary intervention, on those associated with worse adverse events.[18] Higher admission blood glucose level is also related to high burden thrombus and plaque erosion among STEMI.[19] Corroborated and contrasted these findings, our study showed that on admission random blood glucose was increased the risk of adverse cardiac events, however, its risk association was not independent.

Glycated haemoglobin did not significantly change during observation in the DIGAMI-2In DIGAMI-2 trial, but it was associated independently with mortality.[13] Not only in patients with diabetes mellitus, non-diabetic STEMI patients with high glycated haemoglobin levels had endothelial dysfunction and accelerated inflammatory response which is associated with adverse cardiac events following STEMI.[20] Glycated haemoglobin was independently associated with damaged left ventricle diastolic function and elevated filling pressures after STEMI.[21\] However, in a study that included a large number of consecutive STEMI patients with DM who underwent revascularization intervention, glycated haemoglobin was not associated with mortality in any the short or the long term after acute STEMI episodes.[22] Our current study indicated that glycated haemoglobin level was associated with increased odds to develop in hospital adverse cardiac events; however, it did not associate with adverse cardiac events independently.

There is a visit-to-visit fasting blood glucose variability which is proven as an independent predictor of left ventricular unfavorable remodeling in DM patients with STEMI.[23] Furthermore, the glycemic variability which measures the rising and descending acute glucose alteration independently associated with adverse cardiac events in STEMI patients.[24] Higher glycemic variability implicates increased oxidative stress, cytokine release, and endothelial dysfunction which unfavorably influences STEMI outcomes during acute care.[25] These glucose level fluctuations become potential therapeutic targets to reduce myocardial reperfusion injury in STEMI and its related adverse event.[25] Fasting blood glucose alone was independently associated with coronary microvascular obstruction after primary revascularization in STEMI patients without diabetes mellitus.[26] Furthermore, elevated fasting blood glucose level was independently associated with 30-day heart failure and left ventricular systolic dysfunction in patients undergoing primary PCI for STEMI but without diabetes mellitus.[27] Fasting blood glucose also correlated with increased left ventricular end-diastolic

pressure in STEMI patients which was further associated with worse clinical outcome.[28] Incidence of in-hospital adverse events significantly increases in STEMI patients if there are combined elevated random and fasting plasma glucose.[29] Both random and fasting glucose were independent predictors for in-hospital adverse cardiac events and they had good and similar predicting values of in-hospital adverse events.[29] Supporting these findings, in our current study, fasting blood glucose was an independent factor for developing in hospital adverse cardiac events. It surpassed other glucose metabolic index measures, namely random blood glucose and glycated haemoglobin.

This study had several limitations worth mentioning. First, the study design of the retrospective cohort study should be corroborated by a prospective cohort study. Second, the unicenter research should be replaced by including more centers in a multicenter study. Third, the subject number needs to be increased by including more subjects or more centers, and lastly, the follow-up period should be lengthened to detect the long-term consequences of glucose metabolic index disturbance in STEMI.

CONCLUSION

Fasting glucose level was an independent predictor and the most accurate risk factor for adverse cardiac events in the acute event of STEMI, as compared with other gluco metabolic index measures, namely random glucose level, and glycated haemoglobin. Other independent co-variables were age and creatinine level.

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The type of androgenetic alopecia and quality of life (QoL) in male patients

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ABSTRACT

Androgenetic alopecia (AGA) is a nonscarring baldness that mostly affects >50% men worldwide. Hair loss led to psychological difficulties and have a negative impact on the quality of life (QoL). This study aimed to evaluate the relationship between the type of AGA and QoL in male patients. A total of 67 male AGA patients were clinically assessed using the Hamilton-Norwood scale and interviewed using the Hair-Specific Skindex-29 scale to assess QoL. The patients were predominantly in the age group 31-50 years (50.7%), mean age 49.60 years, grade I obese (32.8%), mean BMI 24.93 kg/cm^2, 41.8% with father's AGA history, smoking (62.7%), smoking >100 cigarettes in last 6 months (41.8%), and have hypertension (16.4%) and diabetes mellitus (3%) as concomitant diseases. Based on Noorwood-Hamilton scale, the types of AGA were predominantly type II (25.4%) and followed by type III (16.4%). The results of the Hair Specific Skindex-29 on AGA patients were moderate (58.2%) and severe (41.8%). There were a relationship between AGA type and QoL (p = 0.041) and significant positive correlation between AGA type and QoL (p = 0.020, r = 0.282). In conclusion, patients experienced moderate to severe impact on QoL due to AGA. Thus, every increased in the type of AGA will impact patient's quality of life.

ABSTRAK

Alopesia androgenetik (AGA) adalah kebotakan tanpa jaringan parut yang paling banyak diderita hingga 50% pria di seluruh dunia. Kerontokan rambut menyebabkan masalah psikologis dan berdampak negatif pada kualitas hidup. Penelitian ini bertujuan mengkaji hubungan derajat keparahan AGA dengan kualitas hidup pasien laki-laki. Sebanyak 67 pasien AGA pria dinilai secara klinis menggunakan skala Hamilton-Norwood dan diwawancarai menggunakan skala *Hair-Specific Skindex-29* untuk menilai kualitas hidup. Pasien didominasi kelompok usia 31-50 tahun (50.7%), rerata usia 49.60 tahun, obesitas derajat I (32.8%), rerata BMI 24.93 kg/cm^2, 41.8% dengan riwayat AGA pada ayah, merokok (62.7%), merokok >100 batang dalam 6 bulan terakhir (41.8%), dan memiliki hipertensi (16.4%) dan diabetes (3%) sebagai penyakit penyerta. Berdasarkan skala Noorwood-Hamilton, tipe AGA didominasi tipe II (25.4%) dan diikuti tipe III (16.4%). Hasil *Hair Specific Skindex-29* pada pasien AGA adalah sedang (58.2%) dan berat (41.8%). Terdapat hubungan antara tipe AGA dan QoL (p = 0.041) dan korelasi positif yang signifikan antara tipe AGA dan QoL (p = 0.020, r = 0.282). Simpulan, pasien mengalami dampak sedang ke berat pada kualitas hidupnya dikarenakan AGA. Dengan demikian, setiap peningkatan pada tipe AGA akan berdampak pada kualitas hidup pasien.

Keywords:
androgenetic alopecia;
hair-specific Skindex-29;
quality of life;
risk factors;
age

INTRODUCTION

Androgenetic alopecia (AGA) is a nonscarring baldness that mostly affects up to 50% of men worldwide.[1,2] The AGA onset may be at any age following puberty and increase frequency with age.[1] Approximately 50 to 60% of men are

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affected by the age of 50 years increasing to approximately 80% by the age of 70 years and beyond.[1,3] The etiology of AGA is multifactorial.[1] In men, AGA is an androgen-dependent trait, especially dehydroepiandrosterone (DHEA) levels.[1,3] Considering how lifestyle factors influence hormonal levels greatly, it could be presumed that lifestyle and behavioral patterns may contribute to the occurrence and severity of AGA.[3]

Hair loss led to psychological difficulties and have a negative impact on quality of life (QoL).[4] Loss of self-confidence, lowered self-esteem, low personal attractiveness and social life were reported in male AGA patients.[5,6] People with alopecia are more likely to develop depression and anxiety.[6] Regardless of the extent of the reported psychosocial consequences of alopecia, each investigator used different tools, such as the Skindex-16, the Skindex-29, and the Dermatology Life Quality index and the brief COPE. Among these tools, the Skindex scale was recently used to measure the QoL of patients with hair loss.[7] Gonul *et al*.[8] also reported that AGA patients had significantly higher total Hairdex scores in terms of emotions, functioning, and symptoms, while self-confidence was significantly higher in the alopecia areata patients. Meanwhile, Bade *et al*.[9] reported younger AGA patients were more stigmatized, had poor functioning and emotions stability, but they had more self assuredness. Younger patients seem to retain better QoL despite AGA.[8] However, studies using hair specific Skindex-29 to evaluate the relationship between the type of AGA and QoL in male patients are limited.

MATERIALS AND METHODS

Sample selection

This was cross sectional study conducted in Department of Dermatology and Venereology, University of Sumatera Utara/Universitas Sumatera Utara Hospital, Medan, since July to December 2018 including 67 male with androgenetic alopecia. The diagnosis of androgenetic alopecia was assessed clinically by using Hamilton-Norwood scale. Inclusion criterias were androgenetic alopecia cases with age greater than 18 years, literacy, and absence of psychiatric disorders. Patients with diagnoses other than androgenetic apolecia were excluded in the study. Demographic and detailed clinical data were collected on each patient. Age, gender, body mass index (BMI), family history of AGA, severity of disease were recorded. Informed consent were obtained by each patient for the study. The research was performed in accordance with the principles outlined in the Declaration of Helsinki and approved by the Health Research Ethics Committee of University of Sumatra Utara/H. Adam Malik General Hospital, Medan, Indonesia.

Assessment of androgenetic alopecia

Androgenetic alopecia was classified according to the Hamilton-Norwood scale. Two trained dermatologists blinded to observe subject's hair pattern from two perspectives (side and top), compared the subject's hair pattern with the Hamilton-Norwood baldness scale and selected the best matching stage of the scale through consensus. Subjects were then classified into AGA stages I, II, and IIa, III, IIIa, III vertex, IV, IVa, V, Va, VI and VII, according to the Hamilton-Norwood scale (FIGURE 1). Subjects were then classified into one of three groups to facilitate analysis as mild (AGA grade I and II), moderate (AGA grade IIA, III, IIIA, III vertex, and IV), and severe (AGA grade IVA, V, Va VI and VII), according to the Hamilton-Norwood scale.

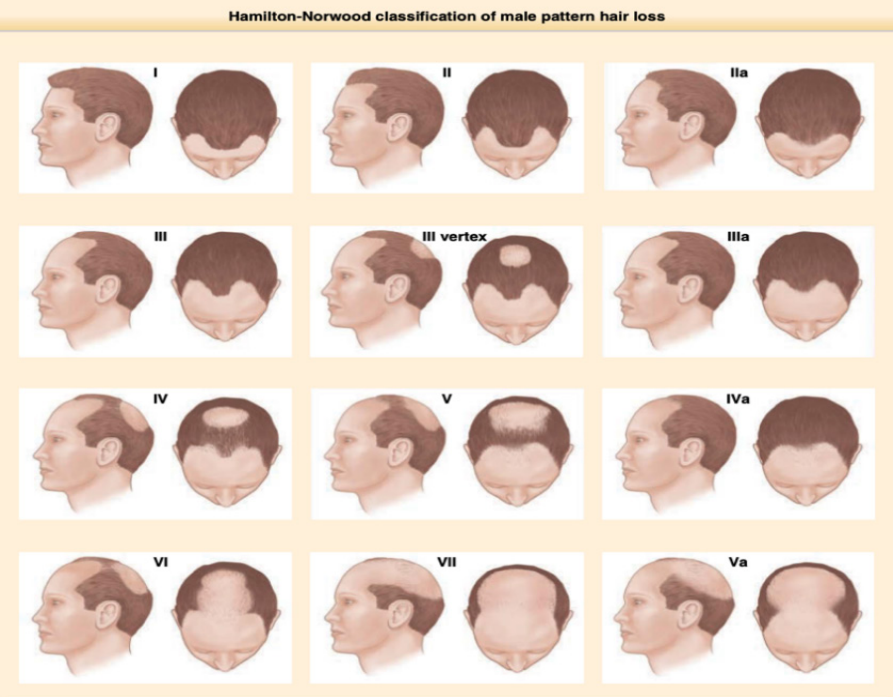

FIGURE 1. The Hamilton-Norwood scale for male AGA.[1]

Assessment of the QoL using the Hair-Specific Skindex- 29

Quality of life was measured by using the Hair-Specific Skindex-29 scale. This scale was originally developed by Chren *et al.*[10] and modified to assess the QoL of patients with AGA. The words 'skin' and 'skin condition' on the Skindex-29 were changed to 'scalp' or 'AGA', respectively, and the Skindex-29 itself was renamed as the Hair-Specific Skindex-29.[10] The Hair-Specific Skindex-29 is a questionnaire that consists of three scales: a symptom scale (7 items), a function scale (12 items), and an emotion scale (10 items) as shown in TABLE 1. Each question will be answered by the patients numerically ranging from 0 (never bothered) to 5 (always bothered). Each answer will be transformed to a linear scale, ranging from 0 (never bothered) to 100 (always bothered). The scale score was the average score from the responded items and the global score was the mean of the sums of each scale. A scale score was the average score from the responded items and a global score was the mean of the sums of each scale. A high score indicates severely impaired QoL, and a low score reflects mild damage in the QoL.[11] Hair-Specific Skindex-29 score was classified into mild (≤ 25), moderate (≤ 32), or severe (≤ 44) impairment of QoL based on Prinsen *et al.*[12]

TABLE 1. Hair specific Skindex-29.[11]

Questions
1. My scalp hurts (Sx).
2. My alopecia affects how well I sleep (Fx).
3. I worry that my alopecia may be serious (Em).
4. My alopecia makes it hard to work or do hobbies (Fx).
5. My alopecia affects my social life (Fx).
6. My alopecia makes me feel depressed (Em).
7. My scalp burns or stings (Sx).
8. I tend to stay at home because of my alopecia (Fx).
9. I worry about getting scars from my alopecia (Em).
10. My scalp itches (Sx).
11. My alopecia affects how close I can be with those I love (Fx).
12. I am ashamed of my alopecia (Em).
13. I worry that my alopecia may get worse (Em).
14. I tend to do things by myself because of my alopecia (Fx).
15. I am angry about my alopecia (Em).
16. Water bothers my scalp (bathing, washing hands) (Sx).
17. My alopecia makes showing affection difficult (Fx).
18. My scalp is irritated (Sx).
19. My alopecia affects my interactions with others (Fx).
20. I am embarrassed by my alopecia (Em).
21. My alopecia is a problem for the people I love (Fx).
22. I am frustrated by my alopecia (Em).
23. My scalp is sensitive (Sx).
24. My alopecia affects my desire to be with people (Fx).
25. I am humiliated by my alopecia (Em).
26. My scalp bleeds (Sx).
27. I am annoyed by my alopecia (Em).
28. My alopecia interferes with my sex life (Fx).
29. My alopecia makes me tired (Fx).

Sx: symptom; Fx: function; Em: emotion

Statistical analysis

Univariate and multivariate logistic regression tests were conducted to determine the relationship between AGA type, age, BMI, smoking habits, and smoking >100 cigarettes in 6 months with quality of life based on the assessment of the Skindex-29 score at a significance level of 5%. Spearman's correlation test was used to determine the relationship between AGA type, age and BMI with Skindex scores in the form of continuous variables with a significance level of 5%.

RESULTS

Patient's characteristics

This study included 67 male patients with androgenetic alopecia. Patients were predominantly age group 31-50 years (50.7%), followed by age group >50 years (46.3%) and the lowest was age group ≤ 30 years (3%), with mean age 49.60 years. Based on body mass index, most of subjects were categorized into grade I obese (32.8%), followed by overweight (28.4%), and normoweight (26.9%), with mean BMI 24.93 kg/cm^2. There were 28 patients (41.8%) with father's AGA history. About 62.7% subjects were smoking and 41.8% subjects were smoking >100 cigarettes in last 6 months. We found that 16.4% subjects have hypertension and 3% subjects with diabetes mellitus as concomitant diseases.

Mean Skindex-29 scores of 67 male subjects were 34.63 ± 9.73, with lowest score was 29 and highest score was 91. Based on Skindex-29 scores, most of subjects severity were moderate in 39 cases (58.2%) and severe in 28 cases (41.8%). Based on Noorwood-Hamilton scale, the types of AGA in this study were

predominantly type II (25.4%), followed by type III (16.4%), IVA (11.9%), and VI (11.9%). The characteristics of subjects with AGA in the study population were shown in TABLE 2.

TABLE 2. The characteristics of male AGA subjects.

Dermographic characteristics	n (%)	Mean (SD)	Median (Min – Max)
Age			
▪ ≤ 30 years	2 (3)	49.60 (10.32)	49 (29 – 70)
▪ 31 – 50 years	34 (50.7)		
▪ > 50 years	31 (46.3)		
Body mass index (BMI)			
▪ Underweight	1 (1.5)	24.93 (3.95)	24.39 (16.26 – 37.76)
▪ Normoweight	18 (26.9)		
▪ Overweight	19 (28.4)		
▪ Obese I	22 (32.8)		
▪ Obese II	7 (10.4)		
Father's history with AGA			
▪ Yes	28 (41.8)		
▪ No	39 (58.2)		
Smoking habits			
▪ Yes	42 (62.7)		
▪ No	25 (37.3)		
Smoking >100 cigarettes in last 6 months			
▪ Yes	28 (41.8)		
▪ No	39 (58.2)		
Hypertension			
▪ Yes	11 (16.4)		
▪ No	56 (83.6)		
Type 2 diabetes mellitus			
▪ Yes	2 (3)		
▪ No	65 (97)		
Skindex-29 scores			
▪ Mild	0 (0)		
▪ Moderate	39 (58.2)	34.63 (9.73)	32 (29 – 91)
▪ Severe	28 (41.8)		
Type of AGA (Noorwood-Hamilton scale)			
▪ I	4 (6)		
▪ II	17 (25.4)		
▪ IIA	3 (4.5)		
▪ III	11 (16.4)		
▪ III vertex	5 (7.5)		
▪ IV	2 (3)		
▪ IVA	8 (11.9)		
▪ V	1 (1.5)		
▪ VA	5 (7.5)		
▪ VI	8 (11.9)		
▪ VII	3 (4.5)		

Hair spesific Skindex-29 domains and global score

Based on the symptoms domain, the mean value was 8.9 with the lowest score of 7 and the highest score of 27. For the emosions domain, the mean value was 11.42 with the lowest score of 10 and the highest score of 28. Meanwhile, the function domain showed a mean value of 14.46 with the lowest score of 12 and the highest score of 91. The mean value of total score was 34.36 with the lowest total score was 29 and the highest score was 91, showed in TABLE 3.

The category of Skindex-29 score

TABLE 4 presented the results of the categorization of the Skindex-29 score. Most of them showed moderate in 39 subjects with a mean Skindex-29 score of 29.92 (SD = 1.2) and severe in 28 subjects with a mean Skindex-29 score of 41.48 (SD = 12.37).

Univariate and multivariate logistics regression

The results of the analysis using the univariate logistic regression test as listed in TABLE 5 showed that there was no significant relation between the independent variable with the quality of life of AGA patients based on the Skindex-29 score assessment (p>0.05).

TABLE 3. Skindex-29 Quality of Life: subscales domain and global score

Domain	n	Mean (SD)	Median (Min – Max)
Symptoms	67	8.9 (3)	8 (7 – 27)
Emotions	67	11.42 (3.06)	10 (10 – 28)
Function	67	14.46 (4.42)	13 (12 – 36)
Overall	67	34.63 (9.73)	32 (29 – 91)

TABLE 4. The category of Skindex-29 scores

Skindex-29 score	n	Mean (SD)	Median (Min – Max)
Moderate	39	29.92 (1.20)	29 (29 – 32)
Severe	28	41.18 (12.37)	36.5 (33 – 91)

TABLE 5. Univariate logistics regression results of AGA type, age, BMI, and smoking behaviour with Skindex-29 scores.

Variables	OR	95% CI	p
AGA type	1.153	0.982 – 1.353	0.082
Age			
▪ ≤ 30 years	0	0	0.999
▪ 31 – 50 years	1.818	0.671 – 4.926	0.240
▪ > 50 years	Ref		
BMI	0.617	0.371 – 1.029	0.064
Smoking habits	1.125	0.411 – 3.079	0.819
Smoking >100 cigarettes in last 6 months	1.387	0.518 – 3.709	0.515

In TABLE 6, the results of a multivariate analysis between AGA type, age, BMI, smoking habits and smoking more than 100 cigarettes in 6 months with quality of life based on the assessment with a Skindex-29 score. The results of the analysis showed that there was a significant relationship between AGA type with the quality of life. The AGA type variable did not show a significant relationship with quality of life when analyzed bivariately (p = 0.082). However, when analyzed together with other variables (multivariate analysis) it showed significant results (p = 0.041, OR = 1.219, 95% CI = 1.008 - 1.475). It meaned that each 1 unit increase in AGA type will increase the odds of quality of life in a more severe direction by 1.219 times.

There was no significant relationship between other independent variables such as age, BMI, smoking habits and smoking >100 cigarettes in 6 months with the quality of life of AGA patients as assessed by the Skindex-29 score, showed in TABLE 6.

TABLE 6. Multivariate logistics regression results of AGA type, age, BMI, and smoking behaviour with Skindex-29 scores.

Variables	OR	95% CI	p
AGA type	1.219	1.008 – 1.475	0.041
Age			
▪ ≤ 30 years	0	0	0.999
▪ 31 – 50 years	1.850	0.585 – 5.853	0.295
▪ > 50 years	Ref		
BMI	0.608	0.347 – 1.065	0.082
Smoking habits	0.545	0.106 – 2.812	0.469
Smoking >100 cigarettes in last 6 months	1.876	0.387 – 9.087	0.434

Spearman's correlation between AGA type, age, and BMI with Skindex-29 score

The data were distributed normally (p > 0.05). By using the Spearman's correlation test, it showed that there was a weak significant positive correlation between the AGA type and the Skindex-29 score (p = 0.020, r = 0.282), showed in TABLE 7. The resulting positive correlation value indicated that every increase in AGA type will be directly increased the Skindex-29 score in AGA patients in this study.

TABLE 7. The Spearman's correlation between AGA type, age, and BMI with Skindex-29 score

	Skindex-29 score	
	r	p
AGA type	0.284	0.020
Age	-0.092	0.457
BMI	-0.150	0.227

DISCUSSION

The present study was evaluated for type of AGA and quality of life in males with AGA. Predominantly, 50.7% subjects in this current study belonged to 31-50 years of age and 46.3% subjects belonged to >50 years of age, with mean age 49.6 years. These findings are important to show that AGA is also part of degenerative diseases. The incidence of AGA gradually increases with age.[9] Similar result was reported by Krupa *et al.*[13] where the prevalence of AGA in an Indian population males aged 30-50 years was 58%. Han *et al.*[11] also reported 46.8% of male AGA belonged to 31-50 years of age and 26.6% belonged to >50 years of age. Meanwhile, Bade *et al.*[9] reported 50% male AGA belonged to 21-30 years of age with mean age of 30.6 years.

Positive father history with AGA was found in 41.8% subjects. Esen Salman *et al.*[14] reported that the frequency of AGA in men with family history of AGA (78.28%) was significantly higher than in men without family history of AGA (39.6%) (p=0.0001). They also reported that AGA frequency in fathers, brothers and second-degree relatives of men with AGA were significantly higher than without AGA (p=0.0001).[14] Chumlea *et al.*[15] reported that men whose fathers had hair loss were 2.5 times as likely to have had some level of hair loss compared to men whose fathers had no hair loss.

Several reports have shown the correlation of lifestyle factors such as obesity, smoking, and concomitant systemic diseases with hair loss.[9,14,16] In our study, most of subjects were grade I obese (32.8%) and followed by overweight (28.4%). Yang *et al.*[17] reported that higher BMI was significantly associated with greater severity of hair loss in men with male-pattern AGA, especially in those with early-onset AGA. In male-pattern AGA with severe alopecia (grade V-VII) had higher BMI than those with mild to moderate alopecia (grade I-IV) (25.1 vs 22.8 kg/m^2, p = 0.01).[17] Meanwhile, Esen Salman *et al.*[14] reported 12% men with AGA were obese and 46% men were overweight. The link between male-pattern AGA and obesity remains unclear. The presence of insulin resistance and up-regulation of insulin-like growth factor-1 in obese subjects are leading to an increased conversion of testosterone to dihydrotestosterone, the principle androgen responsible for male-pattern baldness.[17,18]

In this present study, about 62.7% subjects were smoking and 41.8% subjects were smoking >100 cigarettes in last 6 months. Su *et al.*[19] reported that smokers were at increased risk of having moderate or severe AGA (Norwood types ≥ IV) (OR, 1.61; 95% CI, 1.05-2.46). There were statistically significant positive associations between moderate or severe AGA and smoking status (OR, 1.77; 95% CI, 1.14-2.76), current cigarette smoking of 20 cigarettes or more per day (OR, 2.34; 95% CI, 1.19-4.59), and smoking intensity (OR, 1.78; 95% CI, 1.03- 3.07).[19]

Studies on the association between diabetes mellitus and hypertension with AGA have reported conflicting results.[14,19,20-25] Su *et al.*[19] reported 27.2% male AGA with hypertension and 13.8% male AGA with diabetes mellitus. Meanwhile Ozbas Gok *et al.*[20] reported that there was no significant difference in the rate of metabolic syndrome (included diabetes mellitus) between AGA and control groups (p = 0.135). AGA group had significantly high systolic blood pressure levels than control group (p <0.05). Meanwhile in our study, about 16.4% subjects have hypertension and 3% subjects with diabetes mellitus as concomitant diseases. However, this result should be interpreted carefully because of very small number of patients.

In our study, the types of AGA based on Noorwood-Hamilton classification were predominantly type II (25.4%), followed by type III (16.4%), IVA

(11.9%), and VI (11.9%). Ozbas Gok *et al.*[20] reported that from total of 74 AGA patients, 24 patients (32.4%) were in stage II, 26 patients (35.1%) were in stage III, 17 patients (23%) were in stage III verex, 1 patients (1.4%) was in stage V, and 6 patients (8.1%) were in stage VII according to Hamilton-Norwood classification. Meanwhile Salman *et al.*[14] reported the most common type was type III vertex (24.1%) whereas the least common type was IIIA (0.5%). Type II was the most common type in men aged between 17-29. Type III vertex was the most common type in men aged 30-69 and frequency of type VII increased with age in this group. Type VII was the most common type in men over 70 years.[14]

In our study, the impacts of AGA toward QoL were predominantly moderate in 39 patients (58.2%) and severe in 28 patient (41.8%) using hair specific Skindex-29 scoring. Mean Skindex-29 score was 34.63 ± 9.73. Regardless of the extent of the reported psychosocial consequences of alopecia, each investigator used different tools, such as the Skindex-16, the Skindex-29, and the Dermatology Life Quality index and the brief COPE. Between these tools, the Skindex scale was recently used to measure the QoL of patients with hair loss.[7] Only few studies that reported the impact of AGA toward QoL using hair specific Skindex-29. A multicenter study reported mean global hair specific Skindex-29 score of the AGA patients was 27.3±19.1 (moderate). They also reported that QoL was more damaged if the patient had severe alopecia, a longer duration of AGA, younger age, had received previous non-medical hair care, and visited the hospital for AGA treatment.[11] Jun *et al.*[26] also reported mean composite hair specific Skindex-29 between AA and AGA was moderate (35.7 vs 34.3, respectively). They also reported that AGA patients whose onset age was ≤ 20s and with a disease duration of 1-5 months were more likely to experience lower symptomatic QoL. The differences in the results of this study with other studies may be influenced by age, onset age, disease duration, and previous treatment of the study subjects. Further investigations are needed to determine the effect of these factors on QoL in AGA patients.

We also reported that there were a relationship between AGA type and QoL ($p = 0.041$, OR = 1.219, 95% CI = 1.008-1.475) and also a weak significant positive correlation between AGA type and QoL ($p = 0.020$, $r = 0.282$) in our study. Our findings were consistent with a multicenter study by Han *et al.*[11] have reported that the QoL from the hair specific Skindex-29 score correlated with a severe type AGA ($p < 0.05$). The global hair specific Skindex-29 also significantly correlated with severe type AGA and younger age (< 30 years) ($p < 0.01$).[11] Meanwhile, Jun *et al.*[6] reported that AA and AGA have a significant negative impact on patient QoL. The results suggest that patients with AGA have a significantly decreased QoL.

CONCLUSION

In conclusion, patients experienced moderate to severe impact on QoL due to AGA. Thus, every increased in the type of AGA will impact patient's QoL. It is important that as a clinician, we should consider our AGA patient's psychosocial impact, so that we could offer relevant treatment for the hair loss and also for their emotional distress due to AGA. However, further research is needed to better understand the impacts of AGA and to improve treatment on self-image, psychological functioning and QoL.

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Otoacoustic emission (OAE) features in newborns at Dr. Kariadi Central Genetal Hospital, Semarang, Indonesia

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ABSTRACT

The joint committee on infant hearing (JCIH) recommends the detection and intervention of hearing impairment in newborns. Dr Kariadi Central General Hospital, Semarang, Indonesia is a referral hospital in Central Java province that has implemented an early detection program for hearing loss in newborns. Screening or early detection using otoacoustic emissions (OAE). This study aimed to find out the description of OAE in newborns at Dr. Kariadi Central General Hospital, Semarang. This research was a descriptive analytic using secondary data the OAE results on newborns with or without risk factors (FR) who are treated at Dr. Kariadi Central General Hospital, Semarang in 2020. The result of OAE pass meant that the outer hair cell (OHC) was functioning properly in both ears while refer was if the OHC in one ear or both was not functioning properly. Total 1338 newborns with and without FR whose OAE pass resulted as much as 1297 (97%) and refer 41 (3%). Most risk factors were low birth weight 331 (42%) with the results of OAE pass 312 (94.3%), and refer 19 (5.7%). In conclusion, this study shows that OAE passed in 97% of newborns.

ABSTRAK

Joint committee on infant hearing (JCIH) merekomendasikan deteksi dan intervensi kurang pendengaran pada bayi baru lahir. RSUP Dr. Kariadi Semarang, Indonesia telah melaksanakan program skrining pendengaran pada semua bayi baru lahir, Skrining atau deteksi dini menggunakan *otoacoustic emissions* (OAE). Tujuan penelitian ini untuk mengetahui gambaran OAE pada bayi baru lahir di RSUP Dr. Kariadi Semarang. Penelitian ini adalah deskriptif analitik yang menggunakan data sekunder berupa hasil OAE pada bayi baru lahir dengan atau tanpa faktor risiko (FR) yang dirawat di RSUP Dr. Kariadi Semarang tahun 2020. Hasil OAE p*ass* artinya *outer hair cell* (OHC) kedua telinga berfungsi dengan baik sedangkan r*efer* apabila OHC pada salah satu telinga atau keduanya tidak berfungsi dengan baik. Bayi baru lahir sebanyak 1338 dengan dan tanpa FR dengan hasil OAE *pass* sebanyak 1297 (97%) dan *refer* 41 (3%). Faktor risiko terbanyak adalah berat badan lahir rendah 331 (42%) dengan hasil OAE *pass* 312 (94,3%), dan *refer* 19 (5,7%). Simpulan, peniltian ini menunjukkan bahwa OAE *pass* pada 97% pada bayi baru lahir.

Keywords:
otoacoustic emissions;
risk factors;
newborns;
outer hair cell;
early detection

INTRODUCTION

Otoacoustic emissions (OAE) which is an objective, non-invasive, and practical examination, efficient for newborn hearing screening program. Otoacoustic emissions with a sensitivity of 100% and a specificity of 82-87%.[1] Dr Kariadi Central General Hospital is a referral hospital in Central Java province that has implemented an early detection program for hearing loss in newborns. The results

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of OAE screening can provide data on the prevalence of hearing loss cases and describe the risk factors contributing to hearing loss.

Hearing impairment in infants and children can be caused by maternal and child factors during prenatal, perinatal and postnatal periods.[2] The most common cause of hearing impairment in newborns is congenital abnormalities, with a prevalence of 4-6 infants out of 1000 live births in developing countries. More than 30% of hearing impairment in the prenatal period is caused by diseases, such as measles, mumps, rubella, meningitis, or ear infections. The use of ototoxic drugs in pregnant women can also contribute to the occurrence of the disease. About 17% of perinatal hearing impairment is caused by complications at birth, including prematurity, low birth weight (LBW), birth asphyxia, and neonatal jaundice. Postnatal hearing loss can be caused by ventilator usage, jaundice or hyperbilirubinemia.[1,3,4]

The Joint Committee on Infant Hearing (JCIH) recommended early hearing detection and intervention (EHDI) for the detection and intervention of hearing impairment with the '1-3-6' guidelines. This guideline suggested that screening should be carried out when at the age of less than 1 mo, diagnosis made at the age of 3 mo, and early intervention carried out at the age of 6 mo.[1] Early detection and intervention of hearing impairment are expected to help overcome hearing impairment. This study aimed to describe the result of OAE screening in newborns at Dr Kariadi Central General Hospital Semarang, Indonesia.

MATERIALS AND METHODS

Subjects

This research was a descriptive analytic study. Data were taken from medical records in the form of patient identity, OAE results, and risk factors (RF). The samples were newborn patients treated at Dr. Kariadi Central General Hospital on January 1 to December 31, 2020.

Protocol of study

Infants were screened for hearing impairment with distortion product otoacoustic emission (DPOAE). Otoacoustic emission results can be either a pass or a refer. Pass means that the outer hair cell (OHC) is functioning properly in both ears. Refer if one ear or both are with the refer result. The RF obtained in this study include LBW, premature, asphyxia, hyperbilirubinemia, infants with HIV/AIDS (BIHA), toxoplasma, rubella, cytomegalovirus and herpes (TORCH). If there were multiple FRs then they were included in the group > 1 FR.

Statistical analysis

We analyzed FR using the Chi-square test with p value <0.05 indicates significant.

RESULTS

A total of 1363 infants were screened with OAE and 1338 infants with a pass result of 1297 (97%), refer 41 (3%) as shown in TABLE 1.

TABLE 1. OAE results

	Infants [n (%)]	Pass [n (%)]	Refer [n (%)]
With risk factors	481 (36%)	471 (36%)	10 (24%)
Without risk factors	857 (64%)	826 (64%)	31 (76%)

Most risk factors found were LBW as much as 331 (42%), premature 314 (40%), asphyxia 74 (94%), hyperbilirubinemia 29 (4%), infants with HIV/AIDS (BIHA) mothers 26 (3%), toxoplasma, rubella, cytomegalovirus and herpes (TORCH) 12 (2%). Infants with one risk factor the result of OAE pass 56 (11%), refer 6 (2%). Meanwhile, infants with risk factors >1 with OAE result 425 (83%) pass and 21 (4%) refer. The number of risk factors was not associated with OAE results p = 0.102 (TABLE 3).

TABLE 2. Risk factors analysis

	Pass [n (%)]	Refer [n (%)]	p	CI 95%
1 Risk factor	56 (11%)	6 (2%)	0.102	0.179-1.191
>1 Risk factors	425 (83%)	21 (4%)		

TABLE 3. Distribution of risk factors in newborns and outcomes of OAE

Risk factors	Total [n (%)]	OAE	
		Pass [n (%)]	Refer [n (%)]
BBLR	331 (42%)	312 (94.3%)	19 (5.7%)
Premature	314 (40%)	301 (95.9%)	13 (4.1%)
Asphyxia	74 (9%)	65 (87.8%)	9 (12.2%)
Hyperbilirubinemia	29 (4%)	28 (96,2%)	1 (3.4%)
Baby with aids mother	26 (3%)	25 (96.2%)	1 (3.8%)
TORCH	12 (2%)	10 (83.3%)	2 (16.7%)

DISCUSSION

Speech and language development in children is closely related to hearing function because the ability to hear has a very important role in speech development. The listening process through sound stimulation will affect brain development. Thus the importance of knowledge about early detection of hearing impairment so that intervention can be obtained as early as possible.5 The JCIH 2019 recommends screening for all newborns. Screening was performed on infants within 72 h of discharge from hospital.1

One of the screening tools is OAE which it is used to assess cochlear function. It is a low tone acoustic response to an external sound stimulus received by the OHC of the cochlea. The cochlea acts as an external sound sensing organ. In the cochlea the sound will be selected based on the frequency so it will be transmitted to the auditory nervous system and brain stem, then sent to the brain. In the brain, sound will be perceived. Damage that occurs in external hair cells, for example due to viral infection, ototoxic drugs, reduced blood flow to the cochlea causes OHC to not produce OAE.[6,7]

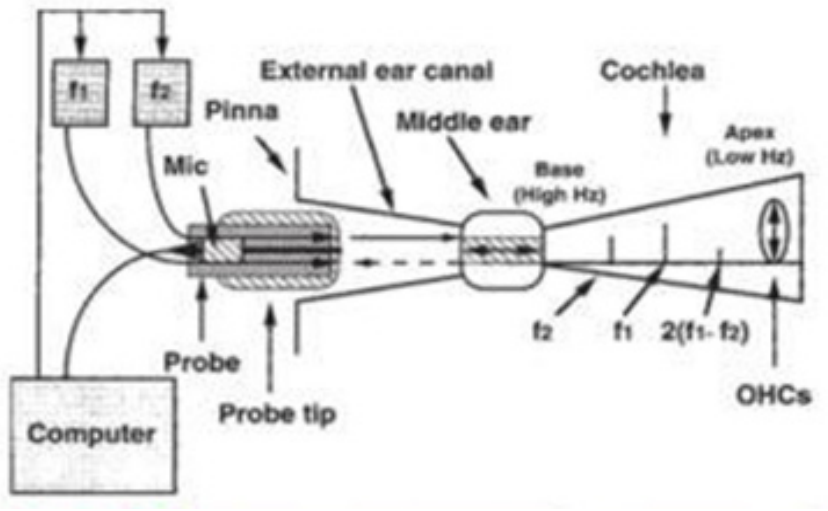

FIGURE 1. OAE mechanism [8]

The method of screening for newborns in Indonesia based on a method developed by the Ministry of Health of the Republic of Indonesia in 2019 (FIGURE 2). Initial screening is carried out with OAE examination after the baby is >24 h old or before being discharged from the hospital. If the first stage of screening results in refer, then at the age of 3 mo should be evaluated with otoscopy, tympanometry, DPOAE, and automated auditory brainstem response (AABR) or brain evoked response auditory (BERA). If the results of the second stage remain the same, a hearing evaluation is carried out with an auditory steady-state response (ASSR).[9,10]

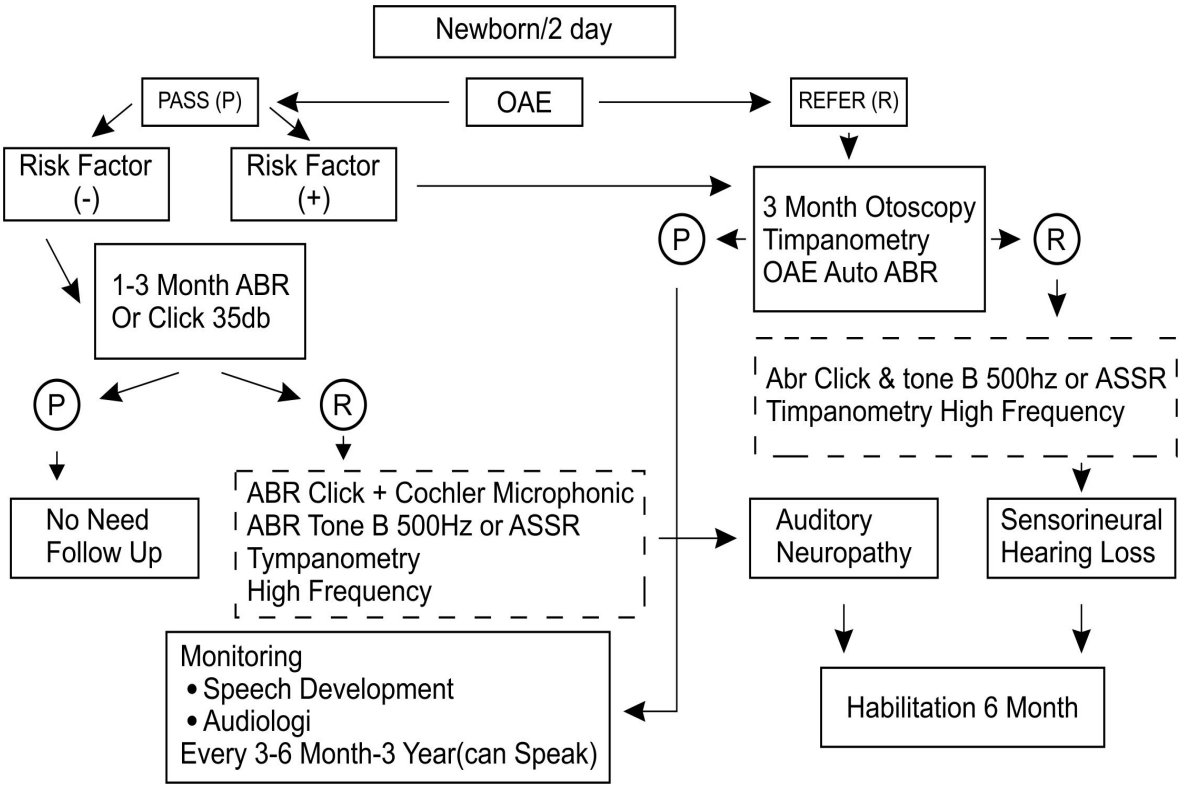

FIGURE 2. Hearing screening in Indonesia [9,10]

The JCIH program (2019) and the Ministry of Health of the Republic of Indonesia (2019) are universal newborn hearing screening (UNHS). Dr. Kariadi Central General Hospital has implemented UNHS where all newborns did the OAE examinations, both babies at risk and without the risk of hearing impairment. Hearing loss in infants and children can be caused by maternal and child factors, it can occur during prenatal, perinatal and postnatal periods. Risk factors for hearing loss in infants aged (0-28 d) are family history of congenital deafness, prenatal infections such as TORCH, anatomical abnormalities of the head and neck, syndrome associated with congenital deafness, LBW (<1500 g), bacterial meningitis, hyperbilirubinemia, severe asphyxia, giving ototoxic drugs, and using a breathing apparatus/mechanical ventilation >5 d in the NICU.5

A total of 1338 infants were examined for OAE in the period January-December 2020, for a total of 41 babies (3%) indicated referral. That could be caused by damage to the OHC or due to technical errors, it could also be due to obstruction in the outer and middle ear. The maximum allowable of fail are 5-10%.11 In this study, 3% of failures were reported, so the results of the OAE that have been carried out show the correct way to use the OAE, and the results of the OAE reference obtained indicate that there is damage to the OHC. The most risk factors found were LBW 331 (42%) then premature 314 (40%). This result is in accordance with previous studies which reported that the most risk factor was LBW 102 (67.7%). Other studies found LBW to be one of the risks of hearing loss in newborn screening.[12]

The results of this study found that there was no relationship between the number of risk factors and OAE results (p = 0.102). The same results were obtained in a previous study which reported that 2,284 infants were not associated with risk factors (p > 0.05).[13,14] Another study linking prematurity, LBW with OAE features in newborns concluded that there was a relationship between LBW and preterm pregnancy with OAE examination results (p = 0.027).[15]

CONCLUSION

Screening for newborns (UNHS) resulted in 1297 passes (97%) and 41 refer (3%). Infants with the most risk factors were LBW 331 (42%) with OAE pass results 312 (94.3%) and refer 19 (5.7%). The number of RF was not associated with OAE results. This study only carried out screening with OAE, did not continue the next screening stage for refer results with otoscopy, tympanometry, OAE, and AABR or BERA and ASSR. Further study can be carried out by screening evaluation with OAE tympanometry and BERA in the next 6 mo and a 2 y cohort study to identify the incidence of hearing loss and speech language.

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Sudden weakness due to thyrotoxic periodic paralysis: a case report

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ABSTRACT

Thyrotoxic periodic paralysis (TPP) is a complication of hyperthyroidism. Lead to sudden hypokalemia and muscle weakness. In most cases, it is found mainly in young adult males of the Asian race. The paralysis is temporary and will return with potassium correction. There are very few cases reported in Indonesia regarding these cases of TPP. In our case, a 44-year old man complained of weakness that started later in the limbs but quickly improved. This case was diagnosed late at first, and uncorrected potassium levels led to recurrent paralysis. Laboratory test results showed severe hypokalemia (potassium level 1.81 mmol/L). The thyroxine level (T4) was 44.12 pmol/L and low serum thyroid-stimulating hormone (TSH) <0.0025 IU/mL.

ABSTRAK

Periodik paralisis tirotoksis (PPT) merupakan suatu komplikasi yang terjadi karena adanya hipertiroid yang menyebabkan hipokalemia dan kelemahan pada otot secara mendadak. Pada banyak kasus ditemukan terutama pada laki-laki muda dengan ras Asia. Kelemahan yang dialami sifatnya sementara dan akan kembali dengan diberikannya koreksi kalium. Laporan kasus yang dilaporkan di Indonesia sangat minim terkait kasus PPT ini. Pada kasus yang kami termukan, laki-laki usia 44 tahun mengeluhkan kelemahan yang diawali kedua kaki kemudian tangan, tetapi membaik dengan sendirinya. Keluhan tersebut dirasakan kembali berulang beberapa hari kemudian. Kasus ini pada awalnya terlambat didiagnosis, sehingga tidak dilakukan koreksi kalium dan terjadi kelemahan berulang. Pemeriksaan laboratorium menunjukkan hipokalemi berat dengan kadar serum kalium 1,81 mmol/L dan kadar tiroksin (T4) tinggi yaitu 44,12 pmol/L dan rendahnya serum *thyroid stimulating hormone* (TSH) <0,0025 IU/mL.

Keywords:
hyperthyroidism;
paralysis;
hypokalemia;
thyrotoxicosis;
T4

INTRODUCTION

Thyrotoxic periodic paralysis (TPP) is a disorder, characterized by abrupt onset of hypokalemia and paralysis.[1] It is more common in men, especially in Asian races, in the ages between the 20s and 40s.[1,2] The clinical of the patient presence hypokalemia and paralysis.[1]The incidence of TPP in Asia is around 1.8%-1.9%.[3] Several conditions can trigger the TPP such as carbohydrate diet, strenuous exercise, stress, and steroid use.[4] Laboratory tests such as potassium level, thyroid-stimulating hormone (TSH), and free thyroxine (FT4) levels help diagnose TPP with limb weakness or paralysis.

CASE

A 44-year-old man complained woke up around 03.00 AM when he wanted to go to the bathroom suddenly felt weak in his legs and could not move his legs. The symptoms slowly showed in his both arms. The patient then tried to move

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both extremities and then recovered for about 2 h. In the afternoon, the patient went to the hospital for a laboratory examination (cholesterol, uric acid, complete blood count), the results were normal. At the first examination, the patient was taking analgesic medication (paracetamol).

One week later, the same complaint appeared when he woke up, after which the patient was taken to the emergency room the next day because he felt the symptoms were getting worse. Several hours before the symptoms appeared, he did not do an excessive activity. The patient said before he slept, he consumed fried rice.

The patient admitted that he felt palpitations, often felt anxious, had frequent sweat, could not sleep at night. But symptoms such as weight lost were denied. This symptom had been felt for about 2-3 mo, but because it was not bothersome so the patient did not try to find doctor. Six months before, he had similar symptoms but healed on their own. The patient denied any history of hypertension, diabetes, and other metabolic diseases. The patient claimed to have a history of kidney stones about 11 years ago. The patient admitted that he rarely had a medical check-up, either a health center or an independent check-up. There was no treatment routinely consumed by the patient before. The patient said there was no similar family history.

Based on the Wayne index as a hyperthyroid score (TABLE 1), a score of 11 was obtained from the patient's signs and symptoms. Interpretation score 11 means equivocal.

TABLE 1. Wayne index

Symptoms of recent onset and/or increased severity	Score	Signs	Present	Absent
Dyspnea on effect	+1	Palpable thyroid	+3	-3
Palpitations	+2	Bruit over thyroid	+2	-2
Tiredness	+2	Exophthalmoses	+2	
Preference for heat	-5	Lid retraction	+2	-
Preference for cold	+5	Lid lag	+1	-
Excessive sweating	+3	Hyperkinesis	+4	-2
Nervousness	+2	Hands hot	+2	-2
Appetite : increased	+3	Hands moist	+1	-2
Appetite : decreased	-3	Casual pulse rate : >80/min	-	-3
Weight increased	-3	>90/min	+3	-
Weight decreased	+3	Atrial fibrilation	+4	-

On physical examination at the emergency room consciousness examination (Glasgow Coma Scale) E4V5M6 was 15, consciousness compos mentis, the blood pressure was 150/100 mmHg, pulse 106x/min, temperature 36.5 ℃. There was no tremor, exophthalmos, or goiter. Meningeal sign examination was negative, cranial nerve examination was within normal limits. Physiological reflexes biceps +2/+2, triceps +2/+2, patella +2/+2, achilles +2/+2. Pathological reflexes were not found. In physical examination showed that the muscle strength in the upper limb 4/5 and leg muscle strength 1/5. One day before

going to the emergency department, he felt fever and take paracetamol.

Laboratory tests in the emergency room found sodium levels of 144.5 mmol/L (normal 136-146), potassium 1.81 mmol/L (normal 3.5-5.1) with an ECG showing the presence of type I AV block (FIGURE 1). The TSH <0.0025 IU/mL (normal 0.35-4.940) was examined, freeT4 was 44.12 pmol/L (normal 9.00-19.05) which led to thyrotoxicosis. Laboratory tests of complete blood, hemoglobin 12.6 g/dL (13.2-17.3), leukocytes 10.17 thousand/mmk (normal 4.5-11.5), erythrocytes 4.65 million/mmk (normal 4.50-6.20), triglycerides 117.0 mg/dL (normal <150), blood sugar at time 111.6 mg/dL (normal 70-140), ureum 43.4 mg/dL (normal 19.0-44.0), creatinine 1.42 mg/dL (0.73-1.18). Initial therapy was infusion of 25 meg KCl in 500 NaCl within 6 h.

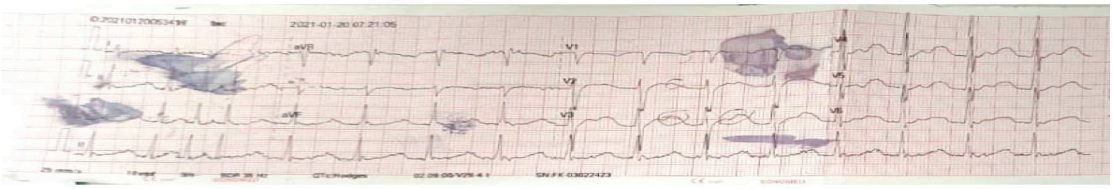

FIGURE 1. ECG

After receiving initial therapy, 5 h later the patient experienced improvement. Motor examination showed muscle strength in the upper limb 5/5 and leg muscle strength 5/5. In addition to hypokalemia therapy, the patient took propylthiouracil (PTU) 100 mg for hyperthyroidism. After giving PTU, follow-up was carried out on a routine outpatient basis once every month at the internal polyclinic. The PTU therapy was still given in this period. Symptoms of hyperthyroidism in the patient such as palpitations, restlessness, tremors, difficulty sleeping but not frequently wer observed. Long-term medication in the form of PTU has still been given up to now. Regular follow-up was carried out every month in the Internal's Clinic to monitor signs and symptoms of hyperthyroidism.

At post-hospital follow-up, tremors were found, weighing 56.5 kg. After two months post-hospitalization and therapy, the patient complained of red urine and sore waist, although the patient was diagnosed with nephrolithiasis. Follow-up three months post-hospital, the patient no longer feels symptoms of hyperthyroidism, no tremor, no restlessness, sleep well.

DISCUSSION

Hypokalemic paralysis is classified into hypokalemic periodic paralysis (HPP) and non-hypokalemic periodic paralysis. Hypokalemic periodic paralysis is hypokalemia due to potassium shift to intracellular. Non-hypokalemic periodic paralysis was by the large amount of potassium deficit that passes through the gastrointestinal or renal.[1] Hypokalemic paralysis periodic can be from family history (familial periodic paralysis/FPP), more common in non-Hispanic Caucasians. Non-familial HPP such as TPP is more common in Asians and Hispanics.[5] The TPP showed hyperthyroidism and also

a rare complication of thyrotoxicosis.[6,7] Any case of hyperthyroidism can lead to TPP, including grave's disease (most commonly). The most common factors that can trigger TPP are consumption of high-carbohydrate foods, high salt/ sodium intake, strenuous exercise, stress, trauma, and medications (diuretics, estrogens, corticosteroids, NSAID, ecstasy).[7] The incidence of TPP is very low and more common in men than women (20:1), even though the incidence of Graves disease is more in women. There is no definite and clear reason for this.[8]

Hypokalemia in the TPP occurs due to a rapid and massive shift of potassium intracellularly.[9] The mechanism of TPP because of hypokalemia and associated paralysis. Progressive muscle weakness to paralysis that occurs due to hypokalemia can be life-threatening but is still reversible. Skeletal muscle is the largest reservoir for potassium in the body (2600 mmol) and has an important role in extracellular potassium homeostasis. The homeostasis process by the Na-K-ATPase pump and the Potassium canal. The K channel regulates the intracellular movement of potassium including inward rectifying (Kir 2.6) and the extracellular movement of potassium.[5,7] Thyrotoxicosis can cause hypokalemia by directly increasing the genetic transcription of the Na-K-ATPase pump thereby increasing the activity of this pump.[10] Thyroid hormone also stimulates beta-2 adrenergic and increases its sensitivity to circulating catecholamines that increase Na-K pump activity.[11] Insulin is known to work by entering blood glucose intracellularly along with the entry of potassium, causing a decrease in potassium levels in the blood.[7]

There are two main examinations on the TPP. Check electrolytes and thyroid values. At the beginning of the examination, the value of potassium is usually less than 3 mmol/liter and can even be 1.1 mmol/liter. When it has entered the healing phase, the periodic paralysis serum potassium can gradually return to normal. The value of thyroid hormone in the blood is high, but TSH will be low.[4] ECG is needed especially if the patient is in hypokalemia for a long time, it induced arrhythmias in cases of TPP.

The occurrence of paralysis is associated with the pathogenesis of intracellular shift potassium. Excessive K into the skeletal muscle cells prevents the muscle cells from being excited, resulting in weakness. The influx of potassium into muscle cells occurs during the rest phase, this may explain why patients experience symptoms at night or dawn during sleep (rest).[12] In this case, the patient consumed fried rice the night before the symptoms, but there's no specific amount of carbohydrate he consumed. Eating high carbohydrates is a trigger factor in the TPP. Hyperinsulinemia plays a role in increasing Na/ K-ATPase activity and inhibiting the efflux from potassium out of muscle cells.[7,8,13] The patient denies any emotional stress due to work or in the family. Emotion, stress, and trauma can be factors that trigger TPP, stress related to hormones. Catecholamines affect Na/K-ATPase activity and inhibit potassium efflux by inhibiting inward-rectifying potassium activity.[14] In this case, an abnormal ECG presence of AV block type 1. Another case report even mentioned acute respiratory failure and ventricular arrhythmias.[9] In another case, the presence of TPP with ventricular tachycardia.[15] The presence of arrhythmias, in this case, can be associated with the occurrence of hypokalemia within a certain time. Low potassium in the extracellular can compensate the body to increase Na channel activity and a buildup of Na in the intracell. Intracell Na accumulation triggers a reduction in Na/Ca exchange and reduces Ca efflux increased

intracellular Ca. Ca overload increases Ca/calmodulin-dependent kinase (CaMKII) activity that can be trigger early after depolarisations (EADs) and induced arrhythmias.[16]

The principle of TPP management is to focus on relieving acute symptoms and preventing recurrences. The patient should be kept in a euthyroid condition to prevent recurrence of paralysis. Recurrent attacks of TPP occur in 60% of patients on anti-thyroid therapy.[8] Potassium is given by intravenous as well as antiarrhythmic propanolol in acute attacks.[6] Identification of the causes of thyrotoxicosis is important for pharmacotherapy. If a known cause such as Graves' disease, a toxic adenoma requiring surgery, needs a definitive therapy such as radioactive iodine or thyroidectomy. Surgery is preferable for cases of Graves with large goiters.[8,13] Education to avoid trigger factors such as maintaining a high carbohydrate diet, strenuous exercise, emotional stress, the use of corticosteroids is important to prevent recurrence.[1,7,13]

In this case report, we described cases of TPP in which the symptoms of hyperthyroidism are not clear. This condition makes it difficult for clinicians to determine further therapy. This article may be helping other clinicians to make therapy in the same case. This report still has limitations, such as a lack of physical examination and follow-up in this case. Urinalysis is needed to see the state of kidney function. A complete thyroid function test may be possible. An EMG may be needed to confirm the cause of the paralysis. Other diagnoses such as metabolic myopathies may be considered which are triggered by exercise, stress, and cold exposure, to be sure about that need for a muscle biopsy. Autoimmune diseases such as myasthenia gravis and GBS can be confused because of similar attacks of paralysis. However, the presence of a low potassium level and the absence of specific clinical myasthenia gravis can confirm the diagnosis of TPP.[17]

CONCLUSION

Thyrotoxic periodic paralysis is a type of hypokalemic periodic paralysis. It is one of the rare cases of complications of hyperthyroidism. It needs to differentiate it from CVA to avoid misdiagnosis. Prevent recurrence of attacks by maintaining euthyroid condition by providing potassium correction therapy and hyperthyroid medication.

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Malignant bilateral ovarian steroid cell tumor without androgenic manifestation: an unusual finding

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ABSTRACT

Steroid cell tumor is a rarest ovarian neoplasm, classified as a pure stromal tumor and mostly is unilateral. Even though this tumor can exhibit malignant behavior but the morphology of cells showed benign characteristics which can become a diagnosis pitfall especially in the frozen section. Moreover patient without any hormonal imbalance or virilizing signs could make the diagnosis process more difficult. Here we reported a case bilateral steroid cell tumor of the ovary in a 42 y.o. unmarried woman without any virilization or hirsutism symptoms. Abdominal ultrasonography and computed tomography (CT) scan revealed a right ovarian solid tumor accompanied by ascites and right pleural effusion. There was significantly increased of Ca 125 level (1138 U/mL) and normal level of testosterone (0.10 ng/mL). Frozen section was done from the right ovarium mass and ascites fluid, the result was benign. From the total abdominal hysterectomy and bilateral salpingo-oophorectomy tissues, histological picture showed diffuse and nests tumor separated by thin fibrous connective tissue with small round centered nuclei, mild atypia, and abundant pale cytoplasm. Large area of necrosis was found especially in the right ovarian tumor, tumor implant to the right fallopian tube and in the uterine serous layer. Periodic acid-Schiff (PAS) stain was negative in more than half tumor cells population. Immunostaining for Melan-A and Calretinin were focally positive, with Ki-67 labeling index ± 5%, and negative for cytokeratin 7 (CK7), cytokeratin 20 (CK20) and smooth muscle actin (SMA). Based on the tumor size, necrosis area, tumor implantation, and immunohistochemistry profiles, we conclude that were malignant steroid cell tumor. Currently, the patient is undergoing postoperative recovery and planned for platinum-based chemotherapy. A careful correlation between clinical and radiological findings, as well as histopathological results, is always essential, as is amply demonstrated by this particular case.

ABSTRAK

Tumor sel steroid adalah sebuah neoplasma ovarium yang paling jarang yang diklasifikasikan sebagai tumor stroma murni dan sebagian besar bersifat unilateral. Meskipun tumor ini dapat menunjukkan sifat ganas tetapi morfologi sel menunjukkan sifat jinak sehingga dapat terjadi kesalahan diagnosis terutama dari potongan beku. Selain itu pada pasien tanpa gangguan ketidakseimbangan hormon atau tanda-tanda virilizing membuat diagnosis lebih sulit. Di sini dilaporkan kasus tumor sel steroid bilateral ovarium pada wanita belum menikah berusia 42 tahun tanpa gejala virilisasi atau hirsutisme. Ultrasonografi abdomen dan *computed tomography scan* (CT scan) mengungkapkan tumor padat ovarium kanan disertai asites dan efusi pleura kanan. Ada peningkatan nyata kadar Ca125 (1138 U/mL) dan kadar testosteron normal (0,10 ng/mL). Pemeriksaan terhadap pemotongan bekuan massa ovarium kanan dengan cairan asites menunjukkan tumor jinak. Dari histerektomi total abdomen dan jaringan salpingo-ooforektomi bilateral, gambaran histologis menunjukkan tumor difus dan bersarang dipisahkan oleh jaringan ikat fibrosa tipis dengan inti bulat kecil di tengah, atypia ringan, dan sitoplasma pucat berlimpah. Area nekrosis yang luas ditemukan terutama pada tumor ovarium kanan, tumor yang berimplantasi pada tuba falopi kanan

Keywords:
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dan pada lapisan serosa uteri. Pewarnaan periodik asam-Schiff (PAS) negatif pada lebih dari setengah populasi sel tumor. Imunostaining untuk melan-A dan calretinin secara fokal positif, dengan indeks pelabelan Ki-67 ± 5%, dan negatif untuk sitokeratin 7 (CK7), sitokeratin 20 (CK20) dan aktin otot polos (SMA). Berdasarkan ukuran tumor, area nekrosis, implantasi tumor, dan profil imunohistokimia dapat disimpulkan bahwa tumor sel steroid tersebut ganas. Saat ini, pasien sedang menjalani pemulihan pasca operasi dan direncanakan untuk kemoterapi berbasis platinum. Korelasi yang cermat antara temuan klinis dan radiologis, serta hasil histopatologis, selalu penting, seperti yang banyak ditunjukkan oleh kasus khusus ini.

INTRODUCTION

Steroid cell tumor is one of the pure stromal tumors of the ovary. The incidence rate is only 0.1% from all ovarian neoplasm which makes it very rare. This tumor is usually benign, unilateral and more than half cases have androgenic symptoms. Only one-third of cases that have malignant behavior and about 10-15% the cases without clinical signs or symptoms of increased hormone levels or asymptomatic.[1] It is widely known that even though the tumor histopathologically was benign. It can not exclude the possibility of malignancy which can be a pitfall especially in frozen section examination.[2]

The following report focuses on a case of malignant bilateral steroid cell tumor of the ovaries diagnosed in a 42 y.o. unmarried woman without any overt androgenic manifestations. There were discrepancies between clinicopathological findings and histomorphological features in the frozen section examination. This was a very rare case, also a diagnostic challenge for pathologists and as far as we know, the first case report from Indonesia.

CASE

Clinical history

A 42 y.o. unmarried woman referred to the Obstetrics and Gynecology Department of Dr. Sardjito General Hospital with chief complaints of abdominal enlargement for 2 mo with abdominal distention. There were no virilization nor hirsutism symptoms. Also no history of vaginal bleeding nor vaginal discharge. In physical examination, there was an abdominal distention due to a fixed huge solid abdominal mass. The upper margin of abdominal mass was 3 fingers above the umbilical, right margin was anterior axillary line, and left margin was midclavicular line. The lower margin of the mass was palpated in rectal examination while uterine was difficult to access. There was also a decreased vesicular sound in the right lung, a sign of pleural effusion. Transabdominal ultrasound examination and abdominal CT scan revealed a right ovarian solid tumor sized 20,1x11.7x16.8 cm^3 accompanied with ascites (FIGURE 1). Laboratory test showed significantly increased of Ca 125 level (1138 U/mL) and normal level of testosterone (0.10 ng/mL). The patient then underewent an exploratory laparotomy with frozen section for the right ovarian mass and ascites fluid, followed by total abdominal hysterectomy, bilateral salpingo-oophorectomy, and sampling of pelvic lymph nodes. Currently the patient is undergoing postoperative recovery and planned for platinum-based chemotherapy.

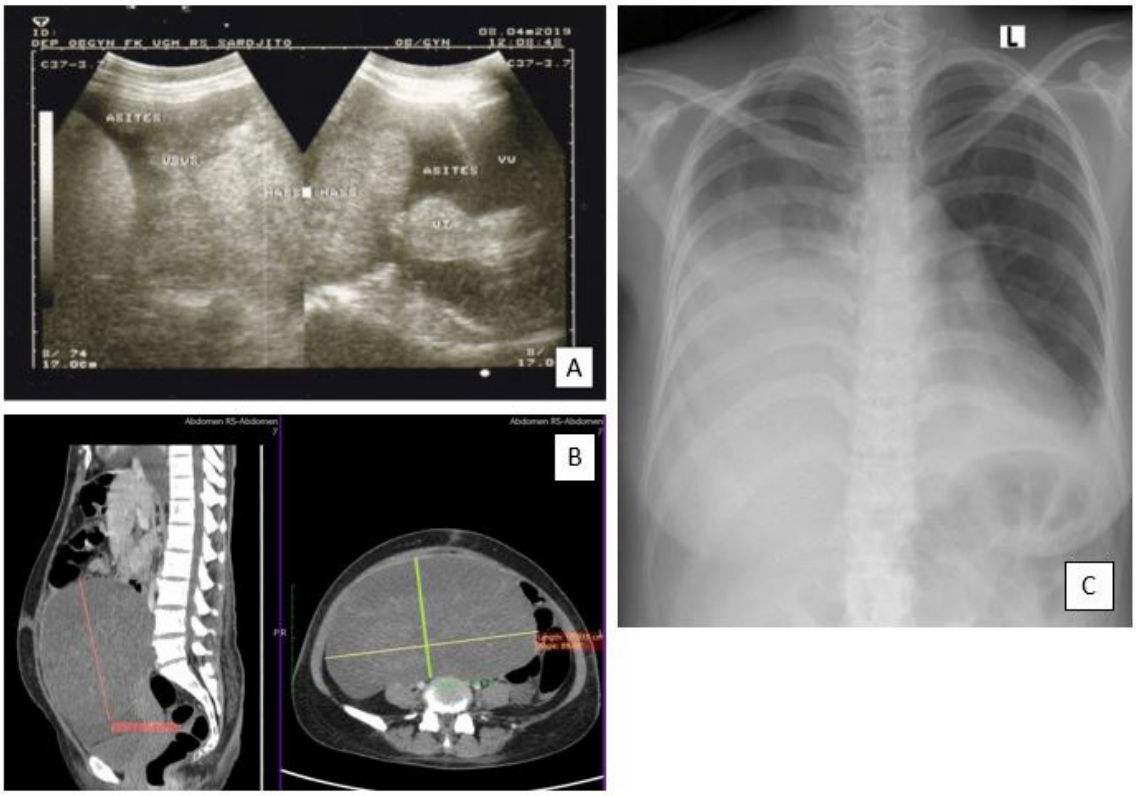

FIGURE 1. Radiological examination (A). Transabdominal ultrasound and, (B). Abdominal CT scan examination revealed a right ovarian solid tumor sized 20x11x15 cm^3 accompanied with ascites. (C). Chest X-ray showed a right pleural effusion.

Pathological findings

Frozen section of the right ovarian solid tumor showed a mass sized 22 x 17 x 8 cm^3, with firm solid cut surface and tan yellowish colored, some with black colored parts (FIGURE 2). The microscopic pictures showed tumor cells arranged in diffuse nests separated by thin fibrous connective tissue with a large area of necrosis. Cells were polygonal-shaped with abundant pale or clear cytoplasm. Small round centered nuclei, some are eccentric, with mild atypia (FIGURE 3). From the ascites fluid sample, microscopically showed hypocellular with 2 – 3 clusters of relatively monomorphic cells with pale cytoplasm and bland round to oval shaped nuclei with mild atypia degree (FIGURE 4). We concluded that the tumor and the ascites fluid were benign. But because of the clinical and laboratory findings led to malignancy, the patient underwent a total abdominal hysterectomy, bilateral salpingo-oophorectomy, and sampling of pelvic lymph nodes for prevention and clinical staging.

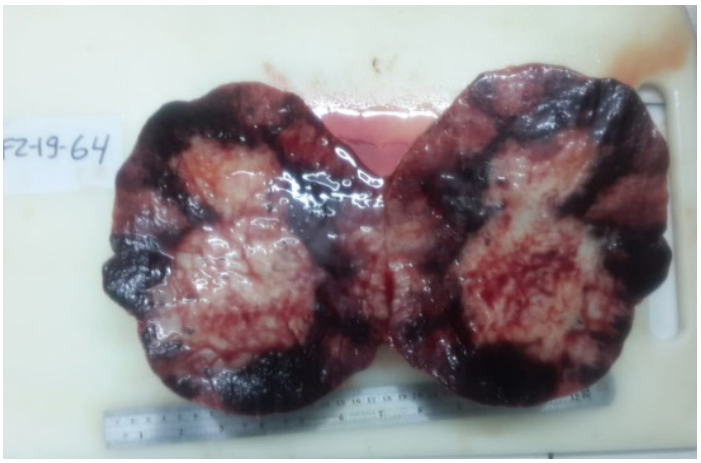

FIGURE 2. Gross appearance of the right ovarian solid tumor in frozen section examination showed solid tumor with tan yellow colored with some black patch

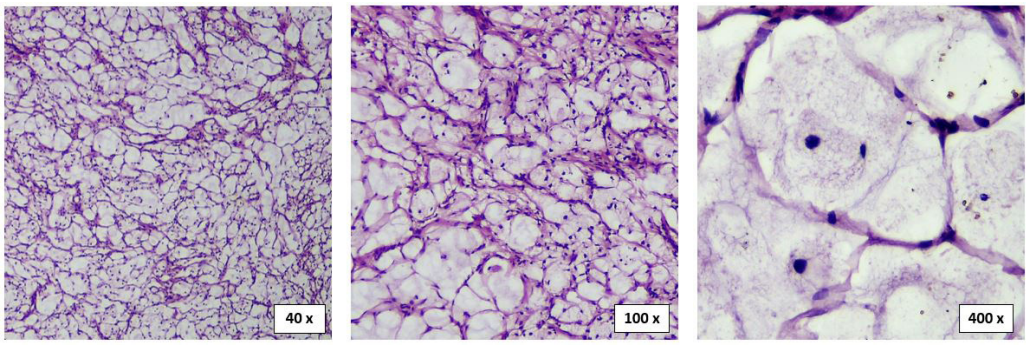

FIGURE 3. Microscopic appearance of the right ovarian solid tumor in frozen section examination: tumor cells arranged in diffuse pattern also in nests with scant fibrous stroma. Cells were polygonal shaped with abundant pale clear cytoplasm. Round small nuclei with mild atypia.

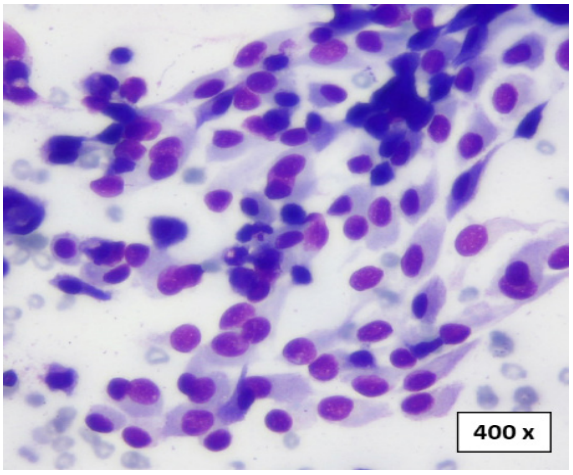

FIGURE 4. Microscopic appearance of the ascites fluid, hypocellular smear with few cluster of mildly atypia cells with pale cytoplasm and bland nuclei.

In specimens of total abdominal hysterectomy, bilateral salpingo-oophorectomy, and sampling of pelvic lymph nodes there were similar right ovarian tumor in the right fallopian tube (FIGURE. 6A), left ovary (FIGURE. 6B). Tumor also implanted in uterine serous layer (FIGURE. 6C). Based on the tumor behavior , we suspected a malignant tendency. Our differential diagnosis was malignant steroid cell tumor (lipid cell tumor), malignant signet ring stromal tumor, and metastasis of signet ring cell adenocarcinoma from gastrointestinal site (Krukenberg tumor). To established the main diagnosis and excluded the differential diagnosis, histochemical staining of PAS and immunostaining of CK7, CK20, Calretinin, Melan-A, SMA and Ki-67 were performed. The results showed that PAS staining was negative in more than half tumor cells population. Immunostaining for Melan-A and Calretinin were focally positive, with Ki-67 labelling index ± 5%, and negative for CK7, CK20 and SMA (FIGURE 7). Clinical, histopathological, histochemical and immunohistochemical findings confirmed the diagnosis of malignant bilateral ovarian steroid cell tumor.

The pleural effusion fluid was a highly turbid fluid approximately 42 mL, also submitted for cytological evaluation. The result showed hypocellular smear with evenly distributed amorphic mass. There were no malignant cells.

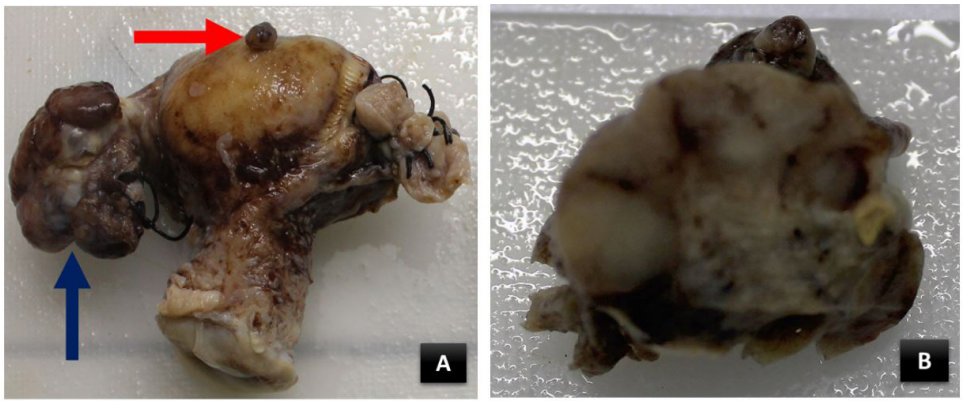

FIGURE 5. Gross appearance of the total abdominal hysterectomy and bilateral salpingo-oophorectomy: (A) Left ovarian mass (blue arrow) and implantation in the uterine serosal layer (red arrow), (B). Cut surface of the left ovarian mass.

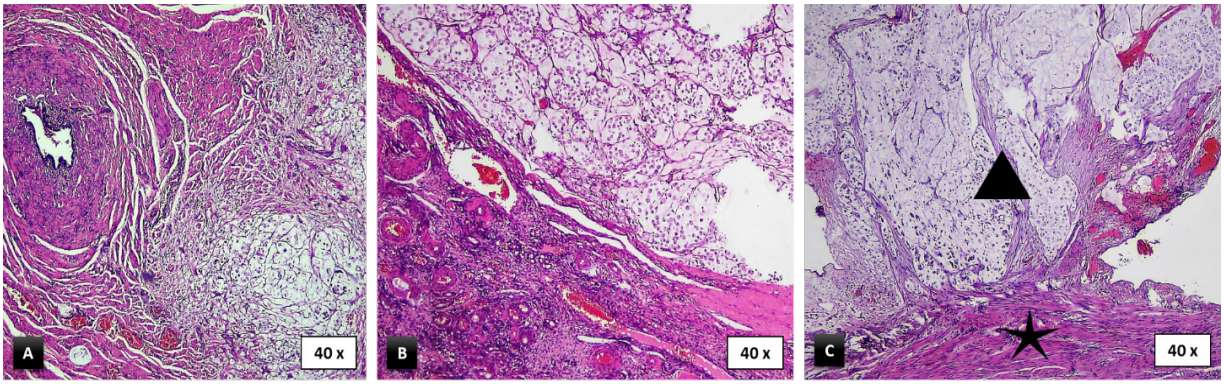

FIGURE 6. Microscopic apperance: (A). Tumor spread to the right fallopian tube, (B). Similar tumor in the left ovary, (C). Implantation of the tumor to the uterine serous layer (★: uterine serous layer; ▲: tumor's implantation).

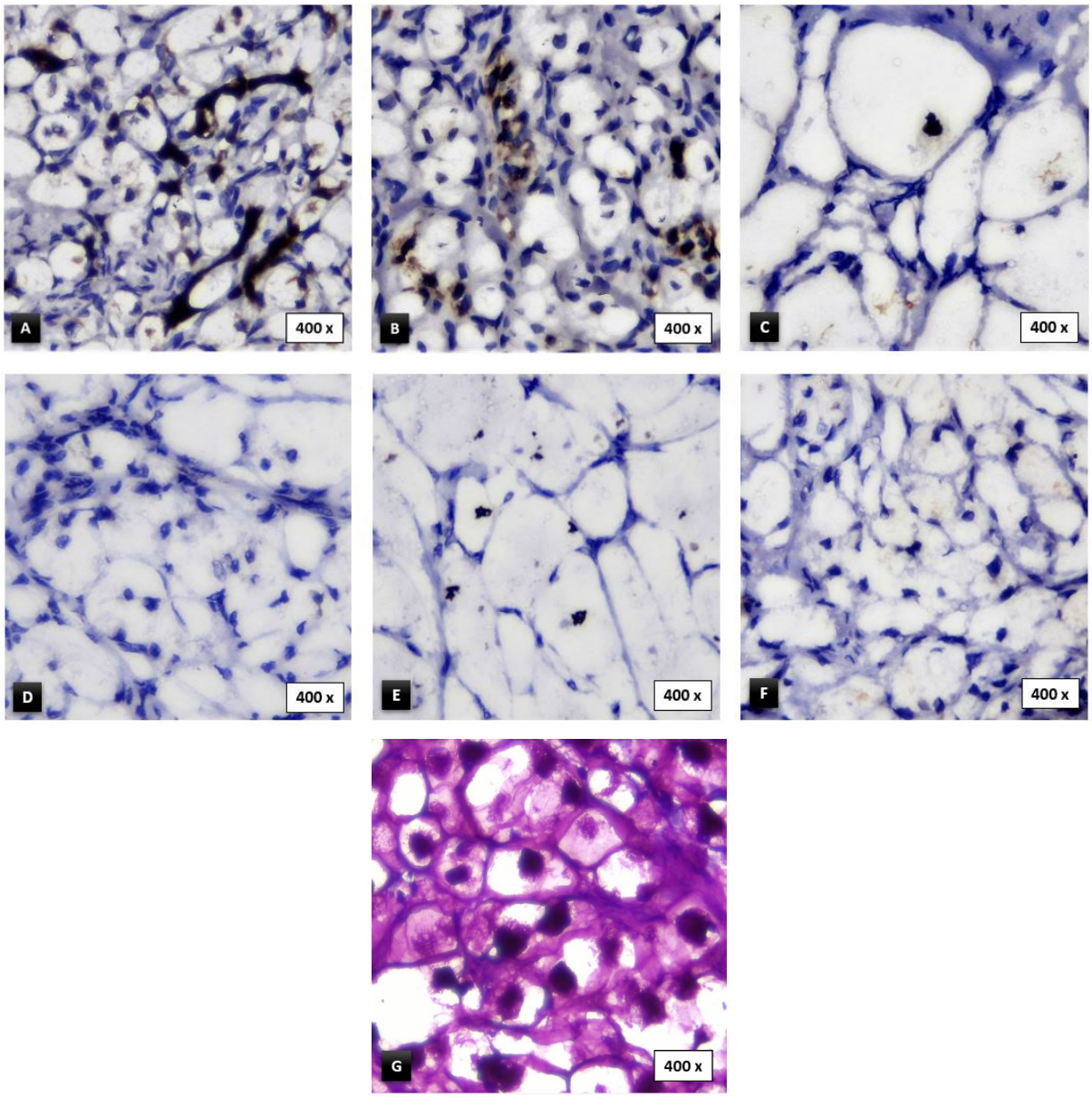

FIGURE 7. Immunostaining of (A) Calretinin, showed focal positivity in nuclei and cytoplasm; (B) Melan-A, showed focal positivity in cytoplasm; (C) Ki-67, low proliferation index; (D) Negative for CK7,(E) CK20 and (F) SMA. Histochemical staining of PAS (G) showed negativity in more than half tumor cells population

DISCUSSION

Ovarian steroid cell tumors composed of cells that resemble steroid hormone-secreting cells (Leydig/lutein/adrenal cortical rest cells) with lack of Reinke crystals, which was the origin of their entity. It was thought to arise from ovarian stromal cells. These tumors occur in a wide age range from premenarchial girls to postmenopausal women, with mean age of 43 years old. This mean age was very similar to our patients' age which was 42 years old. Steroid cell tumors are usually unilateral, with mean diameter is 8.4 cm. The cut surface usually solid, with yellow-orange, brown, red, or black colored, and accompanied by areas of hemorrhage.[1,3]

According to the literature, combined clinical symptoms, microscopic features, and immunohistochemical results

are important to diagnose a steroid cell tumor.[2,6] About one-third of cases were malignant and about 20% of them metastasis to other organs even when the histopathological findings were benign. Because of this reason, it is important to be able to recognize whether the tumor was malignant or not and performed careful follow-up. Hayes and Scully in 1987 identified 5 certain histopathological characteristics that could predict malignant behavior of ovarian steroid cell tumor which are: two or more mitotic figures per 10 high power fields (92% malignant), areas of necrosis (86% malignant), tumor's diameter 7 cm or greater (78% malignant), areas of hemorrhage (77% malignant) and grade 2-3 of nuclear atypia (64% malignant).[1,4-6]

It was difficult to determine the type and behavior of the tumor. Our case also showed benign morphology in the frozen section but evaluation of the total abdominal hysterectomy, bilateral salpingo-oophorectomy, and sampling of pelvic lymph nodes showed tumor implantation to the right fallopian tube and uterine serous layer. Tumor largest diameter was 22 cm with large areas of necrosis also highly indicate the malignant behavior of the tumor.

Because steroid cell tumor is considered as a pure stromal ovarian neoplasm, it will be positive for sex cord-stromal immunohistochemical markers such as calretinin, inhibin, and steroidogenic factor-1. In general, calretinin and inhibin were considered as a sensitive with a percentage of positivity of approximately 100% but less specific marker for ovarian sex-cord stromal tumors and useful to distinguish sex-cord stromal from non-sex-cord stromal tumors. Many studies also report that steroid cell tumor is positive for melan-A. The positivity rate of melan-A in a study by Jones et al. was 86%.[1,4,7] In our case, calretinin and melan-A were both focally positive.

Our case showed negative expression of CK7 and CK 20, these results excluded the diagnosis of metastasis of signet ring cell adenocarcinoma from gastrointestinal site (Krukenberg tumor). In a few literature, other epithelial markers such as AE1/AE3 and CAM 5.2 were reported positive in 30-40% steroid cell tumor. Nevertheless, the staining pattern was predominantly weak and focal.[7] Negative expression of SMA also excluded ovarian signet-ring stromal cell tumor. This result was in line with Jones *et al.*[7] study which stated that most of the steroid cell tumor was negative for muscle-specific actin and SMA. Our case showed low proliferation index (± 5%) but there was more than 2 mitoses per 10 high power fields. PAS staining in more than half tumor cells population was negative due to their lipid-rich cytoplasm.

Before diagnosing a steroid cell tumor, signs of metastasis or other primary mass in other organs need to be evaluated using radiographical imaging because a majority of steroid cell tumors have a more favorable prognosis than the metastatic or malignant primary tumors of the ovary with a similar histomorphology.[7] There were no signs of other primary tumor nor distant metastasis of steroid cell tumor in our case.

Until now, there are no specific tumor markers or imaging techniques for preoperative diagnosis of steroid cell tumors. Ovarian tumor markers such as Ca 125 and α-fetoprotein were generally normal. A significant elevation of these tumor markers also does not indicate malignant characteristics.[3] In our case, the level of Ca 125 level was 1138 U/mL but soon after the surgery it decreased to 90.38 U/mL.

Our case also without androgenic manifestations such as virilization and hirsutism, this was an unusual finding. The level of testosterone in our patient was normal (0.10 ng/mL). About 50% of patients have androgenic symptoms

because the tumor-secreted steroids, mostly testosterone, 10% have estrogenic symptoms, some can show Cushing syndrome or progestational changes, and a minority of 10-15% of patients have no symptoms of increased steroid hormone production.[3]

Because this tumor can be malignant and has aggressive behavior, it should be staged and aggressively cytoreduced. In patients who desire future fertility, conservative surgery with unilateral oophorectomy and proper staging should be performed. The management would be different for women who have completed childbearing, which are total abdominal hysterectomy with bilateral salpingo-oophorectomy with complete surgical staging. Adjuvant chemotherapy were given based on the tumor's histopathological appearance and on its surgical stage. Until now there are no well defined chemotherapy guidelines for steroid cell tumor. Steroid cell tumors with signs of malignancy histologically based on Hayes and Scully's criterias, or were at an advanced stage should be treated with additional postoperative platinum-based chemotherapy.[8,9] In our case, the patient was an unmarried woman but due to signs of malignant behaviour of the tumor and the tumor already affected both of the ovaries, total abdominal hysterectomy with bilateral salpingo-oophorectomy with complete surgical staging were performed with the consent of the patient. After the post-operative surgery, clinician planned to perform postoperative platinum-based chemotherapy.

CONCLUSION

Ovarian steroid cell tumor especially the malignant one is a rare case of all ovarian tumors. Not to mention that only 10-15% of these patients have no symptoms of increased steroid hormone production, making the preoperative definitive diagnosis for this tumor was difficult. The benign appearance of this tumor microscopically but with malignant behaviour are also challenging in this case. A careful correlation between clinical findings, radiological findings, and histopathology is always essential, as is amply demonstrated by this particular case. Moreover, the definitive diagnosis whether the tumor was steroid cell tumor, benign or malignant would very much affect the standar management for the patient. The necessity of a regular follow up must be stressed upon, with the aim of detecting a possible recurrence or evidence of distant metastasis.

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The use of rigid gas permeable contact lens in managing severe anisometropia caused by monocular aphakia following retinal reattachment surgery and high myopia in a 13 year old girl: a case report

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ABSTRACT

Anisometropia if not treated accordingly may cause other issues especially in developing pediatric eyes. This case is a 13 year old female presented with chief complaint of headache and double vision upon wearing prescribed spectacles. Patient had history of high myopia on both eye, amblyopia and spontaneous rhegmatogenous retinal detachment on the left eye. Retinal reattachment and lensectomy surgery were conducted to correct the problem. The surgery was performed without intra ocular lens (IOL) implantation, which left her left eye become high hyperope due to aphakia. Patient was treated with RGP CLs. Final best visual acuity with EDTRS chart was 6/48 on both eyes. Patient reported subjective visual improvement, no headache or double vision.

ABSTRAK

Anisometropia jika tidak ditangani dengan baik dapat menyebabkan komplikasi terutama pada perkembangan mata anak. Dilaporkan kasus, perempuan berusia 13 tahun dengan keluhan utama sakit kepala dan penglihatan ganda saat memakai kacamata. Pasien memiliki riwayat myopia tinggi pada kedua mata, ambliopia dan ablasio retina regmatogen spontan pada mata kiri. Operasi lensectomi dilakukan dengan menempelkan retina kembali untuk memperbaiki masalah. Operasi dilakukan tanpa implantasi IOL, yang membuat mata kirinya menjadi hiperopia tinggi akibat afakia. Pasien diobati dengan RGP CLs. Ketajaman visual terbaik terakhir dengan grafik EDTRS adalah 6/48 pada kedua mata. Pasien melaporkan perbaikan visual subjektif, tidak ada sakit kepala atau penglihatan ganda.

Keywords:
anisometropia;
pediatric;
myopia;
rhegmatogenous;
lensectomy

INTRODUCTION

Visual impairment is a global health problem that has affected an estimated 253 million people worldwide, among those 217 million people have moderate or severe visual impairment and 36 million have blindness.[1-3] From all visually impaired people around the globe, the prevalence of blindness in children is significantly lower than adults. Despite lower in number, prevention and treatment of childhood blindness should remain a priority because of their expected longer remaining lifetime and hindrance in both their visual and general development it may cause.[3-4]

Refractive error is one of the most common cause of visual impairment in children.[5] In some cases, difference in refractive error between the eyes might happen, this is called anisometropia. The

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prevalence of anisometropia in children vary, with most studies reported a prevalence of lower than 10%.[6-9] Although anisometropia is an infrequent finding, it is often related to spectacle intolerance due to aniseikonia.[10]

Nowadays, contact lenses are commonly used for refractive correction, especially in severe anisometropia. Previous studies have reported rigid gas permeable contact lens as an effective device in children visual rehabilitation. Since it can be customized to achieve the desired power, diameter and base curve needed for the small developing eyes.[11-13]

We reported a case of severe anisometropia with unilateral aphakia, amblyopia and low vision with reported spectacles noncompliance. An intra ocular lens (IOL) implantation was not a treatment choice in this situation, since the patient had microcornea of her both eyes. The objective of this report is to illustrate the use of rigid gas permeable contact lens as a treatment option to improve visual acuity in pediatric patients with severe anisometropia.

CASE

A 13-year old female presented with chief complaint of headache and double vision upon wearing spectacles which cause noncompliance. The spectacles prescribed were S-8.00 C-2,00x180 for the right eye and S+10.00 C-1,75x70 for the left eye. Patient had history of high myopia on both eye, amblyopia and spontaneous rhegmatogenous retinal detachment on the left eye 2 years prior. Scleral buckle, vitrectomy, endolaser, lensectomy and injection of silicon oil was conducted to correct the problem. The surgery was performed without IOL implantation due to her microcornea, which left her left eye become high hyperope due to aphakia. A year after the surgery, she was diagnosed with secondary glaucoma and leucoma. The patient then got silicone oil removal and corneal scraped for the band keratopathy. Patient was born 39 weeks of gestation with normal birth weight, with nystagmus present in the first few months of life. There was no history of ocular trauma nor systemic disease. Her guardian denied any similar condition in her family.

Patient's best corrected visual acuity (BCVA) with spectacles were 6/48-2 on the right eye and 6/48-2 on the left eye uncorrected with pinhole. The patient has bilateral nystagmus and microcornea, corneal diameter was 9.20 mm on the right eye and 9 mm on the left eye with band keratopathy. She also has aphakia, decreased light reflexes, retina attached with subtle silicone oil visible at the superior retina of the left eye. Near sight test with spectacles were 1.6M and 1.0M after magnification trial using 20 D spectacles.

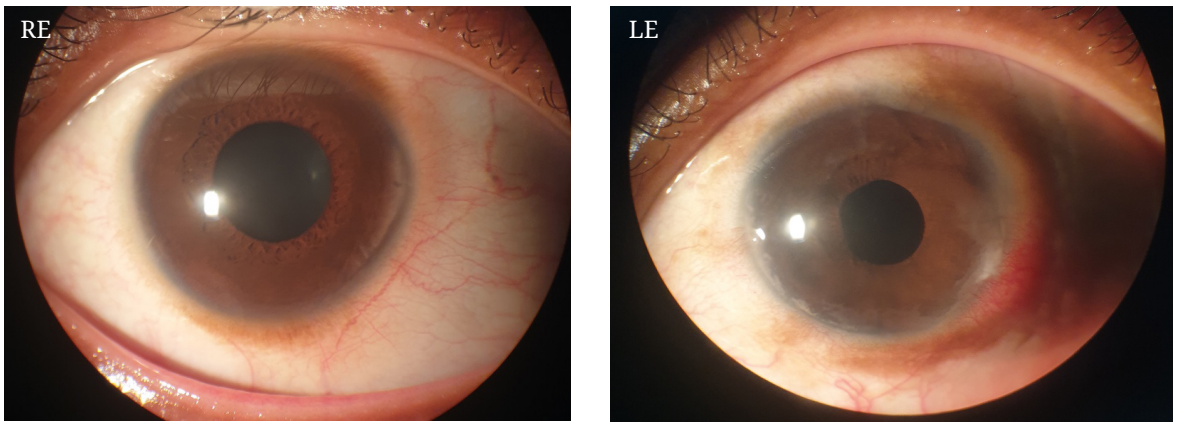

FIGURE 1. The profiles of the cornea of both eyes, as seen from the anterior with slit lamp

After considering her medical history and ophthalmological examination, she was diagnosed with severe anisometropia, amblyopia, nystagmus and low vision. Her amblyopia was likely secondary due to the central nervous system problem since there was congenital nystagmus. In order to correct her underlying issue and improve compliance, she was referred for RGP CLs.

The corneal topography of the right eye showed that the horizontal curvature was 41.70D (8.09 mm) and the vertical curvature was 43.8 D (7.70 mm), while the corneal topography of the left eye showed that the cornea was irregular with the radius of horizontal curvature was 44.8 D (7.53 mm) and the radius of vertical curvature was 46.7 D (7.23 mm).

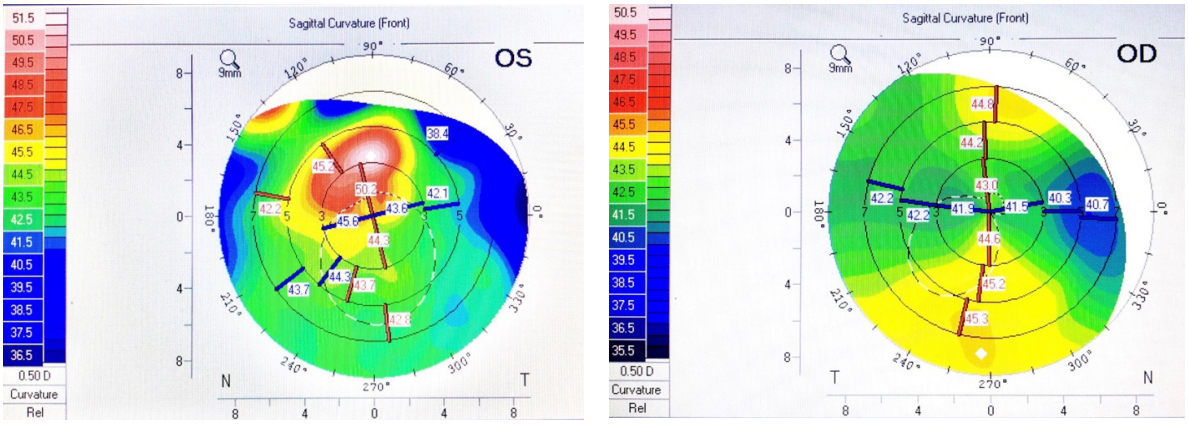

FIGURE 2. The cornea topography of both eyes, as seen from the anterior with slit lamp

Trial fitting for RGP CL was performed using tisilfocon A (Menicon Z α, Japan) lens with base curve 8.00 mm for the right eye and 7.50 mm for the left eye, power spherical -4.00D and diameter 9.20 mm for both eyes. The over refraction with the trial lens revealed no residual errors in the right eye and spherical +10.00D in the left eye. Upon evaluation, there were acceptable up riding centration between blinks on the right eye and good centration on the left eye. Contact lens movement were considerably good, it was 1.5mm upwards while blinking in both eyes. Some decentrations were shown immediately after blink with adequate pupil coverage. The up riding fitting RGP of the right eye was consistence with steep vertical cornea curvature and affected more by tight eyelid. Movement improved after full blink. Best corrected visual acuity was 6/48 on both eyes with EDTRS. Fluorescein test showed with the rule astigmatism on the right eye and acceptable slightly tight fitting with still sufficient movement while blinking on the left eye. Significant corneal with the rule astigmatism, makes the fluorescein pattern could not achieve ideal pattern with central minimal clearance. It could lead to flat band horizontally and pooling at superior-inferior as seen in the right eye, or inferior riding as seen in the left eye.

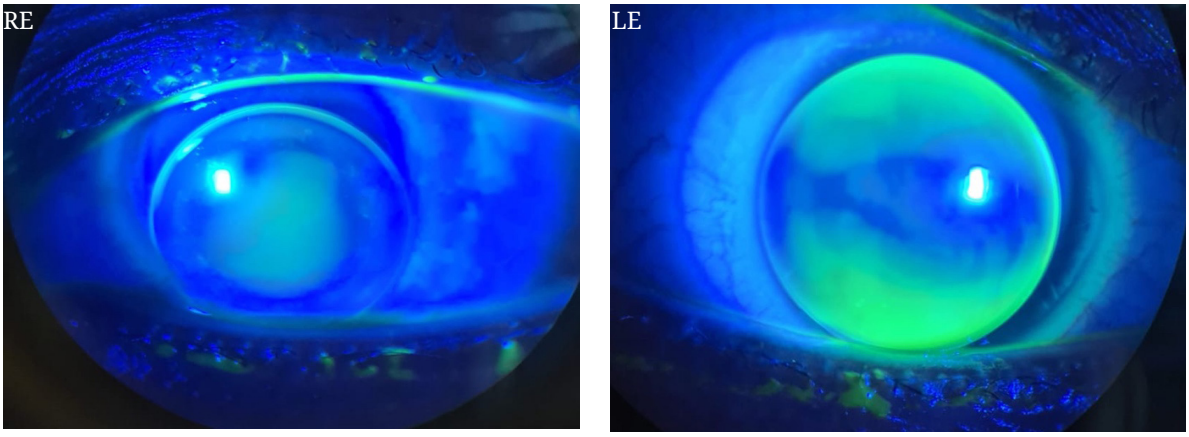

FIGURE 3. Fluorescein test after RGP lens application

The RGP CLs were then ordered with the following specification: base curve 8.00 mm with power -4.00D and diameter 9.20 mm for the right eye and base curve 7.50 mm with power +7.25D and diameter 9.00 mm for the left eye. The patient and caregiver was educated on proper handling of RGP, the importance of routine evaluation and instructed to come for a follow up one week after wearing the contact lenses. After one week of using RGP CLs on both eyes, patient reported subjective visual improvement, better compliance and overall no complaint during CLs wear despite having initial difficulty in use. Best corrected visual acuity using EDTRS chart was 6/48 on both eyes.

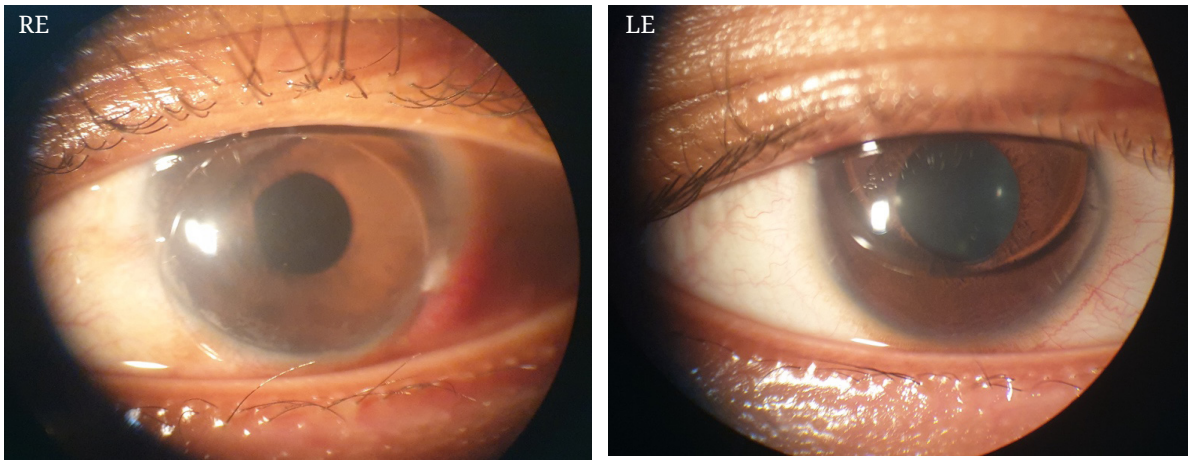

FIGURE 4. The patient wearing the RGP CLs taken immediately after blink, some decentrations were shown but with adequate pupil coverage

Next follow up was one month after using RGP CLs, patient reported broken the right RGP CL. Patient was then fitted for new right RGP CL with the same specification as before. Patient was instructed on gradual use of right RGP CL and educated to blink properly while using prescribed eye lubricant. Patient followed routine evaluation to regularly monitor eye development and complications related to contact lens usage, with the latest being 1 year after using RGP CLs. Best corrected visual acuity after one year with EDTRS chart was 6/48 on the right eye and 6/48 on the left eye. Although BCVA of 6/6 was not achieved, the patient was satisfied with the improvement. There was no complaint of headache or double vision, and better compliance was achieved than when wearing spectacles.

DISCUSSION

Anisometropia is a difference in refractive error between the eyes, where refraction difference of 1 D or greater in spherical equivalent is usually considered as a significant threshold. The prevalence of anisometropia in children vary, with most studies reported a prevalence of lower than 10%.[6-9] In the presence of anisometropia, the visual stimulus is adversely affected as one of the macula is receiving relatively blurred image. The sharper image from the better eye is processed by the visual pathways and the cortex, while blurred image from other eye is suppressed.[6] This will interfere with normal binocular vision and often associated with the development of amblyopia strabismus, aniseikonia and spectacle intolerance especially in children.[9-10]

Amblyopia is a reduction of best corrected visual acuity in one, or less often both eye, caused by abnormal visual input during the early life periode.[5] The cause of amblyopia could be classified as strabismic, refractive, visual deprivation and occlusion.[5,14] Approximately half of amblyopia is secondary to refractive errors and the other half is from other causes such as strabismus.

These conditions are reversible if detected and treated early in life, with compliance being the most critical factor for predicting a successful outcome. A side from compliance, treatment outcome also correlates with the initial degree of anisometropia as they require greater focusing effort to form a clear retinal image. If not, patients may develop low vision, as a visual impairment that cannot be corrected with regular eyeglasses, contact lenses, medicine, or surgery in which visual acuity is 3/60 or worse in the better-seeing eye.[15-16]

In this case, the patient is a pediatric female presented with chief complaint of headache and double vision upon wearing prescribed spectacles which cause noncompliance. Best corrected visual acuity with the new spectacle were 6/48-2 on the right eye and 6/48-2 on the left eye uncorrected with pinhole. While patient's VA with old spectacles were 3/60 on both eyes. Therefore, this was a case of severe anisometropia, caused by monocular aphakia that has caused aniseikonia. Amblyopia and low vision on this case was assumed due to disturbance in visual development centrally as there was congenital nystagmus.

Several modalities are available for the correction of visual impairment in anisometropia. Spectacles are often use as an initial treatment as they are the least invasive and expensive, but fitting and compliance remains an issue since patients may not perceive an improvement in their vision. They may also experience dizziness or even develop headache from spectacle because of the optically induced aniseikonia and asymmetry in lens weight, especially in hight anisometropia.[15-16] Refractive surgery can also be considered as a treatment option for severe anisometropia, since

it reduces dependency on compliance in comparison with other optical devices. However, the accurate refractive stability prediction after surgery is biased by the possible age-related refractive and anatomy changes in pediatric eye development.[12]

Contact lenses are commonly used for refractive correction in severe anisometropia caused by monocular aphakia. In contrast to other types of contact lenses, RGP CLs offers several advantages for the small developing eyes of pediatric patients. They are manufacturedin a wider range of powers which is particularly beneficial for this patient who needs both high minus and plus lenses, considering her severe anisometropia was caused by not only high myopia but also monocular aphakia. In addition, they can also be customized to the specific base curve or diameter needed to accommodate pediatric cornea that is typically steeper in curvature and smaller in diameter than the adult cornea.[13] Previous studies have reported RGP CLs as a safe and effective refractive treatment in pediatric anisometropia for reasons not only mentioned above, but also high oxygen permeability, greater contrast sensitivity, better durability and the ability to correct higher corneal astigmatism which is important to achieve best possible vision and comfort after adaptation.[11,13]

The lens design chosen for this patient was an aspheric lens and the diameter of the lens chosen for fitting was 9.20 mm, which was the only diameter available in the trial lens kit. Precise fitting is important to achieve stable vision and acceptable comfort which improves after adaptation, and should involve the evaluation of both static and dynamic criteria.[17]

In this patient, there was an up-riding centration with lid attachment lens movement and with the rule astigmatism as on the right eye. While there was good centration and with acceptable slightly tight fitting on the left eye. These patterns and movements were consistent with corneal topography profile. The aspheric lens design chosen can minimize lid-lens interaction and provide better centration on astigmatic cornea.[17]

Tisilfocon A was chosen as RGP CL material for both fitting and ensuing treatment. This lens material is a thermoset copolymer derived from fluoromethacrylate and siloxanylstyrene with benzotriazol UV absorber. It has the highest oxygen permeability (Dk) value of 163 barrers, ideal for eyes that require sufficient corneal oxygenation for successful contact lens wear.[17] Oxygen transmissibility becomes more important variable to consider in aphakic contact lens, since higher central thickness required to correct aphakia may compromise corneal oxygen uptake.[18]

Although having many advantages, some disadvantages need to be considered when using RGP CL. The primary disadvantage of RGP lenses is that their oxygen transmissibility decreases in aphakic contact lens. It also takes time for patients and caregiver to become skilled at handling RGP lenses. Studies have reported thatit took patients and caregiver at least 1 month to become skilled at handling RGP lenses. However they eventually became adept at managing RGP lenses over the course of the follow-up period.[19] Studies has also reported that RGP CL has higher rate of lens replacement than other types of contact lens. The higher rate of lens replacement maybe contributed to the greater precision that can be achieved when correcting refractive errors. But it may also reflect a greater tendency to break or lose them.[13] During the 1 month follow up, the patient reported broken right RGP CL. This might be caused by unskilled handling mentioned prior. Educating the patient and caregiver on this matter is important to prevent

lens abandonment, and for patient to properly follow routine evaluation.

CONCLUSION

Treatment for severe anisometropia in pediatric patients vary. Spectacles being the least invasive may cause aniseikonia and compliance problem. While despite reducing dependency on compliance, refractive surgery is invasive and the refractive stability prediction after surgery is biased by possible age-related refractive and anatomy changes in pediatric eye. Intra Ocular Implantation considered not be suitable for a microcornea condition.

RGP CLs is a safe and practical treatment in pediatric patients with severe anisometropia caused by monocular aphakia. It is available in a wider range of powers and can be customized to the specific base curve or diameter suitable for developing pediatric eyes. It also provides superior vision and long-term comfort after adaptation can be expected as patients and caregiver become skilled at handling RGP lenses.

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Pruritus in diabetes mellitus (DM) and its pathophysiology-based treatment

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ABSTRACT

Pruritus is a common complaint of diabetic patients with a substantial impact on financial and health status, but the pathophysiology is unclear and treatment with antihistamines has mostly been unsuccessful. To date, we still do not have guidelines to help us treat pruritus in diabetes mellitus, so we felt the need to review the existing literature to explore possible ways to treat these patients. We collected 85 pieces of literature from various sources such as PubMed and Google Scholar, and independently extracted these data to make this review. While the pathophysiology behind pruritus in diabetes mellitus remains largely unknown, some trials have found a few pharmacological treatments to be effective in alleviating itch in these patients.

ABSTRAK

Pruritus merupakan keluhan umum yang mempunyai dampak yang besar baik secara finansial maupun pada kesehatan pasien diabetes melitus, namun patofisiologinya masih belum jelas dan pengobatan dengan antihistamin seringkali tidak berhasil. Sampai saat ini, pedoman tatalaksana untuk pruritus pada pasien diabetes mellitus masih belum ada, sehingga kami merasa perlu untuk mempelajari literatur yang ada untuk mencari alternatif untuk mengobati pasien diabetes mellitus dengan pruritus. Kami mengumpulkan 85 literatur dari berbagai sumber seperti PubMed dan Google Scholar, dan mengekstraksi data dari literatur-literatur tersebut secara independen untuk membuat tinjauan pustaka ini. Meskipun patofisiologi pruritus pada diabetes mellitus masih belum dimengerti, beberapa penelitian menemukan beberapa pengobatan farmakologik yang efektif.

Keywords:
skin barrier disruption; diabetic neuropathy; oxidative stress; chronic kidney disease-associated pruritus; second line treatment

INTRODUCTION

Pruritus, or commonly known as itch, is a disturbing stimulus that causes the desire to scratch the affected skin area. Pruritus is classified based on its location and its duration. Location-wise, pruritus is classified into generalized and localized pruritus. It is categorized as acute pruritus if it is experienced for up to six weeks. Meanwhile, if it lasts longer than six weeks, it is categorized as chronic.[1,2] Recent epidemiological studies show that chronic pruritus occurs in 22–27% of the population.[1,3]

The International Forum for the Study of Itch (IFSI) classified chronic pruritus into 6 categories: (1) dermatologic, (2) systemic, (3) neurologic, (4) psychogenic/psychosomatic, (5) mixed, and (6) others.[4] Diabetes mellitus (DM), a health issue that has reached pandemic proportions and is also a health problem of dire proportions in Indonesia,[4–6] is one of the systemic disorders that is commonly linked to pruritus.[2] However,

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some authors suggest pruritus in DM as mixed pruritus with the additional role of both dermatologic and neurologic components.[4]

A study conducted on 106 DM patients by Al Mutairi *et al.*[7] reported pruritus as the second most common cutaneous manifestation, affecting 49% of patients. Specific studies on pruritus in DM are limited and hard to compare due to non-homogenous methodology. Most studies did not specify if their results were the point prevalence, life-time prevalence or incidence, of pruritus. In their literature review, Stefaniak *et al.*[4] concluded that pruritus might be experienced in about 18.4% to 27.5% of patients with type 2 DM (T2DM).

Patients with chronic pruritus generally have lower quality of life (QoL).[1,2,4,8,9] The QoL regression in pruritic patients is similar to that in chronic pain patients.[8,10] It has a remarkable impact on the medical and economic condition of the patients. In the United States of America, patients with pruritus have an expenditure ratio of 1.64 times more than the average population. About $90 billion extra per year needs to be spent for treating pruritus.[10] The impact of chronic pruritus is also magnified in diabetic patients. Intense scratching due to pruritus can cause wounds that might lead to diabetic foot ulcers (DFU) and sleep disturbance. DFU is a chronic and devastating complication of diabetes, and around 5–24% of DFU cases will end in loss of limb, usually by means of amputation.[11,12] Long term sleep disturbance has substantial adverse effects on metabolism, disruption in circadian rhythms, and pro-inflammatory responses, and therefore it will further exacerbate the complications of diabetes mellitus.[13] All of the consequences of DFU and sleep disturbance caused by pruritus greatly reduce the QoL of diabetic patients and consequently increase the mortality rate.

Treating pruritus in diabetes should be done to improve the QoL but unfortunately it remains a challenge. There is no proposed mechanism that can accurately explain the pathophysiology of pruritus in diabetes. Underlying pathophysiology of diabetes, the course of the disease, coexisting comorbidities, and medications complicate the issue and tend to make diabetic patients more susceptible to developing pruritus.[4] Pruritus in diabetic patients does not always respond well to the use of antihistamine. Therefore, it is assumed that histamine is not the only pathway that plays a part in pruritus in diabetic patients.[4,14]

Pruritus is a common complaint amongst diabetic patients with a substantial impact on financial and health problems, but the pathophysiology is not clear and treatment with antihistamines has mostly been unsuccessful in many diabetic patients with pruritus. In light of this issue, we would like to understand it better by doing this literature review. We aimed to gather current knowledge of the pathophysiology of pruritus in DM and the possible use of various pathophysiology-based managements, other than antihistamines, in diabetic patients.

MATERIALS AND METHODS

Literature searches were performed on Google Scholar and PubMed without any time restrictions, using the keywords: pruritus, dermatology, DM, and itch. The last search was done on 1st of October 2021. Results from the initial search are reviewed by reading the abstract, discussion, and conclusion to see if they

are relevant to the inclusion criteria, which are as follows 1) Discussing various forms of chronic pruritus related to DM; 2) Talks about the pathophysiology of DM related to the dermatological aspect of it.

Case series, case controls, cross-sectional studies, review articles, systematic reviews, web pages, books, book reviews, reports, editorials, position statements, commentaries, exploratory studies, open-label studies, experimental studies, randomized controlled trials, prospective cohorts, and retrospective cohorts were included in our review. The literature which passed the initial screening underwent a more thorough assessment and independent extraction by all four authors.

A total of 132 articles from Google Scholar and 110 from PubMed were obtained at the start of the search. Seventy-one articles were deleted after manual double checking. A screening was then carried out in which we each independently reviewed the abstracts and conclusions of each literature. Sixty-five literatures were excluded, leaving 106 at hand. A more in-depth review of the literature excluded another 21 and we ended up with 85 literatures.

Among the 85 papers, there is one case series, three case controls, seven cross-sectional studies, 45 review articles, two systematic reviews, one web page, three books, and one book section. The others are one book review, two reports, one editorial, one position statement, one commentary, one exploratory study, two open-label studies, nine experimental studies, one randomized controlled trial, two prospective cohorts, and one retrospective cohort.

DISCUSSION

Diabetes mellitus is a disease characterized by high blood glucose levels resulting from defects in insulin secretion and/or insulin action.[15] Pruritus is often cited as a common manifestation in diabetic patients.[16] There are various conditions associated with the onset of pruritus in diabetic patients, such as skin disorders, diabetic neuropathy, end-stage renal disease, and iatrogenic or secondary to anti-diabetic therapy.[15,16] The prevalence of skin disorders in DM ranges from 30% to 97% in various regions worldwide, but not all of them have pruritus as their symptom.[4,15,17] Pruritus can be experienced by diabetic patients with non-infectious (such as dry skin, prurigo nodularis, lichen planus) or infectious (such as dermatophytosis, candidiasis) skin disorders.[15] Pruritus can also be experienced by diabetic patients for no apparent reason.[18]

Pathophysiology of pruritus in DM is not fully understood. The connection between blood glucose level and pruritus is inconclusive.[16] Ko *et al.*[19] found a connection between high postprandial glucose levels and generalized pruritus in patients with DM. Hillson *et al.*[20] also found a connection between fasting plasma glucose levels and generalized pruritus in both newly diagnosed and untreated DM. Researches with HbA1c have a more mixed result. Neilly *et al.*[21] found no correlation between HbA1c and pruritus, and this finding is echoed by Ko *et al.*[19] as well. However, at the other end of the spectrum, a study by Afsar and Elsurer found that visual analogue scale (VAS) itch score on diabetic patients is strongly related to HbA1c.[22]

Since there are various conditions associated with the onset of pruritus in diabetic patients, it follows that there should be various mediators or pathways in the pathophysiology of pruritus in DM. Before we dive deeper

into the pathophysiology of pruritus in DM, we will first take a look at the pathophysiology of pruritus in general.

Pathophysiology of pruritus in general

Pruritus is a unique sensation. It is encoded by genetically distinct neurons both in the peripheral and central nervous systems. Characteristically, it triggers scratching. Although specific neurons for pruritus have been detected, it is not clear whether all the neurons signal pruritus and only pruritus. Yet, pruritus also interacts with the other sensory modalities (pain, temperature, and mechanical force) at multiple locations, from a particular dermatome to the brain.[23,24] This review summarized our current understanding of the molecular and neural mechanisms of pruritus.

Pruritus is sensed by cutaneous nerve fibers called pruriceptors. Unmyelinated C-fibers, mostly, and thin myelinated Aδ-fibers are responsible for sensing pruritus.[23] Branching terminal fibers of these sensory afferent neurons are found at the epidermal-dermal junction.[14] Some of them reach the uppermost viable layer of epidermis.[23] Sensory nerves are part of an interactive milieu with a plethora of mediators generated from the sensory neurons themselves, neighboring cells, keratinocytes, and the microbiome. There are multidirectional communications between the nervous and immune systems within the skin, perhaps directed against components of the microbiota, which can evoke pruritus.[23,24] Currently known mediators of pruritus, which play different roles in different pruritus conditions, include histamine, serotonin/5-hydroxytryptamine (5-HT), proteases, cytokines-interleukins, peptides (bradykinin, substance P, calcitonin gene related peptide, neurotrophin, opioid peptides), and phospholipid metabolites (cannabinoids, eicosanoids, platelet-activating factor). These mediators are key contributors to and exacerbate pruritus by activating cognate receptors on sensory neurons.[14]

The binding of those mediators with their cognate receptors activates a series of signal transduction systems. Currently, two signal pathways of pruritus have been identified, namely histamine-dependent (histaminergic) signaling pathway and histamine-independent (non-histaminergic) signaling pathway.[14] In the histaminergic pathway, binding of histamine to its cognate receptors, particularly H1 receptor and H4 receptor, promotes activation of phospholipase C (PLC) β3, enhances calcium levels, and irritates lipoxygenase (LOX) and phospholipase A (PLA). These further induce the activation of downstream target transient receptor potential cation channel vanilloid 1 (TRPV1). Pruritus signals through this pathway are relayed via mechanically insensitive C-fibers (CMi), which nerve endings mainly distribute in the dermo-epidermal junction, to their cell bodies in dorsal root or trigeminal ganglia followed by synapsing with second-order neurons in the dorsal horn of the spinal cord.[14,23]

The other signaling pathway, nonhistaminergic, seems to be involved in pruritus which is resistant to antihistamine, such as chronic pruritus.[14,25] Many pruritogens can stimulate this pathway. Binding of pruritogens with their cognate receptors stimulates either protease-activated receptor 2 (PAR-2) or Mas-related G protein-coupled receptors (Mrgprs).

Activation of PAR2 sensitizes PLC, then the downstream targets including TRPV1 and transient receptor potential cation channel ankyrin 1 (TRPA1) are activated. Activated Mrgprs, is then coupled to G beta-gamma protein complex (Gβγ) or PLC or other; then they promote TRPA1/TRPV1 activation. This non-histaminergic signaling pathway is usually mediated by a class of mechanically sensitive C-type fibers (CMHs), which nerve endings mainly distribute in the epidermis. These CMHs also synapses with second-order neurons in the dorsal horn of the spinal cord.[14,23]

Peripheral pruritus nerves synapse with interneurons as the second-order neurons in the dorsal horn of the spinal cord. Interneurons are the gate-keepers of neuronal activity. They have excitatory or inhibitory function to modulate afferent input carried to the projection neurons to the brain. Gastrin-releasing peptide $(GRP)^+$ interneurons and tertiary GRP receptor $(GRPR)^+$ neurons are key to stimulating pruritus circuits in the spinal cord. Binding of GRP to GRPR evokes scratching reaction.[14,23,26] Spinal pruritus signals transmitting process is also elicited by binding of B-type natriuretic peptide (BNP) to its transmembrane natriuretic peptide receptor A (NPRA). BNP-NPRA may serve as the upstream of the GRP-GRPR system to regulate neurotransmission of pruritus in the spinal cord. BNP and glutamate released from primary sensory neurons activate secondary neurons expressing NPRA in the dorsal horn. Then the secondary neurons secrete GRP and activate GRPR of a third neuron and ultimately lead to pruritus perception.[14,23,24]

Complementary to the excitatory circuits, there are numerous interneuron populations in the spinal cord that have an inhibitory function in the pruritus process. For example, inhibitory basic helix loop helix 5 interneurons (B5-I) and somatostatin (SOM) interneurons. Inhibitory interneurons express γ-aminobutyric acid (GABA), as the major neurotransmitter, besides neuropeptide Y (NPY), dynorphin (DYN, a kappa-opioid agonist), and galanin. B5-I inhibits GRP^+ and $GRPR^+$ neuron signaling of pruritus. Loss of inhibition, either by depletion of inhibitory interneurons or the inhibitory neurotransmitters results in intensified pruritus.[23,24,26]

$GRPR^+$ neurons, as the third-order neurons in the dorsal horn of the spinal cord, decussate anteriorly before traveling rostrally along the contralateral spinothalamic and spinobrachial tracts. $GRPR^+$ neurons synapse with thalamic neurons that communicate with various brain regions, including the sensory cortex, insula, and motor cortex, to produce pruritus perception and a strong motor response to pruritus – the scratch.[24,26] In addition to these peripheral inputs, higher-level brain regions provide descending control to spinal cord pruritus neurons. Pruritus is potentiated by serotoninergic neurons from the nucleus raphe magnus (NRM) that directly connect with $GRPR^+$ neurons. Inhibitory interneuron activity in the spinal cord is modulated by noradrenergic neurons in the locus coeruleus modulate. The mid-brain periaqueductal gray (PAG) is activated during scratching and was considered as a pruritus suppressing brain area. GABAergic and dopaminergic (DA) neurons in the ventral tegmental area (VTA) play a role in the central reward processing of pruritus. In general, DA neurons are key neuronal elements in motivation and reward systems, whereas GABA neurons are believed to drive aversion and disrupt reward processes.[23]

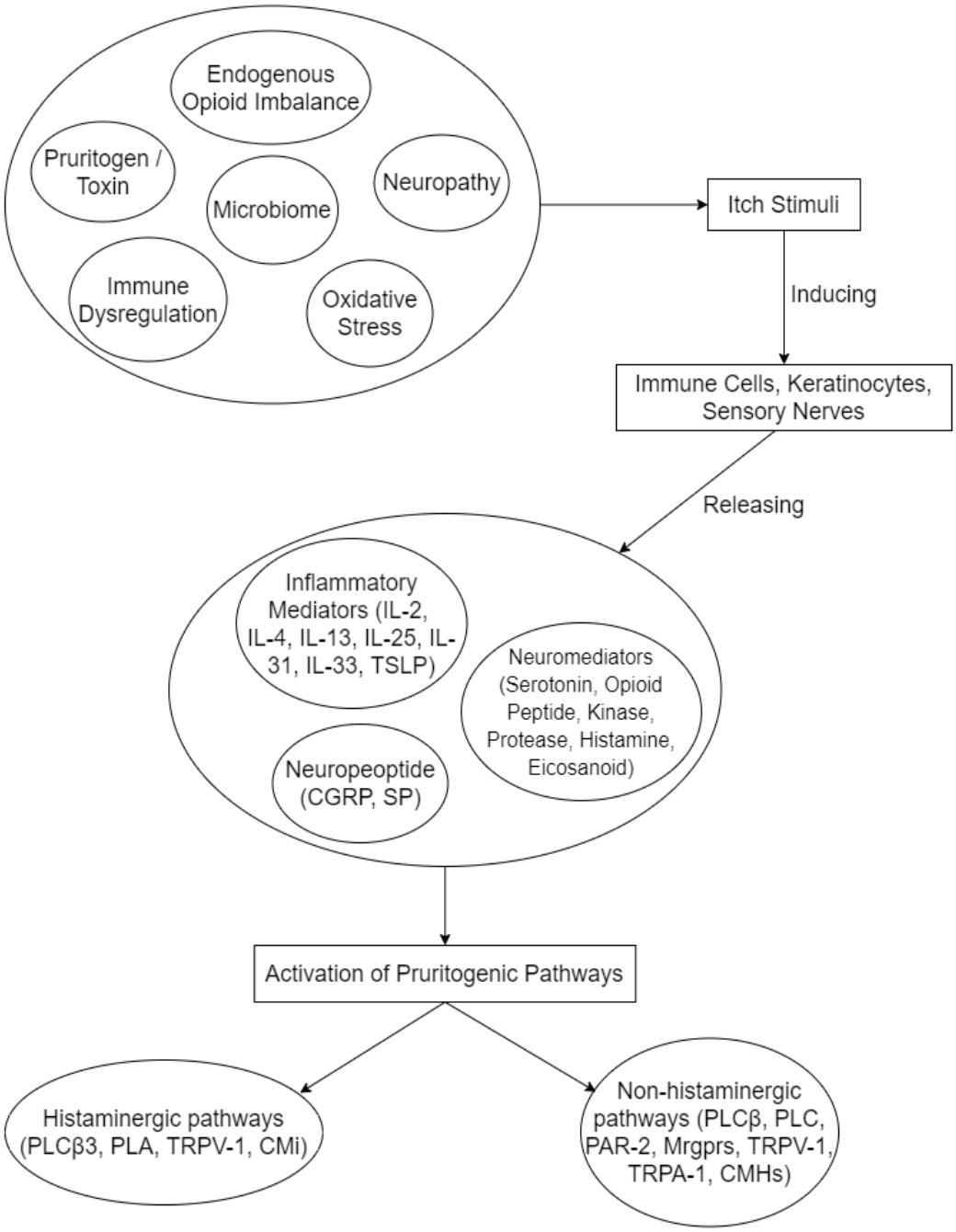

FIGURE 1. Chronic pruritus signaling pathways. **(**IL-2: interleukin-2, IL-4: interleukin-4, IL-13: interleukin-13, IL-25: interleukin-25, IL-31: interleukin-31, IL-33: interleukin 33, TSLP: thymic stromal lymphopoitein, PLCβ3: phospholipase C beta 3, PLA: phospolipase A, TRPV-1: transient receptor potential cation channel subfamily V member 1/vanilloid receptor 1, CMi: mechano-insensitive C nerves, PLCβ: phospholipase C beta, PLC: phospolipase C, PAR-2: protease activated receptor 2, Mrgprs: mas-related G protein-coupled receptors, TRPA-1: transient receptor potential cation channel subfamily A member 1, CMHs: mechanically sensitive C-type fibers)

Another mechanism that might play a role in the pruritogenic pathway would be opioid receptor signaling. Many studies concluded that there is a significant overlap between pain and pruritus neuronal pathways. It is believed that the nociceptive neurons are tasked to create a constant tonus of inhibition of the pruriceptive neurons. Due to this, we experience an urge to scratch every time we feel a pruritus, replacing the pruritus signal with local pain induced by the scratching. Similarly, when pain is reduced pharmacologically, for example by administration of morphine, pruritus often sets in at the area with reduced pain transmission. This mechanism is likely fueled by inhibitory interneurons in the dorsal horn of the spinal cord. This theory is supported by genetic experiment study on mice. When the gene that controls the transcription factors for these inhibitory interneurons was deleted, the mice suffer from severe chronic pruritus.[27,28]

The mechanism of opioid receptors in pruritus is thought to involve upregulations of the central mu-opioid receptors which give rise to feelings of pruritus, while suppressing the peripheral kappa-opioid receptors that reduce pruritus perception. Morphine, which is a mu-opioid receptor agonist, can induce pruritus in humans, while naloxone, a mu-opioid receptor antagonist, can inhibit pruritus instead. Activation of the kappa-opioid receptor system in mice has also been shown to reduce scratching activity induced by substance P, histamine, and morphine. Nalfurafine, a kappa-opioid receptor agonist, has been studied in Japan to treat pruritus in patients with chronic kidney disease (CKD) and chronic liver disease (CLD). The result showed that there is a partial decrease in pruritus intensity without any severe adverse reactions.[27,28]

Pathophysiology of pruritus in DM

The pathophysiology of pruritus in DM is not fully understood, as mentioned before. It seems that the pathophysiology is complex and interconnected. Researchers nowadays believe that dry skin (xerosis cutis) and diabetic peripheral neuropathy are the two main factors associated with pruritus in DM.[4] Disruption of skin barrier underlies the occurrence of dry skin.[29] Therefore, we will focus on the role of skin barrier disruption and diabetic peripheral neuropathy in the pathophysiology of pruritus in DM. Moreover, we will highlight the role of oxidative stress in causing pruritus in DM, as it is believed that oxidative stress plays an important role in the development of complications in DM.[30] At the end, we will discuss about pruritus in diabetic patients with CKD and pruritus secondary to anti-diabetic therapy.

Skin barrier disruption

Skin barrier (SB) is exerted by the epidermis, especially stratum corneum (SC).[29] It protects humans from their surroundings. SB prevents the entry of potential pruritogens like infectious agents and allergens, provides protection against ultraviolet light and oxidative stress, and plays a role in homeostasis, such as humidity of SC.[2,29,31] SB consists of cellular and non-cellular structures.[32] Impaired skin barrier integrity causes excessive water loss and leads to skin dryness. Transepidermal water loss (TEWL), SC hydration, and pH are some of the parameters usually measured to assess skin barrier function.[29]

SB's functions and integrity are disrupted in DM.[4,17,29] A study on physiological changes in the skin of obese, diabetic Japanese men by Ai *et al.*[33] found that there is a significantly higher TEWL and a markedly lower stratum corneum hydration level compared to controls, indicative of dry skin. Skin hydration in diabetic patients is found to be 38% lower when compared to non-diabetic patients.[34] A case-control study

conducted on 52 patients with T2DM by Kim *et al.*[35] also found that SC hydration was significantly lower than their age- and sex- matched non-diabetic control. Moreover, they found a significant decrease of ceramides, free fatty acids, and cholesterol in the SC of DM patients. Triglycerides replaced ceramides as the most substantial SC lipids. Changes in the amount and composition of SC lipids, functioning as a water barrier, leading to skin dryness.[29,36] Decreased sebaceous gland activity is also observed in DM patients and will exacerbate skin dryness.[29]

Dry skin is characterized by a scaly, rough, cracked, and fissured surface.[29,37] In diabetic mice models, impaired epidermal integrity is caused by a certain kind of protein related to tight junction structure, named ZO-1, which was found to be more widely and diffusely expressed, causing hypoplasia and misalignment of the epidermal basal layer.[35,38] Typically, when a dye called lucifer yellow (LY) is applied to normal mice's ears, the stain's distribution would be limited to hair follicles and outer areas of the SC. In diabetic mice, LY stains a wider area of the SC. The quantity of LY positive signals was found 2.7 ± 0.4-fold greater in diabetic mice than in controls.[38] The diameter size of LY dye spots can reach up to 10 μm or more. Epidermal integrity impairment makes the diabetic skin more vulnerable to external substances, such as infectious pathogens and chemical damage. Fungus can easily infiltrate the skin of diabetic patients because their hypha size is about 6–12 μm, smaller than the size of the dye spots.[35,38,39]

The entry of pathogens or chemical agents through the disrupted skin barrier and the skin barrier disruption itself may trigger inflammatory reaction.[40,41] Skin cells, immune cells, and the microbiome can release pruritus mediators that can activate their cognate pruriceptors on the nerve endings. In an inflammatory environment, either histaminergic or nonhistaminergic pathway can be activated, depending on the mediators present. Histamine, released from mast cells, activates CMi that are different to CMHs which are activated with the other pruritogens, as described in the section on the pathophysiology of pruritus in general. The potential action that is formed in the afferent sensory nerves will be conducted to dorsal horn of spinal cord, then projected to thalamus and finally to the somatosensory cortex in the brain, including the motor cortex, to induce perception of pruritus and scratching. The pruritus-scratch cycle can perpetuate skin barrier damage and pruritus. Severe scratch-induced epidermal injury also injures sensory nerve endings that may be rendered hyperexcitable upon regeneration.[29,40]

Diabetic peripheral neuropathy

Diabetic neuropathy is the most prevalent chronic complication of diabetes.[42] It is a diagnosis of exclusion. It will be diagnosed as diabetic neuropathy if no additional causes of neuropathy other than diabetes is found.[42,43] Patients with prediabetes may also develop neuropathies that are similar to diabetic neuropathies.[42] The most common form of diabetic neuropathy is diabetic peripheral neuropathy.[42,44] Risk factors for diabetic peripheral neuropathy include age, male gender, duration of diabetes, uncontrolled glycemia, height, overweight and obesity, and insulin treatment.[45] It is characterized by neurodegeneration of peripheral nerve systems that preferentially targets sensory and autonomic neurons.[46] Among the peripheral nerve fibers, small nerve fibers (e.g., Aδ and C) are the most vulnerable structures. Damage to small nerve fibers is responsible for the development of small fiber neuropathy (SFN) in DM.[47]

A multifactorial pathogenesis of

diabetic peripheral neuropathy is suggested by experimental studies, but the causes remain unknown. A prevailing view of the pathogenesis is that oxidative and inflammatory stress may, in the context of mitochondrial dysfunction and hyperglycemia, damage nerve cells.[42,45,47] Moreover, hyperglycemia is associated with decreased expression of CB_1 receptors in dorsal root ganglia. A decline in CB_1 receptor expression may contribute to the loss of neuroprotective effect of cannabinoids and the neurodegenerative process observed in DM.[1,48] Neurovascular impairment with poor repair processes and endothelial dysfunction also have been implicated in the neurodegenerative process.[44,49] The entire neuron, from the perikaryon (cell bodies) to the terminal, is targeted by diabetes.[46] Biopsy findings reflect a loss of multifocal and focal proximal nerve fibers, but a more severe damage is visible in distal fibers.[45] Degeneration of intraepidermal small nerve fibers and depletion of small nerve fibers surrounding sweat glands were identified.[47,50] Axonal degeneration and segmental demyelination are the main pathological characteristics of neuropathic damage induced by hyperglycemia.[45,46]

Depletion of small nerve fibers surrounding sweat glands cause sudomotor dysfunction. Sudomotor dysfunction is a common feature of diabetic autonomic neuropathy. Autonomic supplies in the skin and subcutaneous tissue have an essential role in blood flow, nutritional delivery, and lubrication modulated by the sudomotor function (sweat gland).[51] Sudomotor dysfunction decreases sweat production. Lower sweat production has a strong positive correlation with the hydration status of the surface of the skin. Lower hydration status will lead to dry skin.[29,42,44,50] Furthermore, pruritus can occur based on the skin barrier disruption caused by dry skin.[52,53]

Meanwhile, damage to the pruriceptive small nerve fibers can result in neuropathic pruritus. Neuropathic pruritus is generally understood as pruritus resulting from neuronal or glial damage without skin alterations.[26] Injured sensory neurons normally have reduced sensation, but in some people, a paradoxical compensatory mechanism may occur. Hyperexcitability may develop in the injured sensory neurons and their surrounding network, generating action potentials in the absence of a stimulus and altering stimulus-response function.[46,54]

Ion channel expression, trafficking, and phosphorylation status within sensory neurons are critical determinants of excitability.[46,54,55] Channels within the injured sensory neurons and the surrounding network can be directly activated independently from receptor recruitment, to excite afferent neurons and produce neuropathic pruritus. For example, voltage-gated sodium channels (Na_v) which are expressed in small-diameter sensory neurons of the dorsal root ganglia, trigeminal ganglia, and sympathetic ganglia. These channels regulate neuronal excitability and action potential propagation by altering resting membrane potential and inactivation thresholds.[26,54] Methylglyoxal, one of the increased reactive metabolites in DM, can post-translationally modify $Na_v1.8$ and TRPA1, which leads to sensory neuron hyperexcitability.[46] Numerous gain-of-function mutations serving to increase neuronal excitability have also been identified as the cause of neuropathic pruritus in patients.[26] Hyperpolarization-activated cyclic nucleotide-gated channels which are important for repetitive firing also act to regulate neuronal excitability, whereas potassium channels act as important breaks on excitability.[46]

Peripheral nerve damage also affects non-neuronal cell types in the

spinal cord. Astrocytes and microglia undergo proliferation, upregulation of cytokine production and release, and morphologic changes called reactive gliosis. Reactive gliosis in both cell types play a role in neuropathic pain by releasing pro-inflammatory cytokines and chemokines, which sensitize pain-coding spinal cord neurons to be more responsive to peripheral stimuli. A similar mechanism whereby gliosis modulates pruritus circuits to promote pruritus perception may be present in neuropathic pruritus.[26,46]

Oxidative stress

Oxidative stress is defined as a disequilibrium status between oxidants (free radicals, reactive oxygen and nitrogen species, reactive metabolites) and antioxidants in cells with the advantage of oxidants. Oxidative stress is linked with DM.[56] Increased glucose levels in DM leads to a failure of oxidative phosphorylation in mitochondria, loss of ATP production, and overproduction of reactive oxygen species (ROS) by mitochondrial electron transport chain. Excessive ROS will damage the nuclear DNA chain. DNA damage activates poly(ADP-ribose) polymerase (PARP). PARP then causes a decrease in glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Decreased GAPDH activity causes an increase in glycolytic metabolites (glyceraldehyde 3-phosphate, fructose 6-phosphate, and glucose) that will activate major mechanisms, namely an increase in glucose metabolism through the polyol pathway, an increase in methylglyoxal which will induce advanced glycation end products (AGEs) formation and AGEs receptor (RAGE) expression, activation of protein kinase C (PKC) isoform β, γ, dan δ, and an increase in hexosamine pathway activity. These mechanisms mediate additional ROS accumulation and inflammation, leading to damage in cells' structures and functions and ultimately DM complications such as skin barrier disruption and neuropathy.[46,56,57] Skin barrier disruption and neuropathy then induce pruritus in DM through the pathophysiological mechanisms that we have described above.

Oxidative stress itself also plays a critical role in both acute and chronic pruritus. Different oxidants induce profound scratching behavior in mice via activation of TRPA1. Oxidative stress-induced pruritus response is largely independent of histamine, consistent with the clinical observation that chronic pruritus associated with oxidative stress is resistant to antihistamine treatment.[58] Another study by *Zhou et al.*[59] also found that oxidative stress plays a critical role in acute and chronic pruritus in the periphery and spinal cord. They suggest that acrolein, which is both a product and initiator of lipid peroxidation, may be a novel endogenous pruritogen during oxidative stress. It has been identified as an endogenous TRPA1 agonist. The role of oxidative stress in inducing chronic pruritus was supported by their result that antioxidants are effective in alleviating chronic pruritus in their mouse model. They also found that antioxidant can scavenge the intracellular ROS, thus interrupting the intracellular pruritus signal transduction in primary pruriceptive sensory neurons.[59]

Pruritus in diabetic patients with CKD

Approximately one-third of DM patients develop complications such as CKD.[60,61] The prevalence of pruritus in CKD patients ranges from 10 – 77%, with an estimated overall prevalence of 55% among patients undergoing dialysis. This phenomenon is known as CKD-associated pruritus (CKD-aP).[62–64] The pathophysiology of CKD-aP is still not well understood at this time, with a lot of conflicting studies. The relation of CKD-aP and DM is not limited to just

being comorbidities. While skin xerosis (which is also a complication of DM itself as we have iterated above) has been traditionally seen as a causative factor in CKD-aP. recent studies have disproved this. Skin xerosis is now believed to be an exacerbating factor instead. Lastly, histamine was also considered the primary pathway for pruritus in CKD-aP, but this too has been disproved and newer studies have shown that CKD-aP is mediated by a nonhistaminergic pathway because it does not have histamine-specific changes like wheals.[27,64]

There are lots of theories out there regarding how the pruritus perception happened, but four of the more popular theories, described by Verduzco *et. al.*[27] are: toxin deposition, peripheral neuropathy, immune system dysregulation, and opioid imbalance. We shall discuss all four theories briefly and compare notes from some studies on them. The first theory, toxin deposition, implicated toxins in CKD patients that accumulate under the skin act as potential pruritogens. Uremic toxins, calcium, vitamin A, phosphate, magnesium, and aluminum have been put forward as potential toxins. It has also been postulated that parathyroid hormone (PTH) levels have something to do with it, because in some patients, parathyroidectomy alleviates the pruritus.[27] However, while this theory has not been fully disproved, there are some researches that refuted this theory. These researches found that the levels of the potential toxins were not consistently related to the presence of CKD-aP, and dialysis or toxin removal did not always alleviate the pruritus.[27,64]

The second theory, peripheral neuropathy, believes that the neuropathy in patients with CKD and DM results in neuropathic pruritus, which happens when diseased primary afferent sensory neurons or diseased interneurons are activated out of proportion to or independent of any pruritogens.[27,46] Neuropathy is common in CKD patients, and it is found that dialysis patients with other neuropathic issues such as restless leg syndrome and paresthesia often have CKD-aP as well.[27]

The third theory, immune system dysregulation, is a lot more complex and involves a lot more pathways and receptors. This theory suggests that microinflammation happens in the skin of CKD patients and possibly also systemically, and the inflammation stimulates pruritus. Supporting this hypothesis would be the high levels of inflammatory markers seen in CKD patients, which includes white blood cell count, T-helper 1 (Th1) cells, C-reactive protein and IL-2.[27] It is noted that in CKD-aP, there is a prominent shift of uncommitted Th lymphocytes into Th1 lymphocytes. This is followed by an increase in cytokines produced by Th1 lymphocytes, such as IL-6, interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α). The release of TNF-α further enhances the expression of Th1 lymphocytes, while IL-6 sets off a cascade known as the TO IL-6/phosphorylated-Bruton's agammaglobulinemia tyrosine kinase (pBTK)/phosphorylated-extracellular signal regulated kinase (p-ERK) signaling-induced pruritus. Moreover, there is also an increase in IL-31, which is a pruritogenic cytokine released by Th2 lymphocytes. On another front, there is indirect evidence that a highly pruritogenic cytokine called leukotriene B4 is also heightened in patients with CKD-aP. Lastly, increased concentration of another pruritogen called β2-microglobin is also found in the serum and skin of patients suffering from CKD.[64]

Low albumin levels and high ferritin levels are also seen in these patients. Further support for this theory includes the fact that the usage of anti-inflammatory medication in these patients has been reported to reduce pruritus. Allergic response may also

be erroneous in patients with CKD-aP. Higher levels of eosinophils, mast cells and the pruritogens associated with these, i.e. histamine and tryptase, are also present in patients with CKD-aP. However, as previously mentioned, the pruritogenic pathway in CKD-aP is non histaminergic.[27] The mast cell tryptase activates proteinase-activated receptor-2 (PAR-2) on pruriceptive nerve afferent fibers. This activation of PAR-2 sensitizes the TRPV1 ion channels downstream in nerve afferents and the transmission of a pruritus signal to the dorsal root ganglion and onto the dorsal horn in the spinal cord. The sensitization of TRPV1 causes the retrograde release of substance P (SP) from nerve endings. SP, in turn, activates dermal mast cells and keratinocytes to release more cytokines, leading to a vicious, self-perpetuating cycle.[64]

The final theory stated by Verduzco *et al.*[27] is the opioid imbalance theory. This hypothesis builds on the theory that the neuronal circuits which transmit pain and pruritus perception overlap substantially. Opioid is a compound that has a role in both. It blocks pain and is known to cause pruritus. Opioid pathways, which have receptors in the brain, peripheral nerves, immune cells, hair follicles, melanocytes, and keratinocytes have been recognized in their role in modulating pruritus. It is suggested that there is an overstimulation of central mu-opioid receptors, antagonism of peripheral kappa-opioid receptors, and an imbalance of stimulation and antagonism of mu- and kappa-opioid receptors in CKD-aP patients, which lead to the pruritus. Studies have also found that there is an upregulation of kappa-opioid receptors in patients with CKD-aP that has a positive correlation with the severity of the CKD-aP. Kappa-opioid receptors activation triggers an antipruritic effect, while mu-opioid receptors trigger pruritus instead. Both kappa and mu-opioids have receptors in the peripheral and central nervous system. They appear on the surface of dermal mast cells, keratinocytes, and in the dorsal horn.[64]

Pruritus secondary to anti-diabetic therapy

Pruritus can be caused by adverse reactions to anti-diabetic drugs, such as the first generation of sulfonylureas, biguanide, and SGLT2 inhibitors. Pruritus pathophysiology in drugs other than biguanide is still not understood.[2] Biguanide causes pruritus by way of cholestatic injury to the liver.[65] The mechanism behind cholestatic pruritus is largely unknown, but it is believed that the existence of liver associated pruritogens in the system of patients with hepatic diseases affects the opioid and serotoninergic system.[27,28] The hepatic diseases in question are usually the ones of cholestatic nature, and patients with hepatitis rarely reports pruritus. The proposed pruritogens for this theory includes, but not limited to: bile salts, bilirubin, gut microbiome, and lysophospatidic acid.[28,66,67]

The role of bile salts in cholestatic pruritus is not well understood and is subject to a lot of conflicting evidence since not all patients with a high level of bile salts complain of pruritus and the removal of bile salts doesn't always produce an attenuation of the pruritus perception. However, while bile salts may not be directly responsible for causing pruritus in cholestatic patients, there is evidence that it might play a role in activating bile salts receptors: Takeda G protein coupled receptor 5 (TGR5) and MrgprX4. Both receptors have been recognized as being located on pruritogenic sensory neurons. Another argument against the role of bile salts as the dominant pruritogen in cholestatic pruritus is the fact that pruritus is more often a feature of early cholestatic disease, when the levels of bile salts are

still relatively low. To top it off, pruritus usually goes away on its own when the patients experience liver failure.[28,66,67]

Bilirubin is found to be elevated in many patients with cholestatic pruritus, much like bile salts. It stimulates MrgprX4. Just like bile salts, the role of bilirubin as a dominant pruritogen in cholestatic pruritus is also shadowed by the same issue in which the plasma bilirubin levels have little to no correlation with pruritus intensity, and pruritus is more often a sign of early disease rather than late stage.[28,66,67]

Gut microbiome was considered as a potential pruritogen in cholestatic pruritus when it was found that treatment with rifampicin improves pruritus in cholestatic pruritus. Supporting this theory is another study that found that probiotics have been shown to exhibit antipruritic effects. However, there is also another study that compared gut microbiota of patients with primary biliary cholangitis (PBC) suffering from pruritus, patients with PBC without pruritus, and healthy volunteers which found that there is no significant difference of the three groups' gut microbiota composition.[28,66,67]

Lysophosphatidic acid (LPA) has been implicated as a potential pruritogen for cholestatic pruritus, but the evidence surrounding its role in pruritus is still mixed. Serum LPA levels are increased in patients with cholestatic pruritus, and a study proved that injection of LPA into the skin of mice initiated a scratch response. Lysophosphatidic acid is produced by phospholipase D Autotaxin (ATX). Serum ATX levels are significantly increased in cholestatic patients with pruritus compared to cholestatic patients without pruritus. However, ATX level also serves as a biomarker for liver fibrosis, where patients with hepatitis also have markedly elevated levels of serum ATX but do not suffer from pruritus. This creates a paradox on the role of LPA and ATX in modulating pruritus in cholestatic pruritus and warrants further studies on their role in it.[28,66,67]

Pathophysiology-based therapy for pruritus in DM

Treatment of chronic pruritus in diabetic patients using antihistamine often does not yield satisfactory results.[14,68] Steroid is also to be avoided in this group due to the unwanted hyperglycemia side effect.[69] Treatment of pruritus in DM must be highly tailored to the individual according to their needs.[40] Conducting a careful history taking and examination, differentiating between localized and generalized pruritus, identifying primary lesions should they exist, and spotting red flag symptoms can help to identify the cause of pruritus and in making treatment choices. Most patients will improve with nonpharmacologic therapy including frequent moisturization, avoiding overbathing, behavioral therapy, and breaking the itch-scratch cycle. If this avenue fails, further investigations are warranted to help guide subsequent treatment with any of the many cause-specific topical and systemic approaches available.[70] In this section, which is divided into nonpharmacologic and pharmacologic therapies, we will discuss some of the therapeutic modalities that have been studied so far.

Nonpharmacologic therapies

Glycemic control

Results from researches to search correlation between glycemic control and pruritus are contradictory. There are some researches that indicates correlation between glycemic control and pruritus (especially with generalized pruritus) as we mentioned above.[19,20,22] However, Neilly *et al.*[19] and Ko *et al.*[21] found no correlation between HbA1c and pruritus.[19,21] Despite the minimal

evidence regarding the connection between hyperglycemia and pruritus in DM, it is still recommended to practice good glycemic control.

Lifestyle changes

The American Diabetes Association (ADA) recommends good skin care and lifestyle changes as the main treatment options for skin complications related to diabetes. The do's include: keeping skin clean, keeping house more humid during the winter, wearing loose and soft clothing, using mild shampoos, moisturizing soaps and skin lotion, checking feet every day for sores and cuts, then treating them right away. Patients may also use wet, cooling, fat moist wrapping. What can not be done are: sauna, irritant contact, frequent washing, very hot bath and showers, scratching, bathing in the winter, and using feminine hygiene spray, ice pack, and alkaline cleanser.[70,71]

Emollients

Emollients come highly recommended by ADA as the main part of the lifestyle changes for diabetic patients experiencing pruritus. Because one of the proposed pathophysiologies for pruritus in DM is dry skin, the use of emollients is believed to be beneficial. It can keep the skin moist, flexible, soft, and increases skin barrier function from external disturbance.[72] The lipid layer of the skin, which is useful in keeping skin hydrated, is also normalized by emollients. Sufficient skin hydration can alleviate the pruritus.[4,73] Application of emollients also reduces the number of penetrated intraepidermal nerve fibers and nerve growth factor (NGF) levels in the mouse skin.[29]

There have been quite a few studies on the usage of emollients to treat pruritus in diabetic patients. The most commonly used active ingredient is urea. One study on the usage of emollient containing 5% urea and 0.2% hydroxyethylpiperazine results in significant reduction in TEWL and desquamation. Additionally, skin hydration also improved. Another researcher published two papers on using emollients to treat xerosis. In the first study, 603 patients were enrolled and 179 were diabetic. They were treated using 10% urea cream for 14 days and the results were a decrease in dryness, callosities, and scaling compared to the baseline. The second study enrolled 30 diabetic patients. They were treated in one foot using 10% urea cream (the other foot was used as control). The result was a decrease in scaling, callosities and dryness.[17]

A RCT with 40 T2DM patients, the treatment group received an emollient that is a mix of 5% urea, arginine and carnosine; while the control group received a glycerol-based emollient. The study compared the results after 28 days and the patients in the treatment group experienced an 89% reduction in skin dryness according to dry skin area and severity index (DASI) scale when compared with the control group. Another study conducted on 54 diabetic patients (a mix of type 1 and T2DM) used an emulsion base with 10% glycerin, 5% urea, 1% lactic acid and 8% paraffin for four weeks. The results show a decrease in dryness and skin fissures with an increase in skin hydration compared with the control group. There are more studies like these, and they utilized a mix of 10% urea and 4% lactic acid, another with 10% urea, alpha-hydroxy acid, allantoin, panthenol, oenothera biennis oil, and centella asiatica extract, which have all shown to reduce dryness.[17] One study compared urea formulations with different concentrations, and the results showed that formulations using 6% urea restored adequate skin barrier function, and the 4% urea formula has a longer duration of action.[17] For patients that have contraindications towards

emollients, a study by Ibrahim *et. al.*, found that the diabetic patients treated using topical clove oil treatment showed significant improvement in pruritus relief.[74]

Other topical agents

Usage of topical agents in relieving pruritus is pretty widespread and there is some evidence that it might help with pruritus in diabetic patients. Agents that might be used include, but not limited to, polidocanol, camphor, menthol, and tannin. However, this method might be less useful in controlling generalized pruritus, since it would be challenging to apply a topical agent to the entirety of a patient's body.[17,70]

Pharmacologic therapies

Gabapentin and pregabalin

Gabapentin and pregabalin have a similar form with an inhibitory neurotransmitter named GABA. Pregabalin, which is newer compared to gabapentin, is a prodrug that is usually more well tolerated than gabapentin. These two agents have been successfully used for treating pain with neuropathic origin, such as diabetic neuropathy.[75,76] Due to the significant role that neuropathy seems to play in the pathophysiology of pruritus in DM, a few studies have looked into using treatments for neuropathic pain for this pruritus.

One such study recruited diabetic patients with stage 4 and 5 CKD and some of them are on hemodialysis/peritoneal dialysis. All of them experienced pruritus that did not get better with antihistamine prescription or UV light treatment. Forty-seven out of 71 (66%) of the subjects reported relief with gabapentin. Sixteen out of the 24 subjects that did not report improvements were put on a course of pregabalin. Thirteen subjects (81%) reported relief with pregabalin. The changes were noticeable fairly quickly, usually after one or two doses or after an increase in dosing. Pregabalin even managed to relieve pruritus in patients that suffer from pre-existing skin condition (pruritus nodularis, chronic eczema and chronic idiopathic urticaria).[64,68]

Antioxidants

Another pathophysiological basis of pruritus in DM that has been proposed is oxidative damage.[4] The role of antioxidants has long been a subject of interest in the care of DM as the state of hyperglycemia promotes auto-oxidation of glucose to form free radicals. Examples of antioxidants that have been studied to this end would be Vitamin C and E, which are believed to lower incidence of T2DM and reduce diabetes complications.[77] Alpha lipoic acid, which plays an essential role in mitochondrial bioenergetic reactions, is believed to modulate and improve insulin sensitivity, as well as protecting insulin receptor from oxidative stress.[77,78] One study used an eight-week course of alpha lipoic acid in diabetic patients with polyneuropathy. They found a significant reduction in pain, burning, numbness and paresthesia since the four-week mark, and an even more significant improvement at eight-weeks.[79] There is also interest in the antioxidant capacities of certain anti-cholesterol drugs like gemfibrozil.[77] Another research utilizing murine models with chronic pruritus conducted by Zhou *et al.*[59] discovered that the systemic administration of N-acetyl-L-cysteine (NAC) and N-tert-butyl-a-phenylnitrone (PBN) significantly alleviates drug induced pruritus, attenuates dry skin related chronic pruritus and suppresses oxidative stress in the skin. However, there is still a lack of studies on the overall safety of these antioxidants.

Cannabinoids

Some clinical studies found that cannabinoids may be beneficial in treating chronic pruritus caused by various skin diseases and systemic conditions. Cannabinoids may have an essential role to play in skin stability as a modulator of the endogenous endocannabinoid system (ECS). ECS is also believed to play a role in some neural mechanisms, including pruritus, pain perception, as well as immunological and inflammatory responses. The ECS modulating function of cannabinoids works on both central and peripheral nervous systems.[80,81] Another study reported that the use of phytocannabinoids in pruritus activates CB1 and Cannabinoid type 2 (CB2) receptors, which have been shown to have antipruritic effects, mediated by inhibiting TRPV-1 receptors.[64]

Cannabinoids are proven to be capable of alleviating pruritus in diseases such as allergic contact dermatitis, asteatotic dermatitis, atopic dermatitis, prurigo nodularis, psoriasis, and even in cholestatic and uremic pruritus. Topical application of physiological lipid cream containing endogenous cannabinoids twice daily reduced pruritus in 38% of patients with uremic pruritus after three weeks. Another study stated that palmitoylethanolamide (PEA) could act as an anandamide (endocannabinoid) activator of CB1 receptors, and when combined with the use of emollients, was found to successfully relieve pruritus in 86.4% patients with prurigo, lichen simplex, and pruritus. A newer study in murine models using peritoneal administration of cannabinoid agonists named WIN 55,212-2 discovered that this agent could ease pruritus induced by high levels of serotonin.[80,81] One RCT that studied the effectiveness of topical cannabinoid oil made from cannabis sativa found that patients treated with the oil showed a significant improvement in pain, cold, and pruritus perception that they experienced after four weeks of treatment.[82] Even though cannabinoids use in DM has been proven to be quite successful in relieving pruritus, more studies should be done, especially studies on a larger population to further determine its efficacy and safety profile.

Antidepressants

The usage of antidepressants as an antipruritic agent in cases that are resistant to antihistamine and topical agents has been reported for over a decade, especially for patients with uremic, cholestatic, and paraneoplastic pruritus.[83] Like we discussed previously, serotoninergic pathways have a role in pruritogenic signaling, and the release of serotonin from mast cells also induces pruritus. Selective serotonin reuptake inhibitors (SSRIs) inhibit pre-synaptic reuptake of serotonin and might dull transmission of nociceptive stimuli through unmyelinated C-fibers. It has been shown to be effective in treating cholestatic pruritus. SSRIs have been advised as a fourth line treatment when standard therapies did not alleviate pruritus.[28]

The mechanism by which other antidepressants reduce pruritus are still unknown, but it is postulated that these drugs work by attenuating central mechanisms that may aggravate pruritus like mood, stress, and neural sensitization in the brain that are common with chronic pruritus. Chronic pruritus is often associated with psychopathology such as anxiety and depression, so antidepressants may improve pruritus by treating the underlying psychiatric issue. A systematic review in 2017 listed fluoxetine, sertraline, amitriptyline, mirtazapine, fluvoxamine, paroxetine, nortriptyline, and doxepin as antidepressants that have been tested and showed success in reducing chronic pruritus of differing types.[28,83] Fluoxetine is contraindicated in DM, but the other

antidepressants might be able to play a role in treating pruritus in DM.

Capsaicin

Some of the pruritogenic pathways that we have discussed in the previous sections featured TRPV1 ion channels. To recap, the activation of PAR-2 sensitizes TRPV1 downstream in nerve afferents, which then transmits a pruritogenic signal to the dorsal root ganglion and onto the dorsal horn in the spinal cord. The sensitization of TRPV1 also causes the retrograde release of SP from nerve endings which will activate dermal mast cells and release more cytokines. Capsaicin targets TRPV1 channels, depriving it from SP.[64]

A RCT comparing capsaicin 0.03% to placebo found that the patients experienced a significant reduction in pruritus compared with the placebo group. Improvement started right away in the first week of usage. However, there is an issue with treatment adherence because of side effects. Application of topical capsaicin in any of its form is inevitably followed by a transient burning sensation which a lot of patients find intolerable.[64,84]

Mast cell stabilizers

Like we previously mentioned, mast cells seem to have a big role in both pruritogenic pathways. Mast cells release a lot of potential pruritogens, which includes, but not limited to, histamine, tryptase, and serotonin. This leads to some trials utilizing mast cell stabilizers to treat pruritus in DM.[64,85] Cromolyn sodium is a mast cell membrane stabilizer that is available in both topical and oral preparations, and both forms have been shown to be effective in treating patients with pruritus due to refractory CKD-aP. However, another mast cell stabilizer, nicotinamide, has not been shown to be better than placebo.[64]

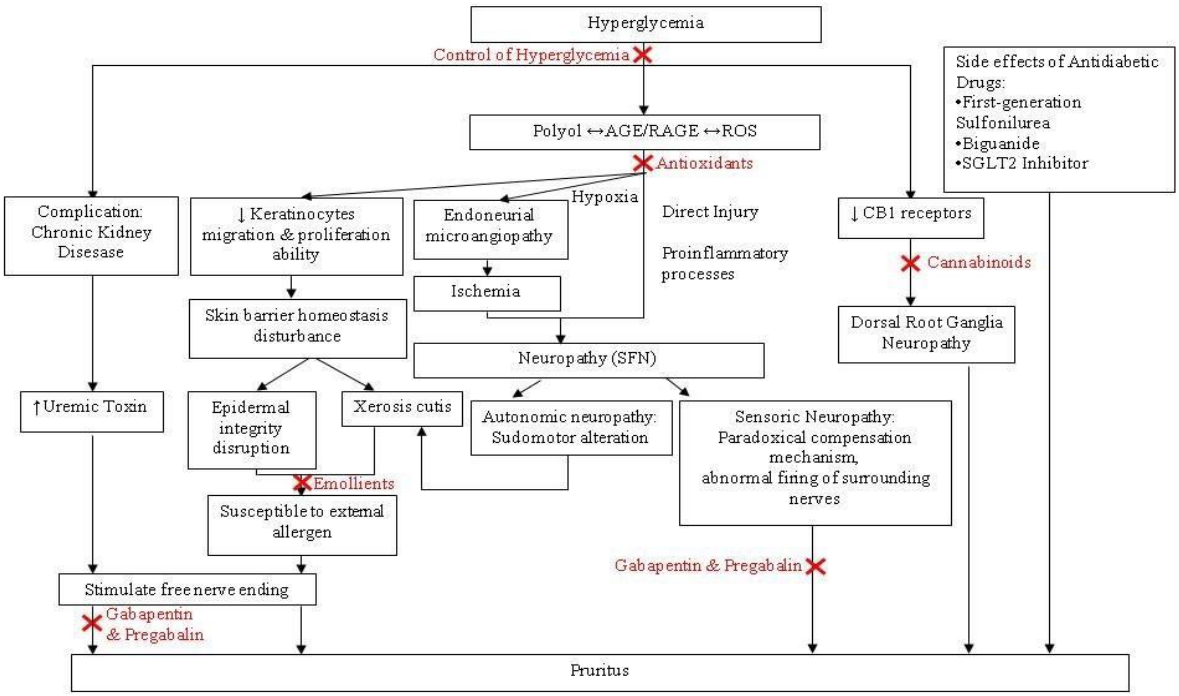

FIGURE 2. Pathophysiology of pruritus in diabetes mellitus and its pathophysiology-based treatment. AGE = advanced glycation end product, RAGE = receptors for AGEs, ROS = reactive oxygen species, CB1 = cannabinoid receptor type 1, SFN = small fiber neuropathy, SGLT2 = sodium-glucose co-transporter-2.

CONCLUSION

The pathophysiology of pruritus in DM is complex and poorly understood. However, a lot of treatment trials have given us more insight on the possible pathologies, while also helping us to understand which treatment might be useful in treating pruritus in DM.

Skin barrier disruption and diabetic peripheral neuropathy are the two main factors associated with pruritus in DM. Oxidative stress, which plays an important role in the development of complications in DM. can also induce pruritus independent of histamine via activation of TRPA1. Meanwhile, pruritus in diabetic patients with CKD and pruritus secondary to anti-diabetic therapy are still not conclusive.

Although there have been no official guidelines regarding the pharmacological treatment for pruritus in DM, we do know that nonpharmacological treatment, such as emollients and lifestyle changes has been the mainstay for treatment and is also recommended by ADA. Some pharmacological treatments have also shown promising results, such as gabapentin, pregabalin, antioxidants, cannabinoids, antidepressants, capsaicin, and mast cell stabilizers. While these treatments have not been fully endorsed by a formal guideline, they might serve us as second line treatment for diabetic patients suffering from pruritus in daily practice. Lastly, further research still needs to be done on a larger population to establish the efficacy and safety of these treatments.

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