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**Acknowledgements:**
Acknowledgements of general support, grants, technical assistance, etc., should be indicated. Authors are responsible for obtaining the consent of those being acknowledged.

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# Role of somatostatin receptor SSRT5-AS1 in predicting biomarkers of primary androgen deprivation therapy on prostate cancer in Indonesian population

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## ABSTRACT

**Introduction: Androgen deprivation therapy (ADT) has been the pivotal strategy for treating advanced prostate cancers. Despite the high efficacy of ADT in prohibiting tumor growth, >50% cases of prostate cancer will develop into an aggressive variant known as castration resistant prostate cancer (CRPC). This study aimed to evaluate the potential role SSRT5-AS1 expression as a biomarker for response to ADT in prostate cancer.**

**Materials and Methods: In total, 36 patients diagnosed with prostate cancer at Dr. Sardjito General Hospital, Yogyakarta, Indonesia were enrolled from 2015 and 2019. The expression of SSRT5-AS1 in primary tumors was quantified using quantitative real-time polymerase chain reaction.**

**Results: The mean age of patients enrolled in this study was 69.07 ± 8.7 years, and the mean of prostate-specific antigen in patients was 141.22 ±112.28 ng/ml. Compared with the median, a higher expression of SSTR5-AS1 had more significant prognostic value than the variable shorter time to CRPC (*p*= 0.043).**

**Conclusion: This study demonstrated that high expression of SSRT5-AS1 is a promising biomarker to predict response to ADT in patients with prostate cancer.**

**KEYWORDS:**
*ADT, prostate cancer, SSRT5-AS1, CRPC, biomarkers*

## INTRODUCTION

Prostate cancer (PCa) is a tumor pathology with the highest incidence in men and is strongly influenced by the hormonal milieu, especially androgens.[1] The use of hormonal therapy for treating PCa dates back to 1941 when American surgeons initiated endocrine manipulation. Ever since then, androgen deprivation therapy (ADT) has become the gold standard for both locally advanced and metastatic PCas.[1,2] After receiving ADT, PCa progresses into a variant that no longer responds to ADT. This variant is termed castration resistant PCa (CRPC).[3] The time required for this variant to develop is known as the time to CRPC. This time reportedly differs among patients with PCa, which has led to emerging issues to find biomarkers to facilitated treatment selection to PCa patients. ADT is associated with several significant side effects that can affect the quality of a patient's life. Consideration must be given for implementing ADT in patients who had benefited less from this therapy, to avoid morbidity associated with androgen derivation.

CRPC is marked by disease progression despite continuous hormonal manipulation, such as with ADT, with a profile that may present a continuous increase in serum prostate-specific antigen (PSA), clinical and radiological progression of pre-existing disease, and appearance of new metastases. Because CRPC is typically unresponsive to ADT and patients show differing time to develop CRPC, the occurrence of this condition is considerably challenging in the clinical scenario. This is because diagnostic options with current biomarkers often lead to over-diagnosis and over treatment owing to limited specific biomarkers to guide clinical decision-making.[4-6] Indeed, new treatment and diagnostic modalities still need to be developed, and significant efforts are being implemented with advances being reached in basic, translational, and clinical research fields. However, diagnostic approaches are still limited, particularly in advanced disease states owing to the heterogeneity and complexity of the disease. Hence, specific biomarkers are required to help in clinical settings to identify early responses to treatment outcomes and to identify patients who are most likely to benefit from ADT.[6]

Notably, PCa has a marked endocrine nature with other non-sex hormones such as somatostatin, which is also related with normal prostate and PCa development. Several studies have reported the role of somatostatin receptor signaling pathway (SSRT) in different types of tumors with SSRT5 expression, which is more pre-dominant in all tissue samples, especially SSTR5-AS1.[7-9] However, the role of SSTR5-AS1 in PCa is still unclear. Therefore, we evaluated the prognostic significance of SSTR5-AS1 overexpression in patients with PCa and time to develop CRPC. The goal of the study was to increase the knowledge of therapy resistant PCa by incorporating novel biomarkers, which could help improve predictive and prognostic models.

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**Table I: Characteristics of patients**

| Variables | Mean (± SD)/ count (%) |
|---|---|
| Age, years | 69.07(±8.7) |
| PSA, ng/ml | 141.22 (±112.28) |
| Time to CRPC, month | 25.7 (±18.36) |
| ISUP Groups (%) | |
| 1 | 5 (13.9%) |
| 2 | 4 (11.1%) |
| 3 | 1 (2.8%) |
| 4 | 9 (25%) |
| 5 | 17 (47.2%) |
| Surgical castration (%) | |
| Yes | 16 (44.4%) |
| No | 20 (55.6%) |
| T staging (%) | |
| T1a | 4 (11.1%) |
| T1b | 2 (5.6%) |
| T1C | 9 (25%) |
| T2a | 2 (5.6%) |
| T2b | 10 (27.8%) |
| T2C | 7 (19.4%) |
| T3C | 2 (5.6%) |
| N staging (%) | |
| Nx | 29 (80.6%) |
| N0 | 4 (11.1%) |
| N1 | 3 (8.4%) |
| M staging (%) | |
| M0 | 18 (50%) |
| M1B | 18 (50%) |
| Comorbidities (%) | |
| Cerebrovascular | 10 (27.8%) |
| Dyslipidemia | 16 (44.4%) |
| ESRD | 7 (19.4%) |
| T2DM | 13 (36.1%) |

ESRD=end-stage renal disease; T2DM:type 2 diabetes mellitus

**Table II: Predictive value time to CRPC**

| Variable | Time to CRPC | *p* value |
|---|---|---|
| High expression of SSTRS-AS1 | 19.40 ± 5.9.55 | 0.043 |
| Low expression ofSSTRS-AS1 | 30.96 + 3.18 | |
| Surgical castration | 32.12 + 3.9 | 0.132 |
| Medical castration | 22.63 + 4.17 | |
| High volume diseases | 26.75 + 3.48 | 0.728 |
| Low volume diseases | 28.67 + 4.7 | |
| PSA > 20 ng/ml | 24.85 + 3.13 | 0.072 |
| PSA < 20 ng/ml | 37.88 + 6.25 | |

*p* values were calculated using Tarone-ware test

## MATERIALS AND METHODS

*Patients*

In total, 36 patients who were diagnosed with PCa from RSUP Dr. Sardjito General Hospital(SGH), Yogyakarta, Indonesia between 2015 and 2019 were enrolled. Patients who received hormonal therapy as the primary treatment were enrolled. All clinical and demographic data were gathered from electronic medical records of SGH. Patients who received local therapy were excluded. This study received approval from the Medical and Health Research Ethics Committee, Universitas Gadjah Mada (KE/0158/02/2020).

The primary endpoint of this study was response to hormonal therapy, described as time to achieve CRPC. CRPC was defined as secondary radiographic or clinical progress of metastases during hormonal therapy or/and increase of PSA values during hormonal therapy after achieving nadir values with a testosterone level of <50 ng/ml. Clinical staging was determined by unified tumor, node, and metastases criteria according to the EAU 2021 guidelines[5] and using the digital rectal examination, magnetic resonance imaging, computed tomography with contrast, or bone survey.

The pathological results on this study were categorized according to the 2005 International Society of Urological Pathology (ISUP) score that is currently being used as a risk stratification parameter in the 2021 EAU guidelines of prostate cancer.[5]

*Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)*

Methods previously described by Indrawarman et al. were used.[10] In addition, this study was conducted in compliance Helsinki Declaration, and the study was registered with International Standard Randomized Controlled trial register (ISRCTN) under the reference no 24834343.[11]

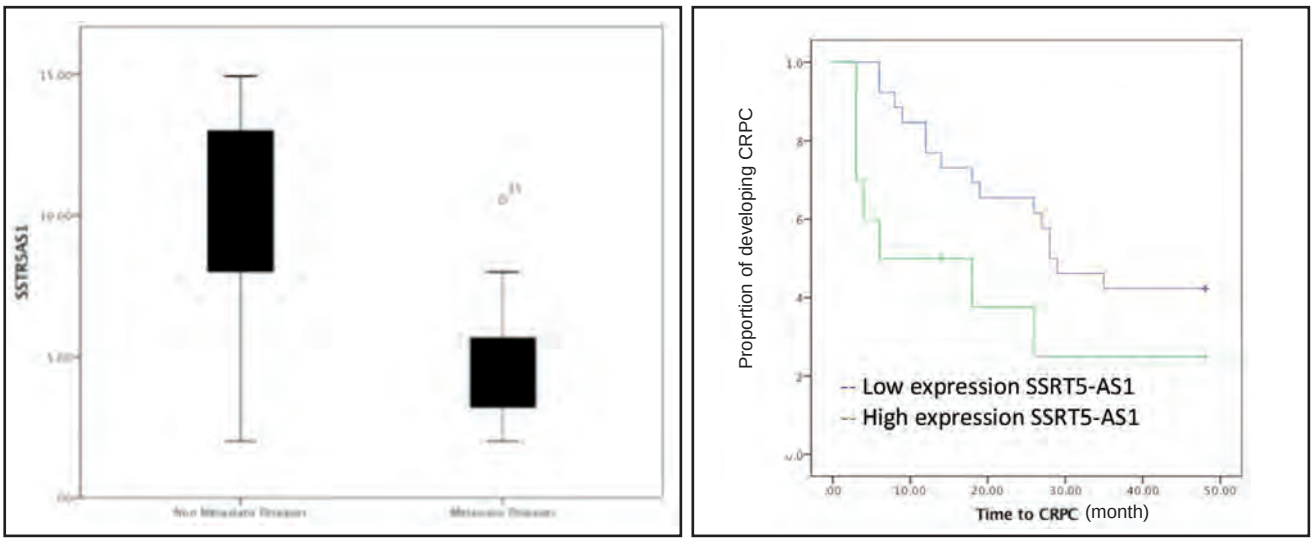


**Fig. 1:** The expression of SSRT5-AS1 in patients with prostate cancer with no metastases and bone metastases at diagnoses ($p$< 0.001) (left) and Kaplan-Meier estimates of time to CRPC in patients with prostatic cancer who received ADT for expressions of SSRT5-AS1 (right).

RNAs were extracted from formalin-fixed and paraffin-embedded biopsied samples of PCa tissues and two additional samples of benign prostatic hyperplasia (normal references). Hybrid-R™ Isolation Kit (GeneAll, Seoul-South Korea) was used to extract total RNAs, and NEXpro™qRT-PCR Kit (NextPro, Seoul-South Korea) was used to assess SSRT5-AS1 expression. The primer pair sequences used for the quantification were as follows: 5'-ACTACAGGTGCCATCAGACC-3' (forward) and 5'-GGTGTGCTGGAAAAGGGTCC (reverse). Amplification conditions comprised an initial denaturation step at 95°C for 10 min, followed by 40 cycles at 95°C for 20 s, at 55°C for 40 s, and at 72°C for 60 s. The extension step was conducted at 72°C for 5 min. Amplification of samples using q-PCR was performed using BiONEERExicycle™ 96 (BioNEER, Daejeon, South Korea). RB1 and TP53 expression was determined by the cycle threshold values and was normalized using GADPH, as previously described.

## RESULTS

The mean age of patients was 69.07 ± 8.7 years, and the mean PSA was 141.22 ± 112.28 ng/ml. Most patient who was enrolled were classified to have high risk, with 47.2% patients being in ISUP 5 group and 25% being in ISUP 4 group. In this study, patients were castrated using LHRH agonist approximately 55.6% of enrolled patients, and 44.4% patient were surgical castrated.

To further examine the association of SSTR5-AS1 with tumor spread, we evaluated SSTR5-AS1 expression in both patients with non-metastatic disease and patients with metastatic diseases.

At diagnosis, the expression SSTR5-AS1 in the metastatic disease group was significantly lower than that in the non-metastatic disease group ($p$<0.001).

However, the metastatic status in this study showed similar time to CRPC after receiving castration (Tarone-ware p= 0.728). The expression of SSTR5-AS1 was also similar between patients with PSA value at diagnosis >20 ng/ml and those with PSA value <20 ng/ml. However, time to CRPC in patients with PSA > 20 ng/ml reached faster than in other groups. However, results showed no statistically significant correlation in PSA level and SSTR5-AS1 expression.

In addition, compared with median, higher expressions of SSTR5-AS1 had more significant prognostic value than shorter time to CRPC (Figure 1) (mean: 19.40 + 5.95, $p$ = 0.043) compared to patient with lower expression of SSTRS-AS1 (Figure 1). Meanwhile, the method of castration, diseases volume according CHAARTED study, and PSA value >20 were not statistically significant predictors of time to develop CRPC in this pilot study (Table II).

## DISCUSSION

Somatostatin receptors (SSTRs) are commonly expressed on neuroendocrine tumors (NEDs), and their expression has been correlated with disease prognosis in various types of cancer.[11-13] To function biologically and activate it's signaling pathway, somatostatin needs to bind with five somatostatin receptor family gene products (SSTR1 to SSTR5).[11-12] The development of NED and SSTRs over expression by CRPC has been associated with negative or worse prognosis.[14-16] However, the context of NED in PCa is still debatable owing to the mechanism by which it develops and the clinical significance. However, some studies have reported the presence of neuroendocrine cells in PCa.[17-18]

The methods of castration did not show statistical significance. This finding was consistent with that reported by a previous study.[5-6,19] In addition, this study also revealed that metastatic volume by charted study and the initial PSA value did not show statistical significance. This result may be attributed to small sample power.

However, patients with high SSTR5-AS1 expression had shorter time to CRPC after receiving ADT as single therapy. Thus, this indicated that these patients benefit less from single ADT. Our study is the first to evaluate SSRT5-AS1 in a clinical setting for predicting the time CRPC in Asian population. Previously, Ramnarine et al. reported that patients with high expression who underwent prostatectomy plus adjuvant ADT had worse metastasis-free survival than those with low expression of SSTR5-AS1. However, for patient who did not receive ADT as adjuvant therapy had similar metastasis-free survival.[20]

Remarkably, the overexpression of SSTR5 has been indicated as a potential oncogenic factor, presenting as either aggressiveness or high proliferation rate in adenocarcinomas of the lung, squamous cell carcinomas of the lung, and small lung cancer.[21] Mass et al. reported a decrease in SSTR1–4 expression and overexpression of SSTR5, which possibly resulted from the evolution of tumor cells to elude cell cycle control by somatostatin, which can subsequently act as a growth advantage. However, the knowledge about the role of SSTR5 in PCa is still very limited and its therapeutic potential remains unknown.[20-21]

## CONCLUSION

To investigate the prognostic value of SSTR5, we measured the expression of SSTR5-AS1 in patients with PCa treated with ADT. Our results showed no apparent correlation between the expression of SSTR5-AS1 with disease metastasis and PSA score. However, our results illustrated a correlation between the expression of SSTR5-AS1 and time to CRPC, which indicated that the expression of SSTR5-AS1 could be considered as a biomarker to predict response to therapy in PCa.

Currently, there is no standard for the cut-off of overexpression of SSTR5-AS1 that can be used as a biomarker for disease progression. Future studies with larger populations are needed to validate and conform our findings. Additionally, further population studies should consider ethnicity in sample characteristics to determine if this biomarker is limited to the Javanese population.

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# Social determinants of health protocol adherence among adults during COVID-19 pandemic in Yogyakarta, Indonesia

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**ABSTRACT**

**Introduction: Coronavirus disease (COVID-19) must be controlled by involving all stakeholders, including the community. Community protocol compliance with COVID-19 health guidelines is essential. This study assessed the social determinants of health on community protocol compliance with COVID-19 health guidelines among adults in Yogyakarta, Indonesia.**

**Materials and Methods: This study was a mixed-method study of 461 adults from February through May 2021 in Yogyakarta Province. We collected data through an online survey, focus group discussions, and in-depth interviews. Logistic regression was used to analyze the results.**

**Results: Most respondents (86%) always wore masks, followed social distancing (51.8%), and washed their hands regularly (99%). Subjects older than 45 years, women, and community leaders demonstrated greater compliance with COVID-19 health protocols compared to other people. On the other hand, the occupation has become a healthy lifestyle practice indicator.**

**Conclusion: Gender, age, educational level, economics, and social status were determinants of health protocol adherence among adults in Yogyakarta. Therefore, health providers need to consider social determinants for health promotion approaches and COVID-19 prevention and control strategies.**

**KEYWORDS:**
*guidelines adherence; health promotion; protocol compliance; social determinant of health*

## INTRODUCTION

Following the discovery of two cases in Indonesia on March 02, 2020, coronavirus disease 2019 (COVID-19) has been spreading rapidly. The World Health Organization (WHO) data on July 7, 2021, showed a total of 2,379,397 confirmed cases in Indonesia, with 62,908 deaths.[1] The official Indonesian government website (covid19.go.id) listed the highest number of cases in Java Island, concentrating on the Special Capital Region of Jakarta/*Daerah Khusus Ibukota* (DKI) Jakarta, followed by West Java, Central Java, and East Java.[1] Since the beginning, the Indonesian government has implemented several strategies and policies to combat the pandemic. The strategy is to form a task force to promote social restrictions and handle testing, tracing, as well as treatment campaigns to prevent the spread of the disease.[2] Indonesia also started vaccinations in January 2021. As of July 7, 2021, 14 million people received complete vaccinations.[3] That number covered 7% of the national target.[1,4] This effort was still not optimal in preventing the spread of COVID-19.[5]

Preventive measures, such as the COVID-19 health protocols aimed at prevention of direct contact with infected or possibly infected persons, wearing masks, avoiding mass gatherings, regular washing hands, avoiding touching face, as well as disinfecting surfaces.[6] Denford et al.[7] claimed that community levels of adherence vary depending on their perceptions regarding their potential exposure to the virus, the benefits or requirements to adopt the measures, and their faith in the effectiveness of the measures. Studies have also found that sociodemographic factors, such as gender, educational level, occupational types, and age were associated with adults' adherence to COVID-19 health protocols.[8,9]

Studies regarding community adherence to COVID-19 prevention measures in Indonesia are scarce. A study in Central Java showed that community adherence in Indonesia varied based on protocols used. The results showed that 31% of respondents abided by stay-at-home protocols. The protocol adopted by most respondents was handwashing with soap (88.2%).[10] A study in Bali showed that community adherence to health protocols was associated with perceived social norms, perceived benefits, and gender.[11] These studies showed how social determinants become factors that contribute to health protocol adherence in Indonesia.

The WHO defines social determinants as "non-medical factors that influence health outcomes." Socioeconomic and political context may indirectly affect health outcomes through changes in individual health behaviours, material circumstances and psychosocial factors.[12] Therefore, contextual factors are important in explaining health outcomes and behaviours, including the adherence to health protocols in preventing the spread of COVID-19.

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By July 2021, the Special Region of Yogyakarta was among the top ten provinces with a high prevalence of COVID-19 sectors in Indonesia. Approximately 2.9% of COVID-19 cases in Indonesia had occurred in that province.[1] Yogyakarta Province is also one of the primary tourist destinations in Indonesia. The pandemic has severely impacted the socio-economic conditions there. Exploration of community adherence to health protocols in the province, especially regarding socially determinant factors, can help local governments improving the adherence and prevent the spread of COVID-19 in the community. The outcome may also provide insights into the essential social determinants affecting protocol adherence in vital tourism industries. Therefore, this study aimed to assessing the social determinants of adherence to health protocols among adults in the Special Region of Yogyakarta, Indonesia.

## MATERIALS AND METHODS

A mixed-method study with an explanatory sequential design[13] was conducted in Yogyakarta Special Province, Indonesia, in the first half of 2021. The population enrolled were adults aged 18 to 59 years old, from Sleman, Kulon Progo, Gunung Kidul, Bantul, and Yogyakarta City. This paper is the second publication of research entitled Policy Analysis of the Social Action of the Healthy Lifestyle, or *Gerakan Masyarakat Hidup Sehat* (GERMAS).

A cohort of 461 people volunteered for the study. We collected data via an online survey using Google Form, focus group discussions, and in-depth interviews. The online survey was conducted during February–May 2021, with 499 people completed the survey. Unfortunately, 50 people did not match the inclusive criteria. They may not have lived in from Yogyakarta Special Province, or they were less than 18 or over 60 years old. Thus, 50 respondents were excluded, and we included 449 respondents for further analysis. The minimum sample needed was 385 respondents.

We developed the questionnaire with content validation. We delivered the online questionnaire through Instagram and WhatsApp messaging applications. The questionnaire covered the five points of health protocols to prevent COVID-19, including physical distancing, hand washing, mask-wearing, mobility restriction, and crowd avoidance; and the sociodemographic variables include gender, age, education level, economic status, occupational, and social status. The questions were favourable and unfavourable.

We categorised the educational level into low (elementary and junior high school), middle (senior high school), and high (diploma and higher education). Low economic status defined as monthly income below three million rupiahs (approximately $208 USD); middle economic status when their income ranging between three to six million rupiahs, and high economic status for those with monthly income above six million rupiahs (approximately $416 USD) monthly income. While for occupational type, we categorize them into four groups, namely: students (including diploma/higher education students), formal workers (employers, teachers, lecturers, police, and military officers), non-formal workers (traders, entrepreneurs, labourers, farmers, and fishers), as well as not working (stay-at-home parent, retired, and not working). This study conducted logistic regression for the quantitative data ($p<0.05$).

In addition, the focus group discussion and in-depth interview guidelines were developed according to the survey results. We conducted focus group discussions to elaborate and confirm the quantitative data. The qualitative informants were among stakeholders from various sectors, including provincial and districts officers, such as health officers, the provincial planning board of Yogyakarta, community leaders, as well as the Indonesian Society for Health Promotion and Education of Yogyakarta. The ethical committee of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, approved this research protocol (KE/FK/0310/EC/2021). Informed consent were obtained from all the participants.

## RESULTS

Most respondents were women (79.7%) and over 45 years old (39.9%). Only 29.4% of respondents were community leaders, as noted in Table I. Table I shows that most respondents had high levels of education (63.7%), low economic status (58.8%).

Most respondents complied with health protocols. Mask use (86.6%) and hand washing (94.6%) were the health protocols with the highest level of compliance in Yogyakarta. One-third of the respondents removed their masks while talking and some did not wear their masks properly (Table II).

Table II shows maintaining physical distancing, staying at home, and avoiding crowds had the lowest scores for health protocol adherence among adults in Yogyakarta.

The qualitative data showed that many community stakeholders promoted the health protocols. In this pandemic, all the stakeholders (government and private sectors) were actively involved in health protocol promotional activities.

> "…Most stakeholders were involved in the health protocol campaign…They were not limited to the health officer only…" (In-depth interview, health promotion officer)

There were many sectors participate in the health matters during COVID-19 pandemic. The health promotion division of the provincial health office had empowered the existing network for health protocols and the healthy lifestyle campaign. There were massive social media campaign through the WhatsApp group of the local leader, Instagram, and YouTube. In addition, the provincial and district level Yogyakarta government issued many regulations related to the health protocols for COVID-19 prevention and control.

This study illustrates a significant correlation between sociodemographic variables, e.g., sex, age, education level, occupation, and social status, with health protocols that covered physical distancing, mask wearing, restricted mobility, as well as avoiding crowds ($p<0.05$). In contrast, there was no significant correlation between socio-demographics and handwashing behaviour. In this study,

**Table I: Demographic Characteristics of the respondents**

| Respondent characteristics (n = 449) | f | % |
|---|---|---|
| Gender | | |
| Men | 91 | 20.27 |
| Women | 358 | 79.73 |
| Age | | |
| 18–35 years old | 138 | 30.7 |
| 36–45 years old | 132 | 29.4 |
| >45 years old | 179 | 39.9 |
| Education level | | |
| Low | 21 | 4.7 |
| Middle | 142 | 31.6 |
| High | 286 | 63.7 |
| Economic status | | |
| Low | 264 | 58.8 |
| Middle | 157 | 35.0 |
| High | 28 | 6.2 |
| Occupation | | |
| Unemployed | 151 | 33.6 |
| Formal worker | 239 | 53.2 |
| Informal worker | 37 | 8.2 |
| Student | 22 | 4.9 |
| Social status | | |
| Citizen | 317 | 70.6 |
| Community leader | 132 | 29.4 |

**Table II: Health protocol adherence**

| Variable | Respondent | % |
|---|---|---|
| Wearing a mask | | |
| Wears mask | 389 | 86.64 |
| Wears two-layer fabric mask or medical mask | 435 | 94.65 |
| Wears their mask while talking | 300 | 66.82 |
| The mask covers mouth and nose | 367 | 81.74 |
| Handwashing | 448 | 99.78 |
| Physical distancing | 248 | 55.23 |
| Stays at home | 284 | 63.25 |
| Avoids social gathering | 287 | 63.92 |

almost all respondents (99.78%) had good hand washing practices.

Table III shows that sex, age, education level, occupation, as well as social status were the social determinants of health protocol compliance among adults in Yogyakarta. Education levels and age were the most substantial social determinants of health protocol compliance in Yogyakarta. Respondents who were over 45 years old had the highest compliance level toward health protocols.

Social status, represent by the community leader, contributed to the health protocol compliance. Respondents who were community leaders have high levels of compliance toward health protocol. Community leaders are public figures. Hence, the pleasant habit and practices of the community leaders will lead to good habits among people. We excluded the handwashing variable from Table III because of the correlation analysis result. There was no significant correlation between sociodemographic and handwashing behaviours.

## DISCUSSION

This study showed that the Yogyakarta community had high compliance with health protocol. Also, there were social determinant of the health protocol compliance, such as education level, age, social status, sex, as well as social status. The socio ecological model suggests assessing social determinants to investigate the health status and health behaviour.[14] Moreover, many factors, including social determinants of health, contribute to the health status and health behaviours.[15] In line with the previous study, this result proved that age and gender were the social determinants of the community adherence toward health protocol among adult in Yogyakarta.[8,9] Moreover, the level of community compliance toward health protocol was varied.[7] Even though most people had high levels of compliance, one-third of respondents did not obey the social restrictions. Many stakeholders supported the health protocols campaign in Yogyakarta, both from government and private sectors. Indeed, they were provided infrastructure to support the health protocol practices. The participation of various stakeholders in the public health intervention will lead to the program's sustainability.[16]

**Table III: Logistic regression of the association between sociodemographic factors, including mask use, physical distancing, restricted mobility, and avoiding crowds**

| | Physical distancing | | | Avoiding crowds | | | Restricted Mobility | | | Use a mask properly | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | OR | *p*-Value | 95% CI | OR | *p*-Value | 95% CI | OR | *p*-Value | 95% CI | OR | *p*-Value | 95%CI |
| Sex (vs male) | | | | | | | | | | | | |
| Female | 1.67 | 0.03 | 1.05 - 2.66 | 1.61 | 0.05 | 1.00 - 2.56 | 1.64 | 0.04 | 1.03 - 2.61 | | | |
| Age (vs 18-35 years old) | | | | | | | | | | | | |
| 36-45 years old | 1.42 | 0.15 | 0.88 - 2.30 | 1.38 | 0.20 | 0.85 – 2.24 | 1.11 | 0.67 | 0.68 – 1.80 | | | |
| >45 years old | 3.03 | 0.00 | 1.91 - 4.81 | 2.23 | 0.00 | 1.39 – 3.56 | 2.10 | 0.01 | 1.31 – 3.36 | | | |
| Education (vs low) | | | | | | | | | | | | |
| Middle | | | | | | | | | | 3.39 | 0.04 | 1.06 – 10.86 |
| High | | | | | | | | | | 2.3 | 0.12 | 0.80 – 6.73 |
| Occupation (vs unemployed) | | | | | | | | | | | | |
| Formal worker | 0.41 | 0.00 | 0.27 – 0.63 | 0.49 | 0.00 | 0.32 – 0.77 | 0.66 | 0.06 | 0.43 – 1.02 | | | |
| Non-formal worker | 0.86 | 0.70 | 0.40 – 1.83 | 0.73 | 0.42 | 0.33 – 1.58 | 0.91 | 0.82 | 0.42 – 1.97 | | | |
| Students | 0.32 | 0.02 | 0.13 – 0.81 | 0.29 | 0.01 | 0.12 – 0.72 | 0.37 | 0.03 | 0.15 – 0.91 | | | |
| Social status (vs Citizen) | | | | | | | | | | | | |
| Community leader | 1.71 | 0.01 | 1.12 – 2.60 | | | | | | | | | |

COVID-19 has influenced all aspects of life, just as many factors have determined community compliance with health protocols. Therefore, public health intervention is considered a social determinant of health.[17,18] Public health intervention and need assessment are essential.[19]

## CONCLUSION

This study concludes that education level, age, and social status were the social determinant of health protocol compliance. Government should adjust strategies to improve health protocol compliance according to target characteristics, such as education level, age, and social status.


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## CONFLICT OF INTEREST

We declare that there is no conflict of interest in this study.

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# Improvement of quality in clinical care for patients with benign prostatic hyperplasia: Cost effectiveness analysis

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**ABSTRACT**

**Introduction: Currently, St. Carolus Hospital (SCH), Jakarta, Indonesia is using a combination therapy for patients with benign prostatic hyperplasia (BPH) based on the clinical practice guidelines (CPG). In this study, we used two methods of administering combination therapy, namely, the standard method and the modified method. To date, no research has been conducted to reduce the cost burden of BPH medication without reducing the quality of service. Thus, this study aimed to compare the clinical outcomes and quality of life (QoL) of the modified therapeutic method with those of the standard therapeutic method and perform cost-effective analysis of the two therapeutic methods available at SCH.**

**Materials and Methods: The study design used was a retrospective cohort. Data were obtained from medical records at SCH and interviews. Decision tree analysis was used for this study based on clinical outcomes and costs. Clinical outcomes and costs were compared between the standard and modified therapy models. Interviews were conducted to obtain cost data from a societal perspective. Then, the data were analyzed using SPSS statistics program and MS Excel.**

**Results: A total of 100 BPH patients met the inclusion criteria. The mean age in the standard therapy and modified therapeutic method groups was 66.92 (SD±6.67) and 67.10 (SD 8.49) years, respectively. At the start of therapy, the mean international prostate symptom score (IPSS) in the standard method group was lower than that in the modified group (15 vs 17), but the mean Qmax in both groups was the same (9 ml/s). In addition, the mean QoL score in both groups was 4 (not satisfied). At the end of therapy, there was an improvement in the IPSS, Qmax and QoL was observed in both groups. In the Mann Whitney statistical test, there was no significant difference in IPSS and QoL was found between the standard therapeutic method group and the modified therapeutic method group (P = 0.07 and P = 0.498). In the unpaired T test, there was a significant difference in Qmax was found between the standard method group and the modified method group (*p* = 0.039, 95%CI, -3.20529 to -0.8769). The effectiveness of standard therapeutic methods and modified therapy methods is 82% and 90%, respectively. The average cost of standard therapeutic methods is greater than that of modified therapeutic methods per visit. Furthermore, the average cost-effectiveness ratio of the modified therapeutic method is lower than that of the standard therapeutic method.**

**Conclusion: The Modified therapeutic method has better results regarding the maximum urinary flow rate compared with the standard therapeutic method. The modified therapeutic method is also more cost-effective than the standard therapeutic method. This study can be used as the basis for service standards in hospitals and national health technology assessments as a policy direction for the national health insurance benefit package.**

**KEYWORDS:**
*Benign prostate hyperplasia, combine therapy, cost, cost-effective analysis*

## INTRODUCTION

The prevalence rate of lower urinary tract symptoms (LUTS) worldwide varies widely, and it is significantly increasing with age. The number of patients with LUTS worldwide is approximately 2.3 billion people or 45.8% of the world population in 2018, which shows an 18.4% since 2008.[1] The incidence of benign prostatic hyperplasia (BPH) in Indonesia remains unknown, but based on the data obtained from Cipto Mangunkusumo Hospital (RSCM) from 1994 until 2013; a total 3,804 cases of BPH with a mean age of the patients 66.61 years have been reported. In addition, Hasan Sadikin Hospital reported 718 cases of BPH, from 2012 to 2016, with a mean age of patients 67.9 years old.[2]

The treatment of choice is a major factor contributing to the cost of treatment from medical therapy (a combination of alpha blockers and 5-alpha reductase inhibitor (5-ARI)) to invasive action, such as transurethral resection of prostate.

The combination of alpha blocker and 5-ARI has been proven to be more effective than monotherapy based on studies from several countries.[3,4]

The high prevalence rate of BPH can cause a huge economic burden and medical costs to the authorities. The cost of BPH treatment in the UK is estimated around £ 180 million annually, of which 60% are due to complications from BPH. Long-term treatment will also increase costs, thereby causing a heavy burden to the government and society.[5]

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In addition, cost-effectiveness analysis (CEA) has been conducted to evaluate the BPH medical therapy in several American and European countries, which shows that combination of pharmacological therapy is more cost-effective than monotherapy.[3,4,6-9]

At present, St. Carolus Hospital (SCH), Jakarta, Indonesia is using combination therapy for patients with BPH, based on the clinical practice guidelines (CPG). Thus, in this study, we used two methods of administering combination therapy. Based on the CPG, in the standard method, the combination drug is given for 6 months every day and routine follow-up is conducted every month. On the contrary, in the modified method, the drug is given every day for 3 months, and then the administration of drug is continued intermittently for 3 months, and follow-up is conducted once a month in the first 3 months and then once at the end of 6 months.

In this study, we compare the objective clinical outcome and quality of life (QoL) of the modified therapeutic method with those of the standard therapeutic method and perform cost-effective analysis of the two therapeutic methods available at SCH.

## MATERIALS AND METHODS

The study design used was a retrospective cohort. Data were obtained from the medical and financial record of SCH. Interviews were also conducted with BPH patients in regard to their international prostate symptoms score (IPSS) and QoL. Decision tree analysis was performed in this study with financing and clinical outcomes as parameters. The inclusion criteria were as follows men above 50 years who were diagnosed with BPH from urologist, and currently taking Tamsulosin (0.4 mg) and Dutasteride (0.5 mg). We excluded patients with prostate cancer or other diseases that can cause LUTS except BPH. All patients who refused and didn't complete the routine follow up were also excluded. Then we divided the patients into standard therapy method group (Group 1), and modified therapy method (Group 2). Clinical outcomes and costs were then analyzed between the two groups.

## RESULTS AND DISCUSSION

*1. Respondent Characteristics*

BPH is a benign enlargement of the prostate in elderly men. Patients aged 60-69 and 70-79 were the largest age group included in this study at SCH .All respondents in Group 1 showed moderate symptoms, whereas in Group 2, 49 patients showed moderate symptoms, and one people had severe symptoms (IPSS > 20). Group 1 had fewer patients with IPSS (16-19) compared with Group 2 (44% vs 68%, Table I). The quality of life (QoL) of patients in both groups at the beginning of therapy showed dissatisfaction and unhappiness toward their condition. In all 86% of the patients in Group 1were "mostly dissatisfied" with their condition, whereas only 60% of the patients in Group 2 were "mostly dissatisfied" with their condition. However, a total of 20 patients (40%) in Group 2 reported "unhappiness", whereas only six patients (12%) in Group 1 reported "unhappiness". The majority of patients in both groups had an initial maximum urinary flow rate (Qmax) of < 10 mL/s (86% in Group 1, 66% in Group 2) (Table I).

2. Clinical Outcomes and Effectiveness

Group 1 was given standard combination therapy for BPH (Tamsulosin 0.4 mg q.d. and Dutasteride 0.5 mg q.d.) continuous for 6 months, and routine follow-up was conducted every month. On the contrary Group 2 was given modified combination therapy using the same drugs, but the administration of the drug was continued for the first 3 months and then every 2 days for the last 3 months. Follow-up was also conducted monthly for the first 3 months, and at the end of 6 months. The data during each follow-up was collected and analyzed the basis of clinical improvement and costs.

Post Therapy IPSS, QoL and Qmax

Improvement on IPSS was observed in both groups. A total of 37 people in Group 1 (74%) had an IPSS of 4-7, and only 13 (26%) patients had an IPSS ≥ 8. Meanwhile, 41 patients (82%) in Group 2 had an IPSS of 4-7, and 9 patients (18%) had an IPSS ≥ 8. Forty-seven patients in Group 2 showed improvement in IPSS compared with Group 1 (50 patients, 94% vs 100%) with different mean improvements (7.22 vs 8.20). Based on the statistical analysis, using Shapiro-Wilk test to evaluate the normality of post therapy IPSS, we found that the data were nonparametric in both groups. We then analyzed the data using Mann Whitney test to compare both groups ($p = 0.077$). $p > 0.05$ indicates no significant difference between IPSS of groups 1 and 2. (Table II)

At the end of therapy, Group 1 and Group 2 showed a "mostly satisfied" QoL (52% vs 50%). Mixed feeling and dissatisfied QoL were also observed in both groups, but most of the patients showed improvement. Forty-seven patients (94%) in Group 1 showed improvement in QoL, whereas 46 patients (92%) in Group 2 showed improved same mean improvement. Normality test using the Shapiro-Wilk test on post-therapy QoL showed that the data were nonparametric ($p<0.001$). Therefore, statistical analysis was conducted using the Mann Whitney test with a significance value of 0.498. $p$ value > 0.05, indicates no significant difference in QoL between two groups. (Table II)

The Maximum urinary flow rate (Qmax) at the end of therapy in both groups mostly showed Qmax > 10 mL/s. The number of patients that had Qmax > 10 mL/s was greater in group 2 than in group 1 (90% vs 82%). The number of patients that had improved Qmax during 6 months of therapy was 41 (82%) in Group 1, and 45 (90%) in group 2 with different mean improvement (4.19 vs 5.20; Table III). Normality test on post-therapy Qmax showed normal distribution of data. We used unpaired T-test with P = 0.039. P < 0.05, indicates significant difference in Qmax after therapy between the two therapy groups. (Table II).

Effectiveness of Therapy

Effectiveness of therapy can be seen from the improvement of IPSS, QoL score, and Qmax. Effectiveness of each parameter in both groups can be seen in Table III.

**Table I: Age group of distribution, initial IPSS, QoL, Qmax of BPH patients in the standard therapeutic method and modified therapeutic method at St. Carolus Hospital**

| Characteristics | Standard Method | | Modified Method | |
|---|---|---|---|---|
| | Total | % | Total | % |
| Age | | | | |
| 50-59 | 4 | 8 | 12 | 24 |
| 60-69 | 30 | 60 | 17 | 34 |
| 70-79 | 14 | 28 | 20 | 40 |
| ≥ 80 | 2 | 4 | 1 | 2 |
| Initial IPSS | | | | |
| 8-11 | 0 | 0 | 1 | 2 |
| 12-15 | 28 | 56 | 14 | 28 |
| 16-19 | 22 | 44 | 34 | 68 |
| ≥ 20 | 0 | 0 | 1 | 2 |
| Initial QoL | | | | |
| Mixed about equally satisfied and dissatisfied | 1 | 2 | 0 | 0 |
| Mostly dissatisfied | 43 | 86 | 30 | 60 |
| Unhappy | 6 | 12 | 20 | 40 |
| Initial Qmax | | | | |
| < 10 mL/s | 43 | 86 | 33 | 66 |
| ≥ 10 mL/s | 7 | 14 | 17 | 34 |

**Table II: IPSS, QoL, Qmax at the end of the therapy in the standard therapeutic method and modified therapeutic method at St. Carolus Hospital**

| Post Therapy | Standard Method | | Modified Method | |
|---|---|---|---|---|
| | Total | % | Total | % |
| IPSS | | | | |
| 0-3 | 0 | 0 | 0 | 0 |
| 4-7 | 37 | 74 | 41 | 82 |
| ≥ 8 | 13 | 26 | 9 | 18 |
| Changes in IPSS | | | | |
| Improved | 50 | 100 | 47 | 94 |
| Not Improved | 0 | 0 | 3 | 6 |
| QoL | | | | |
| Pleased | 9 | 18 | 7 | 14 |
| Mostly satisfied | 26 | 52 | 27 | 50 |
| Mixed about equally satisfied and dissatisfied | 10 | 20 | 9 | 22 |
| Mostly dissatisfied | 5 | 10 | 7 | 14 |
| Changes in QoL | | | | |
| Improved | 47 | 94 | 46 | 92 |
| Not Improved | 3 | 6 | 4 | 8 |
| Qmax | | | | |
| < 10 mL/s | 9 | 18 | 5 | 10 |
| ≥ 10 mL/s | 41 | 82 | 45 | 90 |

**Table III: Effectiveness of standard therapy and modified therapy method**

| Effectiveness | Standard Method | Modified Method |
|---|---|---|
| IPSS | 7.22 | 8.20 |
| QoL | 1.88 | 2.06 |
| Qmax | 4.19 | 5.20 |

**Table IV: Direct cost, indirect cost, and the average cost between the standard and modified therapy groups**

| Cost | Standard Method | Modified Method |
|---|---|---|
| Direct Cost | Rp 278,866,476 | Rp 219,814,417 |
| Indirect Cost | Rp 18,000,000 | Rp 12,000,000 |
| Total | Rp 296,866,476 | Rp 231,814,417 |
| Average Cost in 6 Months | Rp 5,937,330 | Rp 4,636,288 |
| Average Cost / Visit | Rp 989,555 | Rp 927,258 |

**Table V: ACER and ICER based on IPSS, QoL and Qmax**

| Method | ACER | | | ICER | | |
|---|---|---|---|---|---|---|
| | IPSS | QoL | Qmax | IPSS | QoL | Qmax |
| Standard | 822,345 | 3,158,154 | 1,415,672 | | | |
| Modified | 565,401 | 2,250,625 | 891,251 | -1,327,593 | -7,228,007 | -1,290,715 |

Group 2 has shown to be more effective with regard to of IPSS, QoL and Qmax improvement compared with group 1.

*3. BPH Clinical Service Cost*

The Cost of BPH clinical services at SCH includes direct cost and indirect cost. Direct cost includes medical and non-medical cost, whereas indirect cost includes consumption cost, transportation and cost incurred by caretakers of patients. Cost difference was found between the two groups. The cost in Group 2 was cheaper than that in Group 1, both direct cost and indirect cost. In Group 1, the total cost of illness reached was Rp 296,866,746 in 6 months and the average cost per visit was Rp. 989,555. By contrast, in Group 2, the total cost of illness reached was Rp, 231,814,417 in 6 months and the average cost per visit was Rp. 927,258 (Table IV).

Based on the higher level of effectiveness in QoL, and the lower cost of medical services in Groups 2 compared with Group 1, we performed CEA which was expressed in the form of average cost-effectiveness ratio (ACER) and incremental cost-effectiveness ratio (ICER). The ACER of Group 2 was smaller than that of Group 1. Therefore, group 2 can be considered as more cost-effective compared with Group 1. Based on the indicators of clinical quality, including IPSS, QoL and Qmax, the ICER Group 2 was -1,327,593, -7,228,007, and -1,290,715 respectively. This result indicates the improvements in the effectiveness and cost savings of modified therapeutic methods (Table V).

## DISCUSSION

BPH is a benign enlargement of the prostate in elderly men. Patients aged 60-69 years and 70-79 years were the largest age group included in this study at SCH. LUTS caused by BPH are progressive complaints, which occur at the age of 70 years or more.[4] Some researchers reported that BPH began at the age of 50. Patients with BPH will then be assessed on the basis of the degree of complaint and QoL using the IPSS, and the maximum urinary flow rate using uroflowmetry. At present, medical therapy is given for patients with moderate or severe complaints. The Indonesian Association of Urologists recommends daily combination therapy for 6 months among patients with moderate to severe symptoms.[2] However, long-term treatment will cause a large increase in costs which can cause a heavy burden to the government and society.[5] Therefore, finding a cost-effective therapy without losing the quality of the clinical outcome is necessary.

The initial severity of complaints by the patients determines the treatment option, cure rate, and complications that will occur in the future. In Europe, 63% patients with BPH come with moderate to severe complaints.[4] In several other studies, improvement in complaints was observed after combination therapy.[8,10] This finding is consistent with the result of this study, where patients in combination therapy showed improvement in complaints. However, modified therapeutic methods provide greater improvement when compared with standard therapeutic methods.

In this study with regard to the QoL, patients in both groups at the beginning of therapy showed dissatisfaction and unhappiness toward their condition. This situation caused the patients to come for treatment. This result is also in accordance with several other studies, that is people with BPH come for treatment if it interferes with daily life and activities.[8] QoL has improved after receiving either standard therapy or modified therapy. The improvement in QoL showed almost the same percentage in both groups. Early combination therapy reduces prostate growth progression and reduces the incidence of acute urinary retention; thus, giving early combination therapy will improve the QoL patients with BPH.

The maximum urinary flow rate (Qmax) examined by uroflowmetry in both groups showed an inadequate flow of urine (obstruction). Obstruction was found in 86% of patients in the standard method group and 66% of patients in the modified method group. Qmax in both groups improved after combination therapy. The modified therapeutic method group showed a higher Qmax improvement than the standard therapeutic method group within 6 months of clinical observation.

The high prevalence rate of BPH causes a huge economic burden and medical costs. The cost of BPH treatment in the United Kingdom is approximately £ 180 million annually, of which 60% are due to complications from BPH. Medical costs were also found to be 1.1 billion USD in the US per year.[10] Evaluation of BPH medical therapy in several American and European countries shows that combination medical therapy is more cost effective than monotherapy.[3,4,6-9]

The medical costs of the modified therapeutic method are less than that of the standard method. The cost per visit of the modified therapeutic method is also less than that of the standard therapeutic method. CEA is the conversion of cost and effectiveness in the form of a ratio. CEA is expressed as the ACER and ICER. The effectiveness of this study based on the clinical quality indicators shows different values. The maximum urinary flow rate isan objective indicator of clinical quality compared with other indicators, and it showed a significant difference in statistical tests in favor to the modified therapeutic method. Given its better effectiveness and lower clinical service costs, the ACER in the modified method group is smaller. ACER does not show real

economic value compared with ICER.[12] The ICER is more important than ACER, because it shows the degree of cost effectiveness of an intervention. In addition, the ICER of the modified therapeutic method is more cost-effective, where one clinical quality effectiveness value can reduce costs by Rp. 890,225.

Research on clinical quality and cost provides an understanding of the importance of coordination and collaboration with clinicians. Management encourages peer groups or medical staff to perform therapy in accordance with CPG; conduct clinical evaluations or audits of high-volume, high-risk, and high-cost cases; and monitor and evaluate the applicable CPG in hospitals. Therefore, collaborative care will be created in hospitals. Our study shows the importance of the role of head management in the hospital for clinical values in Continuous Quality Improvement. Hospital leaders can encourage cross-functional team performance among health professionals by setting budgets on clinical services. Data on the successful cost-effective health services can be used as an adequate promotional value.

Moreover, a comprehensive understanding of the application of pharmacoeconomic studies is necessary. Cost-effectiveness analysis is widely used to compare two or more health interventions that have different degree of effectiveness.[13-15] The existence of the HTA (Health Technology Assessment) team and the committee for quality improvement in hospitals are necessary because it can ensure that the dimensions of quality run well in the hospital. Understanding the quality of services related to clinical quality is a common need for clinicians and management in accordance with the dimensions of health service quality. The Institute of medicine defines six dimensions of health service quality: patient safety, effectiveness, efficiency, patient-centered, punctuality and equity. The modified BPH therapy method that is conducted in this study also pays attention to other quality dimensions such as patient safety, where patients are monitored in the same way as standard therapeutic methods. Another dimension of quality that can be observed is patient centeredness, where this study can also answer patients desire not to take medication every day for life. Therefore, the modified therapeutic method conducted in this study for patients will be low-cost service to be implemented in the future. This modified therapeutic method will be great help to the National Health Insurance (JKN) of Indonesia with regard to of balancing cost-effectiveness while maintaining service quality.

Ensuring the quality of hospital health services based on the dimensions of health service quality, requires directors of hospital with meta-leadership quality. Quality of service must be the indispensable part of the hospitals. This way, a hospital with services that are affordable, feasible and of good quality will be achieved.

## CONCLUSION

Clinical quality indicators, including IPSS, maximum urinary flow rate, and QoL have improved in both combinations therapeutic methods. The modified therapeutic method was more effective in improving clinical IPSS and maximum urinary flow rate than the standard therapeutic method.

A significant relationship was found between the maximum urinary flow rate in the standard therapeutic and the modified therapeutic groups.

The total and average cost of therapy for clinical services of patients with BPH in the modified therapeutic methods was less than that of the standard therapeutic methods. Furthermore, the cost-effectiveness ratio of the modified therapeutic method group was less than that of the standard therapeutic method group.

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# Triple-action of the standardized antidiabetic polyherbal extract; Synacinn™ through upregulation of *GLUT4* and inhibition of *DPP(IV)*, α-amylase, and α-glucosidase activity

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## ABSTRACT

**Introduction: Synacinn™ is a standardized polyherbal supplement for diabetes mellitus which is formulated from *Andrographis paniculata, Curcuma xanthorrhiza, Cinnamomum zeylanicum, Eugenia polyantha,* and *Orthosiphon stamineous*.**

**Materials and Methods: This study aimed to elucidate the antidiabetic potential of Synacinn™ on three specific actions, including 1) the insulin sensitivity and glucose transport on dexamethasone-induced insulin-resistance 3T3-L1 adipocytes, 2) the inhibitory capacity on postprandial enzyme activity (*α-amylase and α-glucosidase*), and 3) the inhibitory activity of hepatic *DPP(IV)* enzyme.**

**Results: Results showed that insulin resistance of 3T3-L1 adipocytes may be developed by prolonging the exposure of 1µg/ml of dexamethasone for >48 hours. The insulin-resistance condition was minimized by the treatment of 10 µg/ml of Synacinn™ which significantly improved the insulin-stimulated glucose utilization by 10.6%. Meanwhile, insulin-stimulated glucose utilization in normal adipocytes was also attenuated by 9.2%. At the cellular level, Synacinn™ attenuated glucose utilization mainly by upregulating *GLUT4* protein expression by 1.71 fold. Additionally, Synacinn™ is a potent inhibitor for the activity of *α-amylase* and *α-glucosidase* with $IC_{50}$ of 0.467 mg/mL and 0.245 mg/mL, respectively. Synacinn™ also controlled the glycemic index through inhibition of hepatic *DPP(IV)* enzyme with $IC_{50}$ of 1.11 mg/mL.**

**Conclusion: Results suggested that Synacinn™ reduced diabetes mellitus through sensitizing the cellular glucose utilization, reducing the postprandial carbohydrate degradation, and inhibiting the hepatic *DPP(IV)* enzyme function.**

## INTRODUCTION

In recent decades, new drugs and drug classes have become available for type-2 diabetes mellitus ($T_2DM$) patients that act at different sites of actions including sulfonylureas, meglitinides, biguanides, thiazolidinediones, *DPP(IV)* inhibitors, and *α-amylase* and *α-glucosidase* inhibitor.[1,2] Physicians would prescribe these drugs based on the level of glucose and hemoglobin 1C (HB1C), which may include a single or combination of oral therapy drugs.[1] Despite advanced research on drug development, DM therapies require lifelong drug consumption to control the glycemic condition at a healthy level. Unfortunately, these drugs have limitations and unwanted side effects. For example, metformin increases glucose uptake in body tissues and inhibits gluconeogenesis in the liver, but it causes gastrointestinal problems,[2] hepatotoxicity[3] and is not suitable for patients with kidney problems.[4] Meanwhile, sulphonylureas, an insulin release stimulator, is only ideal for T2DM patients.[5] Potentially natural therapies derived from the plants (single compounds, a group of compounds or whole extract) have become a popular choice to reduce and prevent the DM traditionally.[6,7]

Synacinn™, a traditional polyherbal supplement is recommended for the treatment of DM, and has symptoms including tiredness and high blood glucose level. Synacinn™ is formulated from five herbs, including *Andrographis paniculata, Curcuma xanthorrhiza, Cinnamomum zeylanicum, Eugenia polyantha,* and *Orthosiphon stamineous*. Qualitative and quantitative HPLC fingerprinting of this formulation has been critically developed as reported by Zainol et al.[8] Synacinn™ contains gallic acid, catechin, rosmarinic acid, curcumin, cinnamaldehyde, and andrographolide which are known as therapeutic agents against various diseases. Herb-drug interaction analysis also recommended that Synacinn™ could be consumed separately from a drug known to be metabolized by all tested CYP450 enzymes.[9] It is believed that the synergistic outcomes of this combination involved multiple mechanisms, ultimately in covering all the possible effects of DM in the body. Synacinn™ at 250 (b.i.d.) mg $kg^{-1}$ normalizes the blood glucose level, total glyceride, and cholesterol in STZ-induced rats.[10] It also protects the liver, kidney, and pancreas from the damage caused by STZ administration.[10] However, the fundamental mechanism behind the antihyperglycemic event is still unknown. This study investigated the reversal of insulin-resistance conditions using an *in-vitro* model developed by the acute exposure of dexamethasone (DEX) on 3T3-L1 adipocytes.

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Subsequently, the utilization of glucose and intracellular protein expression was assessed. Furthermore, Synacinn™ was also examined for its ability to inhibit the postprandial enzymes α-amylase and *α-glucosidase* as well as hepatic *DPP(IV)* activity.

## MATERIALS AND METHODS

*Materials*

Standardized water extract of Synacinn™ was supplied by Naturemedics Laboratories Sdn. Bhd. Terengganu, Malaysia. DEX,3-isobutyl-1-methylxanthine (IBMX), insulin,and rosiglitazone (ROS) were purchased from Sigma Aldrich (St. Louis, MO, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Invitrogen (Carlsbad, CA, USA). Mouse 3T3-L1 preadipocyte was purchased from American Type Culture Collection, Manassas, USA. Dulbecco's Modified Eagle's medium (DMEM), fetal bovine serum (FBS),and penicillin strep (PS) were purchased from Gibco, Life Technologies (Rockville, MD, USA).

*Cells maintenance and differentiation*

3T3-L1 preadipocytes were cultured and maintained between 80% and 90% confluency in DMEM supplemented with 10% of FBS and 1% of PS. To initiate differentiation, two-days post confluent cells were incubated with differentiation medium (DMEM supplemented with 10% FBS, 0.5 mM IBMX, 2 mM DEX, and 1.7 mM insulin). After 48–72 h, spent media was replaced by DMEM supplemented with 10% FBS and 1.7 mM insulin. Differentiated adipocytes were maintained in DMEM until day ten.

*Cytotoxicity assay*

All procedures were referred to Ismail et al.[11] with slight modifications. Preadipocytes were treated with Syancinn™ ranging from 5 to 10000 µg/mL diluted in DMEM for 24 h. MTT solution was added to each well and incubated for 4 hours at 37°C. The developed formazan was dissolved in 200µL of DMSO and analyzed using microplate reader (Biotec, ELx 808, Vermont, USA) at 570 nm via the KC Junior program. Treatment was conducted in six replications. Untreated cells were used as controls.

*Induction and validation of insulin resistance inadipocytes*

Fully differentiated adipocytes were serum-starved and treated with1 µM of DEX diluted in DMEM for 24, 48, and 72 h. Upon completion of insulin-resistance induction, cells were treated with 50 µM ROS for 48 h. The development of insulin resistance was considered successful if the glucose utilization was significantly lower ($p<0.05$) than that of the control.

*Synacinn™ treatment on insulin-sensitive and insulin-resistance adipocytes*

Prior to Synacinn™ treatment, cells were starved for 3 h in the basal DMEM. Normaland insulin-resistance adipocytes were treated with Synacinn™ at concentrations of 1, 10, and 100 µg/mL with and without the 1 µg/mL of insulin. After 48 h of incubation, spent media were collected, and glucose utilization was assayed using the Cobas C111. Subsequently, cells were lysed,and the supernatant was collected and stored at –80°C for protein analysis. ROS was used as a positive control. Treatment was conducted in triplicates.

*Western blotting*

Samples (30µg) were separated by electrophoresis, transferred and blocked by the 5% of skimmed milk. Primary antibody anti-*GLUT4* (1:2000) (PA519621, Thermo Scientific), *IRS-1* (1:2000) (PA11057, Thermo Scientific), *PI3K* (1:2000) (4257P, Cell Signaling Technology), *AKT* (1:2000) (4691P, Cell Signaling Technology), were added for overnight incubation at 4°C with the continuous shaking. Then, the membrane was incubated with a secondary antibody-AP conjugate (1:7500) (0031210, Thermo Scientific) for 1 h at room temperature. The developed band was scanned and quantified using ImageJ.

*DPP (IV) inhibitor assay*

*DPP(IV)* inhibition assay was conducted according to the *DPP (IV)* inhibitor screening assay kit (Cayman; 700210). Reaction was initiated by mixing 30µL of assay buffer, 10µL of *DPP(IV)* enzyme, 10µL of samples, and 50µL of substrate solution. The mixture was incubated for 30 min at 37°C. Fluorescence reading was obtained by using an excitation wavelength of 355 nm and an emission wavelength of 458 nm with a multimode reader (Varioskan Flash, Thermo Scientific).

*α-Amylase inhibitor assay*

The activity of α-amylase was assayed according to the manufacturer protocol (Abcam; ab102523) with modification. A total of 5 µL of 0.5U/µL Aspergillus oryzae α-amylase was preincubated with 45 µL of samples for 30 min at 37°C. Total amount of 100 µL reaction mix was added to each reaction and mixed carefully. The mixtures were allowed to react for 20 min, followed by the measurement of absorbance at 405 nm. Acarbose was used as a positive control. Percent of inhibition was calculated by using Equation(1).

$$\%inhibition = \frac{^{OD}control - ^{OD}sample}{^{OD}control} \times 100 \quad (1)$$

*α-Glucosidase inhibitor assay*

The inhibitory activity of *α-glucosidase* was conducted according to the procedures provided by the QuantiChromTM *α-Glucosidase* Assay Kit (DAGD-100) with modifications. About 10µL of 1.0U/mL of *α-glucosidase* from baker's yeast was preincubated with 10µL of samples for 15 min at 37°C. A total of 200µL of working reagent was added and allowed to react for 30 min. The reaction was measured at 405nm. Acarbose was used as a positive control. Percent of inhibition was calculated usingthe Equation(1) mentioned above.

*Statistical analysis*

All data were expressed in mean ± standard error (SEM). Statistical analysis was performed using SPSS program with one-way ANOVA and Tukey test. Significant differences were considered asp< 0.05.

## RESULTS

*Effect of Synacinn™ on insulin-resistance in-vitro model*

Development of insulin-resistance adipocytes by dexamethasone

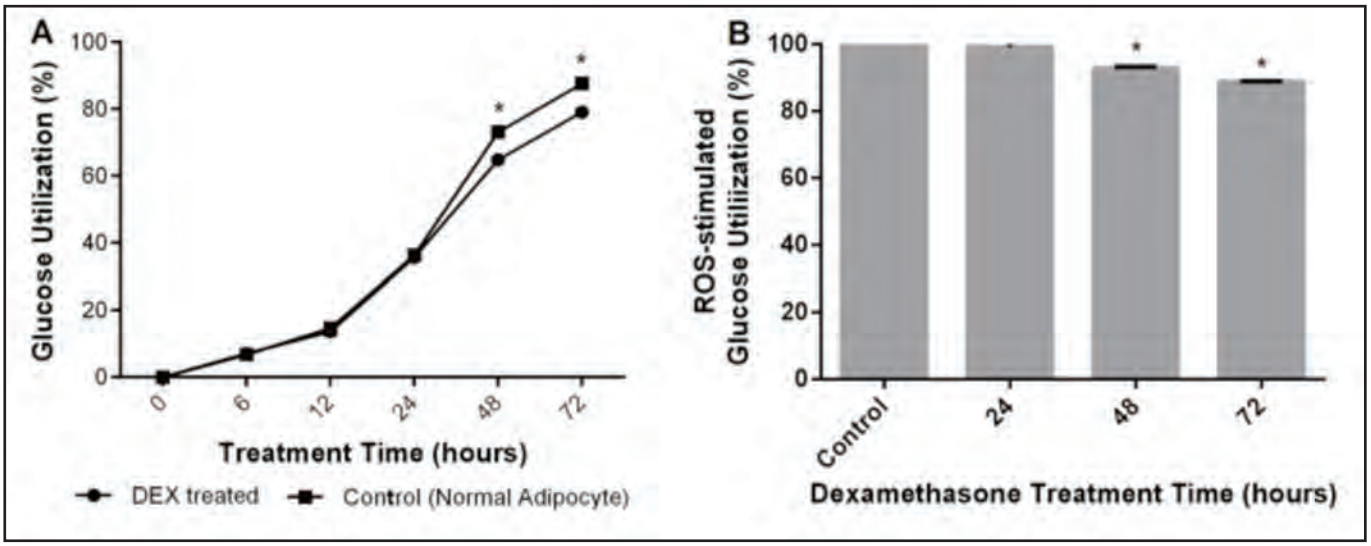


**Fig. 1:** A)Insulin-stimulated glucose utilization during the induction of insulin resistance by DEX. [*] Significant differences ($p< 0.05$) of 8.3% and 8.5% were quantified at 48 h and 72 h, respectively, as compared to control. Treatment was carried out in triplicates. B)Validation of insulin-resistance model by rosiglitazone (ROS). Significant differences of 7.1% and 11.2% were measured at 48 h and 72 h of treatment, respectively.

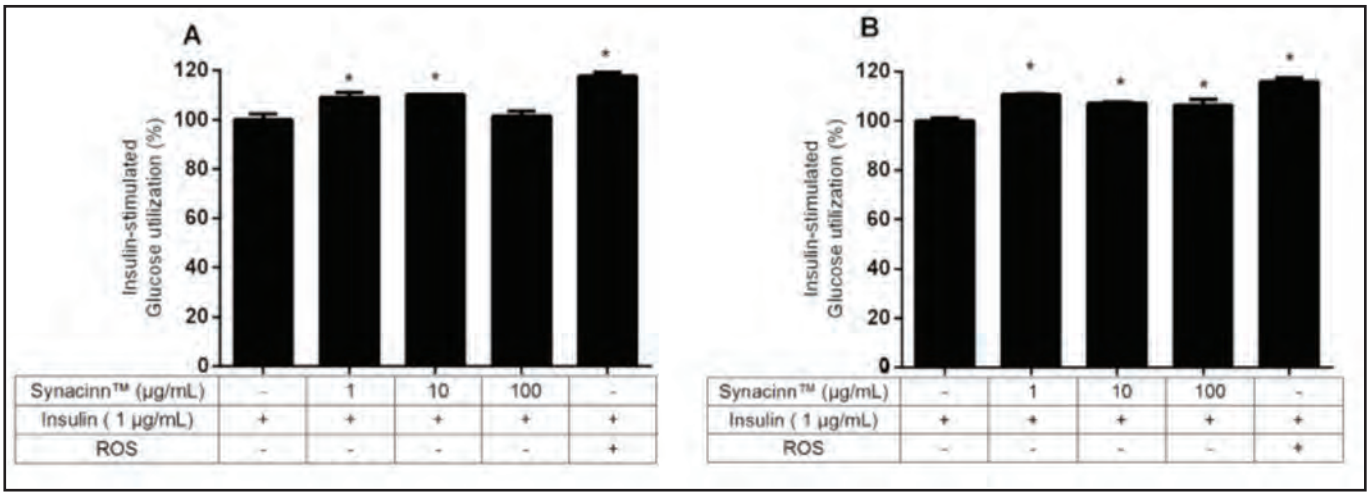


**Fig. 2:** Effect of Synacinn™ on insulin-stimulated glucose utilization. A) Effect of Synacinn™ on glucose utilization in normal adipocytes. B) Effect of Synacinn™ on the glucose utilization in insulin resistant adipocytes. Rosiglitazone (ROS) was used as a positive control. [*] Significance was considered as $p< 0.05$.

Induction of insulin resistance by DEX was carried out in the presence of insulin, which manifests the condition inthe human body. As illustrated in Fig 1 (A), treatment of 1µM DEXfor 72 h showed a time-dependent inhibition on glucose utilization. The presence of DEX partially disturbed the glucose utilization starting at 48 h of treatment, and continuously inhibited until the end of the experiment. Significant differences ($p < 0.05$) of 8.3% and 8.5% were quantified at 48 h and 72 h of DEX treatment, respectively, as compared to the control (normal adipocytes).

In addition, following the induction of DEX, the insulin-resistance condition was validated in the presence of 50µM ROS as presented in Fig 1 (B).The ROS-stimulated glucose utilization was consistent with the results in Fig 1. Significant differences were observed starting at 48 h (7.1%) and 72 h (11.2%).

Synacinn™ sensitized the insulin-stimulated glucose utilization in normal adipocytes and insulin-resistance adipocytes

Glucose utilization stimulated by insulin is predominant in insulin-sensitive tissues like muscles and adipose. In this study, normal adipocytes and insulin-resistance adipocytes were treated with Synacinn™ for 48 h, and the glucose concentration in spent media was measured to estimate the utilization of glucose by cells. As in Fig. 2 (A), insulin-stimulated glucose utilization in normal adipocytes was

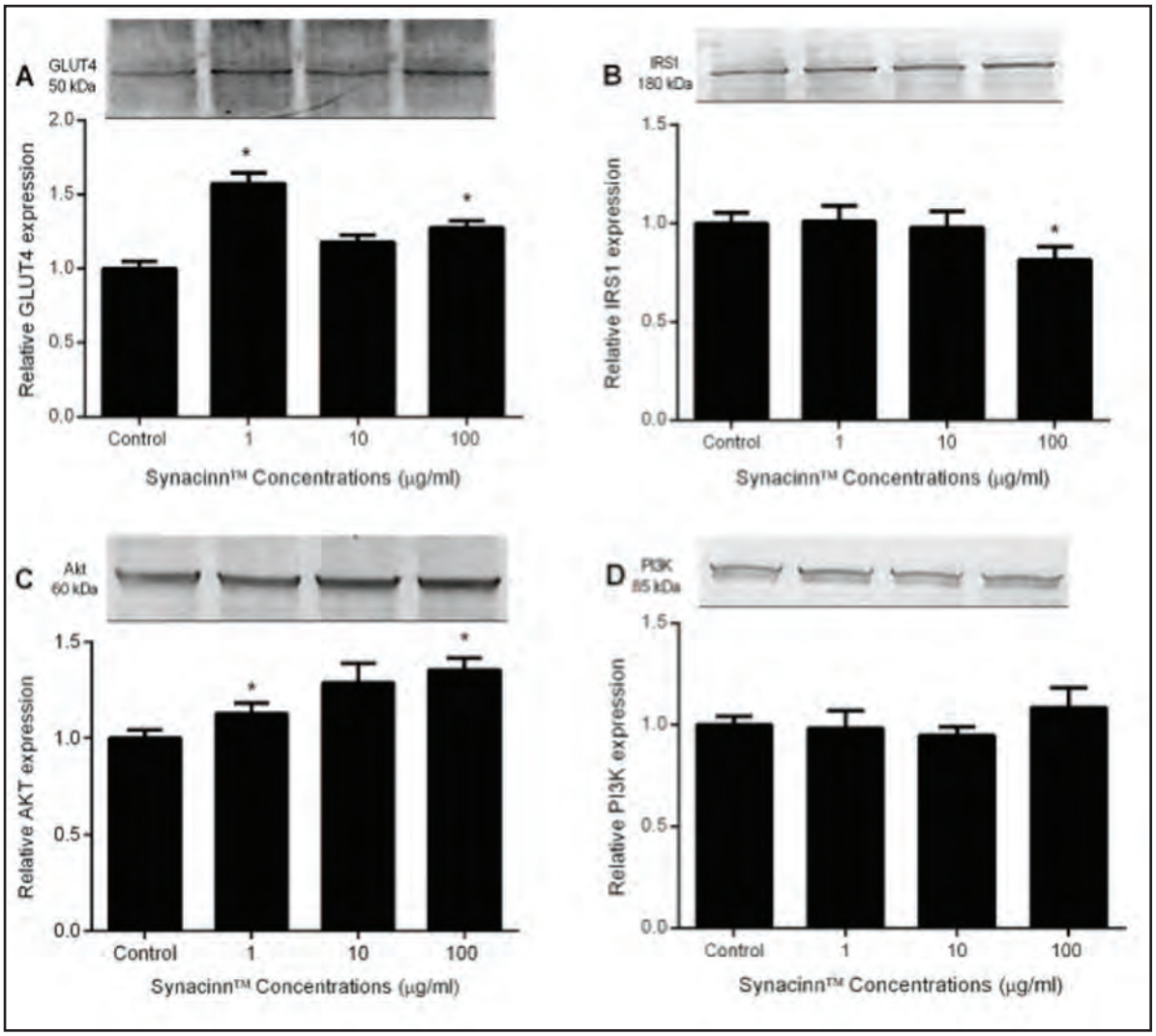


**Fig. 3:** Effect of Syancinn™ on the expression of insulin signaling pathway proteins in normal adipocytes. A) Relative expression of *GLUT4*, B) relative expression of *IRS-1*, C) relative expression of *AKT*, and D) Relative expression of *PI3K*. [*]Significance was considered as $p< 0.05$.

significantly increased during the treatment of Synacinn™. At a concentration of 1 and 10 µg/mL, Synacinn™ significantly ($p< 0.05$) attenuated glucose utilization by 9.2% and 10.2%, respectively. However, total of 100 µg/mL failed to increase the glucose utilization. As expected, ROS as positive control enhanced the glucose utilization by 17.6%.

The effect of Synacinn™ on glucose utilization was further investigated using DEX-induced insulin-resistance adipocytes. Fig.2 (B) demonstrates that Synacinn™ in the presence of insulin restored the impaired glucose utilization process. In comparison with the control (insulin only), significant improvement ($p< 0.05$) on glucose utilization was observed with 10.6%, 7.2%, and 6.3% increment for 1, 10, and 100 µg/mL of Synacinn™ treatment, respectively.

Synacinn™ increased glucose utilization through upregulation of GLUT4

Previously, it was discovered that Synacinn™ enhanced glucose utilization in normal and insulin resistant adipocytes. Further analysis of the expression of proteins related to the insulin signaling pathway has shown that Synacinn™ treatment on normal adipocytes enhances the expression of *GLUT4* and *AKT*. The total *GLUT4* expression was markedly increased during the treatment of all concentrations with 1.55-, 1.17-,and 1.28-fold for 1, 10, and 100 µg/mL,respectively (Fig.3A). Meanwhile, the expressions of AKT were increased by 1.13-, 1.29-, and 1.22-fold during treatment of similar doses of Synacinn™ (Fig.3C). No changes in the expression of IRS-1 and *PI3K* were detected upon Synacinn™ treatment (Fig.3B and Fig.3D).

The treatment of Synacinn™ on insulin resistant adipocytes showed that Synacinn™ treatment in the presence of insulin specifically improved the expression of total *GLUT4* (Fig.4). Treatment of 1, 10, and 100 µg/mL Syancinn™ significantly increased the total *GLUT4* expression by 1.39-, 1.71-, and 1.59-fold, respectively (Fig.4A). However, the changes of expression of *IRS-1*, *AKT*, and *PI3K* were not significant during Syancinn™ treatment.

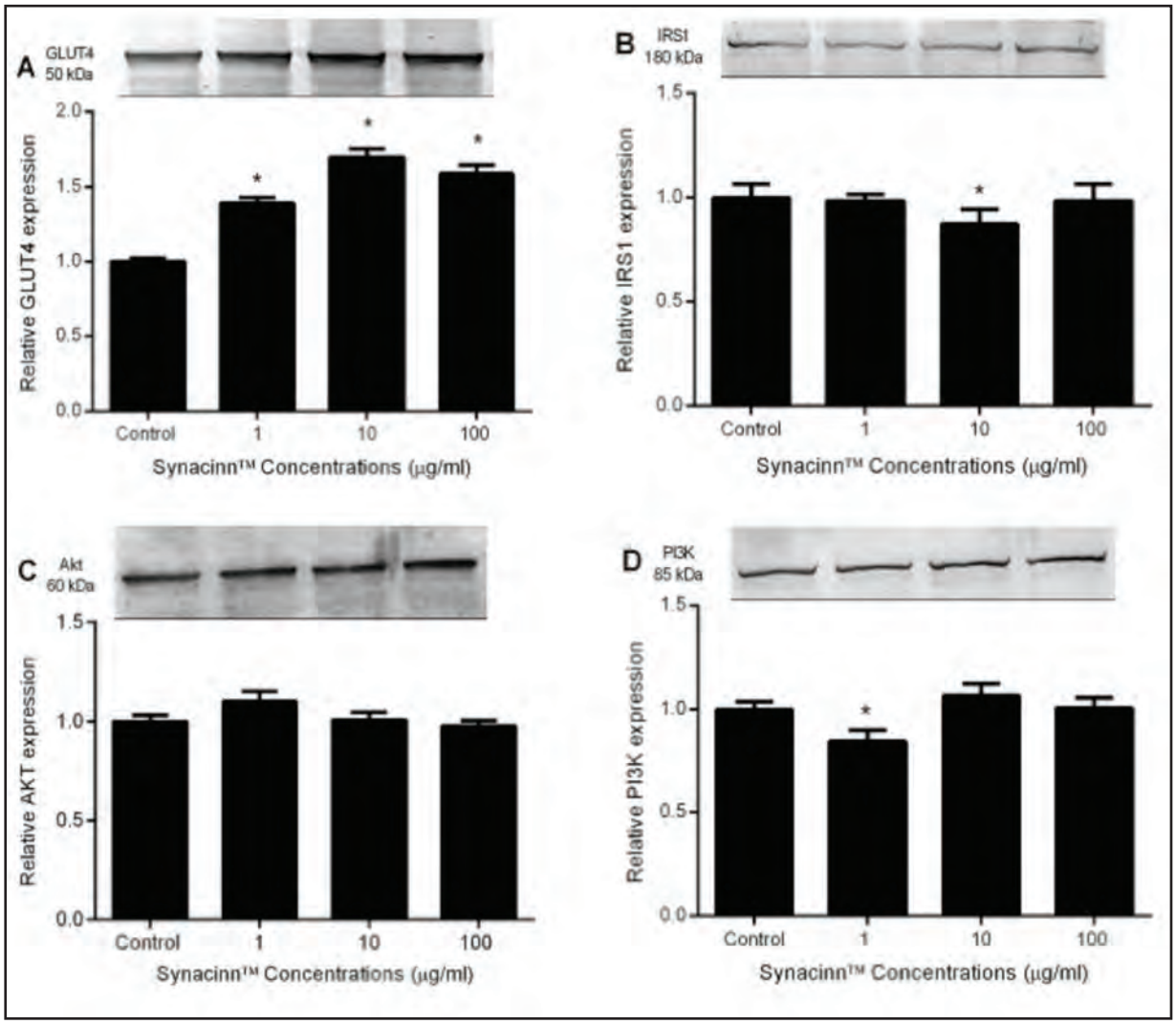


**Fig. 4:** Effect of Syancinn™ on the expression of insulin signaling pathway proteins for insulin-resistance adipocytes. A) Relative expression of *GLUT4*, B) relative expression of *IRS-1*, C) relative expression of *AKT*, and D) relative expression of *PI3K*. [*] Significance was considered as $p< 0.05$

*Effect of Synacinn™ on the inhibition of DPP(IV), α-amylase, and α-glucosidase activities*

Another route of antidiabetic therapies is by inhibiting DPP(IV) enzyme activity from converting glycogen in the liver to glucose, which in turn will increase the glycemic index in the blood. In this study, adose-dependent inhibition trend was observed during the treatment of Synacinn™ on *DPP(IV)* activity (Fig 5). At the highest tested concentration, 4 mg/mL, Synacinn™ inhibited 94.3% of its activity with $IC_{50}$ of 1.11 mg/mL.

The antidiabetic effect of Synacinn™ was further analyzed on the inhibition activity of the postprandial enzymes *α-amylase* and *α-glucosidase* as presented in Fig. 6. A dose-dependent inhibition was achieved by Synacinn™ toward the activity of both enzymes. At the highest concentration of 4 mg/mL, Synacinn™ inhibited 98.5% of *α-amylase* activity and 96.6% for *α-glucosidase* with $IC_{50}$ of 0.467 mg/mL and 0.245 mg/mL, respectively.

## DISCUSSION

Synacinn™, a polyherbal supplement for DM, is formulated from five different types of Malaysian herbs, including *A. paniculata, C. zeylanicum, C. xanthorrhiza, E. polyantha,* and *O. stamineus*. It is believed that this combination triggers a synergistic mechanism ultimately to overcome all the possible effects of DM. In a recent study, *in-vitro* pharmacodynamics tests were conducted to identify the antidiabetic potential of the standardized extract of Synacinn™. We discovered that this novel polyherbal formulation is a multifunctional mediator for DM with the following abilities: 1) increases the glucose utilization in normal and insulin-resistance adipocytes through upregulation of *GLUT4*, 2) inhibits the activities of the postprandial enzymes, and 3) inhibits *DPP(IV)* enzyme activities.

To investigate the mechanism of action of Synacinn™, the in-vitro insulin-resistance model that mimics T2DM was

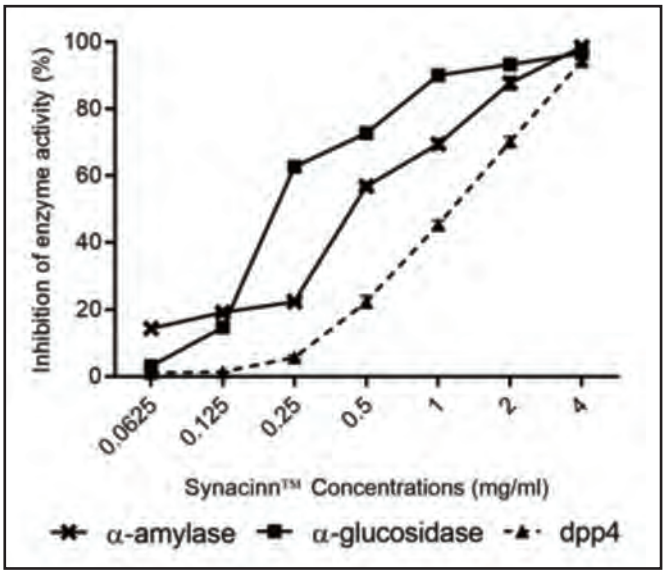


**Fig. 5:** Effect of Syancinn™ on the activity of hepatic *DPP(IV)*, *α-amylase*, and *α-glucosidase*. Dose-dependent inhibition was observed for the *DPP(IV)* enzyme activity with a maximum inhibition at 4 mg/mL (94.3%) ($IC_{50}$ = 1.11 mg/mL) For *α-amylase* and *α-glucosidase*, the maximum inhibition were 98.5% ($IC_{50}$ = 0.467 mg/mL) and 96.6% ($IC_{50}$ = 0.245 mg/mL), respectively.

developed by treating the *3T3-L1* adipocytes with 1 µM DEX with the presence of insulin for 72 h. During the development of insulin resistance, we discovered that the disturbance of insulin-stimulated glucose utilization was initiated at 48 hand worsened at 72 h of DEX treatment. This condition was further validated by 50µM ROS, which produced similar results. In contrast with Sangeetha et al.[12] in the absence of insulin, 50% reduction in glucose uptake during the insulin-resistance state was achieved as early as 24 hours. The differences suggested that insulin might have a protective mechanism toward the early stage of DEX-induced insulin resistance. However, the protective effect was later diminished ata longer treatment period. In addition, no phenotypic changes such as cell death, changes in size and shape (data not shown) were observed during this period, suggesting that the inhibitory effect of glucose utilization was due to the resistance imposed on the cells. ROS has been reported to improve insulin sensitivity in in-vitro insulin-resistance models, such as *3T3-L1* adipocytes, human embryonic kidney 293 *(HEK 293)*, and $C_2C_{12}$ skeletal muscle cells.[12-14] As a *PPARγ* ligand, ROS binds specifically and activates the *PPARγ* nuclear receptor. The activated *PPARγ* binds to the retinoid X receptor and forms a complex. This complex will assist the transcription of another gene especially in insulin signaling and adipogenesis pathway.[15] In isolated fetal rat primary brown adipocytes, ROS treatment has correspondingly increased the expression of *IRS-1* and *IRS-2Tyr* phosphorylation, which subsequently activates the *PI3K* and *AKT* proteins. The mRNA level of *GLUT4* was not changed, but ROS increases the translocation of *GLUT4* to the plasma membrane resulting in a significant increase of basal and insulin-stimulated glucose uptake.[16] ROS was also shown to improve glucose transport in vastus lateralis muscles and adipocytes of Goto–Kakizaki diabetic rats and independently increase the expression of PKC-ζ/λ without the improvement in the activation of *PI3K* and *PKB*.[17]

Synacinn™, a standardized polyherbal supplement, is designed to reduce DM and its complications. In a recent study, it was confirmed that Synacinn™ increases glucose utilization in normal adipocytes and reverses DEX-induced insulin resistance in *3T3-L1* adipocytes. Interestingly, lower concentrations of Synacinn™ were more potent in sensitizing insulin-stimulated glucose utilization. Even though the improvement was not as good as ROS within the tested period, it was postulated that for a longer time, Syancinn™ exhibited a promising effect as a glucose-lowering agent. Among the phytochemicals in Synacinn™ that have such an effect on adipocytes are gallic acid and andrographolide.18-20 Meanwhile, 5µM rosmarinic acid increases glucose uptake by 86% in L6 rat myotubes.[21]

In insulin-responsive tissue like muscles and adipose, glucose transportation is regulated by a cascade of intracellular phosphorylation event insulin signaling pathway. Cellular analysis on the insight of DEX-induced insulin resistance identified dephosphorylating several downstream proteins in the insulin signaling pathways including *IRS-1, PI3K,* and *AKT,* which subsequently inhibit the translocation of *GLUT4* from the cytosolic compartment to the cellular membrane.[22,23] In this study, we discovered that the effect of Synacinn™ was dominant in restoring glucose transportation rather than repairing the insulin signal transduction. The restoration of glucose utilization in adipocytes was in fact, mainly stimulated by upregulation of *GLUT4* level, and not by other downstream proteins in the insulin signaling pathway, such as *IRS-1, PI3K*, and *AKT*. The expression of *GLUT4* was hyped up to 1.5-fold suggesting that more glucose will be transported into the cells. Even though Synacinn™ does not improve *IRS-1, PI3K*, and *AKT* expression in insulin-resistance adipocytes, it is postulated that these proteins' activity is sufficient to enhance *GLUT4* activity. Similar results were demonstrated by 10µM gallic acid showed the enhancement of *GLUT4* translocation without any stimulation on the *AKT* and *AMPK* phosphorylation.

In this study, we also discovered that Synacinn™ controls the glycemic index through inhibition of *DPP(IV)* enzyme. Dose-dependent inhibition of the activity of this enzyme was observed (Fig. 6), where the optimum concentration was at 4 mg/mL.Curcumin, gallic acid, and rosmarinic acid have shown potential as natural sources of *DPP(IV)* inhibitor.[24] The inhibition of *DPP(IV)* enzyme activity influences the blood glucose level by prolonging the half-life of active glucagon-like *peptide-1 (GLP1)* to stimulate the insulin secretion, increase beta-cell mass, inhibit glucagon secretion, reduce the rate of gastric emptying,and induce satiety.[25]

The control of postprandial hyperglycemia may be achieved by slowing the absorption of glucose through the inhibition of the carbohydrate hydrolyzing enzymes (*α-amylase* and *α-glucosidase*).Inhibitors of these enzymes delay carbohydrate digestion and prolong the overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial blood glucose rise.While most individual herbs in the formulation have

been reported to inhibit carbohydrate hydrolyzing enzymes, Synacinn™as in polyherbal combination exertsgreater potential as an inhibitor for *α-amylase* and *α-glucosidase* with $IC_{50}$ of 0.467 mg/ml and 0.245 mg/ml. In comparison, the binary water-ethanolic extract of Andrographis paniculataexhibited higher $IC_{50}$ for *α-amylase* (35.7 mg/mL) and *α-glucosidase* (4.63 mg/mL).[26]

## CONCLUSION

To summarize, the multifunctional standardized polyherbal formulation, Synacinn™, modulates hyperglycemic control through three specific mechanisms of action including:1) enhancing cellular glucose transportation through upregulation of $GLUT_4$, 2) inhibiting postprandial enzymes activities,which delay the degradation and absorption of polysaccharide, and 3) altering the gluconeogenesis process in the liver by inhibiting hepatic *DPP(IV)* enzyme activities.


## ACKNOWLEDGMENT

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# Vascular remodeling and association with inflammation in the heart of obesity model

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## ABSTRACT

**Introduction: Obesity alters several metabolic activities, subsequently leading to the development of cardiovascular diseases. The insulin resistance-induced obesity stimulates vasodilatation and vasoconstriction imbalance, which ends up in cardiac vascular remodeling. Therefore, we aimed to investigate the effect of obesity in cardiac diseases with a focus on inflammatory mediators associated with endothelial dysfunction.**

**Materials and Methods: Rats (3 months old, weighing 200 g) were divided into control (n=6) and the obese groups, which included rats fed on a high-fat diet (HFD, n=6 in each sub-group) for 1 month (OB1), 2 months (OB2), and 4 months (OB4). Then, the rats were sacrificed, and their hearts were harvested for histological quantification as well as the quantification of the mRNA expression of inflammatory mediators, eNOS, and ppET-1 by reverse transcriptase-polymerase chain reaction (RT-PCR). Sirius Red staining was performed to assess vascular remodeling, while immunohistochemistry of CD68 was performed to assess the localization of macrophage.**

**Results: HFD-induced obesity was significantly manifested in the obese groups relative to that in the control group. It was followed by an increase in the mRNA expression of inflammatory mediators in the obese groups when compared to that in the control group. Long-term obesity promoted vascular remodeling, which was noted in the OB4 group, along with downregulation of the eNOS mRNA expression and the upregulation of the ppET-1 mRNA expression.**

**Conclusion: Obesity associated with inflammation and vascular remodeling in the heart.**

**KEYWORDS:**
*Obesity, inflammatory mediators, vascular remodeling, eNOS, ppET-1*

## INTRODUCTION

Obesity is a condition marked by the presence of excess fat tissues in several areas of the body that results in a high body mass index (BMI). It has been known that people with BMI >30 are categorized as obese and that they may be at a greater risk to several health problems.[1,2] The prevalence of obesity has increased over several past decades in both developed and developing countries. In 2013, more than 50% of the population in Oceania, North Africa, and the Middle East countries were recorded as obese.[3] According to The Indonesian National Basic Health Research, the prevalence of people with BMI score >27 in Indonesia has increased since 2007, namely by 10.5%, 14.5%, and 21.8% in the years 2007, 2013, and 2018 respectively.[4,5]

Obesity is associated with heart failure, atrial fibrillation, sudden cardiac death, and myocardial steatosis.[6,7,8,9] High BMI correlates with a high level of fat mass and free fatty acid (FFA), which has a severe effect on the cardiovascular system.[10] An accumulation of adipose tissue increases the release of adipokines[11] and pro-inflammatory cytokines, such as IL-6 and TNF-α,[12] which results in the infiltration of immune cells.[13] Hypertrophy and hyperplasia of the adipose tissues lead to lipotoxicity and alter lipid metabolism. An increase in the lipid metabolism promotes macrophage infiltration and activation mediated by nuclear factor kappa-light-chain-enhancer of the activated B cells (NF-κB) pathway. An increase in the level of FFA, cardiomyocyte fatty acid uptake exceeds the mitochondrial oxidative capacity, ultimately forming inclusions of lipid within the cardiomyocytes, also known as cardiac steatosis. This condition leads to lipotoxicity of the hearts.[14]

Inflammation contributes to the development of early endothelial dysfunction that leads to the development of atherosclerotic plaque. The acute phase protein and inflammatory mediators in circulation aggravates myocardial fibrosis and endothelial dysfunction. Imbalance vasodilator and vasoconstrictor agent are regulated by phosphatidylinositol 3-kinase-dependent (PI3K) and mitogen-activated protein kinase, respectively, in vascular endothelial tissues. Measuring the diameter and blood flow in coronary arteries is regarded as the gold standard to assess endothelial function.[15,16] This study was performed to explore the effect of obesity in the cardiovascular system with a focus on inflammatory mediators associated with endothelial dysfunction.

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## MATERIALS AND METHODS

*Animal study*

This study was conducted according to the ethical approval of the Ethical Committee of Medical Research and Health of Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada (ethical expediency number KE/FK/0385/EC/2019). The rats were maintained at 25°C–30°C with 50%–60% humidity, under a dark-light cycle of 12:12 h.

Sprague–Dawley rats (age: 3 months, weight: 180–200 g) were divided into 4 groups, namely control group that received standardized food (Control, n = 6) and obese groups that were fed with a high-fat diet (HFD). The obese groups consisted of rats with obesity for 1-month (n = 6, OB1), obesity for 2 months (n = 6, OB2), and obesity for 4 months (n = 6, OB4) groups. Lee Index (($\sqrt[3]{}$weight(grams))/naso-anal length (mm)) was used to determine the level of obesity. At the due date, the rats were sacrificed, and their hearts were harvested. The area under the coronary sulcus was immersed in an RNA preservation solution (Favorgen, FARSS100) for RNA extraction, and the apex was immersed in 4% paraformaldehyde.

*RNA Extraction and cDNA synthesis*

The heart of rat was extracted according to the procedural technique described by the manufacturer of the Genezol RNA Solution (GENEzol™, Cat. No. GZR100). Then, 3000 ng of total RNA was used to synthesis the cDNA. The synthesis of cDNA was performed using the cDNA Synthesis Kit (SMOBio, RP1400) with the PCR condition of 25°C for 10 min, 42°C for 50 min, and 85°C for 5 min.

*Reverse transcriptase PCR (RT-PCR)*

Assessment of the mRNA expression of inflammatory mediators was done using CD68 (forward 5′-TGTGTCCTTCCCACAAGCAG-3′ and reverse 5′-AAGAGAAGCATGGCCCGAAG-3′), NFκB (forward 5′- CACTCTCTTTTTGGAGGT-3′ and reverse 5′-TGGATATAAGGCTTTACG-3′), and MCP-1 (forward 5′-GCTGTAGTATTTGTCACCAAGCTC-3′ and reverse 5′-ACAGAAGTGCTTGAGGTGGTT-3′). The mRNA expression of eNOS was assessed using forward 5′-CCGGCGCTACGAAGAATG-3′ and reverse 5′-AGTGCCACGGATGGAAATT-3′) and the ppET-1 performed using forward 5′- GTCGTCCCGTATGGACTAGG-3′ and reverse 5′-ACTGGCATCTGTTCCCTTGG-3′). PCR conditions were denaturation at 94°C for 10 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min, followed by the final extension phase end step at 72°C for 10 min. The RT-PCR was performed by mixing cDNA, Taq master mix (Promega, GoTaq Green, M7122), and primers. The PCR products were analyzed on 2% agarose gel along with a 100-bp DNA ladder (Bioron, Germany, Cat. No. 306009). The internal control used HRPT-1 (forward 5′- AGACGTTCTAGTCCTGTGGC-3′ and reverse 5′- ATCAAAAGGGACGCAGCAAC-3′) and β-actin (forward 5′-GCAGATGTGGATCAGCAAGC-3′ and reverse 5′- GGTGTAAAACGCAGCTCAGTAA-3′), was used to normalize the expression.

*Immunohistochemistry staining of CD68*

The samples were cut into 4-µm-thick paraffin sections and placed in coated-object glass, followed by deparaffinizing with xylene and rehydration with grading alcohol. Next, the antigen was retrieved using heat-induced antigen retrieval methods, followed by blocking peroxidase with 3% H202 in the PBS solution. Then, the slides were incubated with the blocking serum for 20 min and incubated with mouse 1st polyclonal antibody anti-CD68 (Abcam®, ab955; 1:300) overnight. On the following days, the slides were incubated with antibodies (Biocare Medical®, STUHRP700L10), and diaminobenzidine tetrahydrochloride (DAB) (Biocare Medical®, STUHRP700L10). The results were captured under a light microscope (Olympus CX22®) through the Optilab software with 400x magnification.

*Statistical analysis*

Data were analyzed using the SPSS 22 software, and the normality test was performed by using Shapiro–Wilk. Normally distributed data were analyzed using One-way ANOVA and independent t-test. The significant value was determined if the *p*-value was less than 0.05.

## RESULTS

*HFD feeding stimulated obesity in conjunction with the severity of cardiac inflammation*

According to the Lee Index, at the end of study, we showed that HFD feeding for 1, 2, and 4 months significantly caused obesity in Sprague-Dawley rats ($p = 0.000$). Starting at week 8, the body weight increased gradually, which was noted in OB2 ($p = 0.009$) and OB4 ($p = 0.006$) when compared to that in the control group. At week 12 and week 16, a remarkable escalation of the body weight in OB4 was noted to that in the control group ($p = 0.000$).

With an increase in the body weight, the inflammatory cytokines were upregulated. Long-term obesity provoked cardiac inflammation, which was obvious in the OB4 groups. The mRNA expression of NFκB, MCP1, and CD68 in the OB4 group was markedly elevated when compared to that in the control group ($p < 0.05$). However, the mRNA expression of NFκB and MCP1 in the OB1 group was not statistically different when compared to that in the control group (Fig. 1).

*Obesity enhanced cardiac vascular remodeling via an imbalance of vasodilator and vasoconstrictor agent*

Finally, to evaluate the functional alteration induced by obesity, our results suggested a significant difference between the control and OB groups ($p < 0.01$). This alteration was accompanied by the downregulation of eNOS mRNA expression and the upregulation of ppET-1 mRNA expression in obese groups, particularly in the OB4 group ($p < 0.05$) (Fig. 2).

## DISCUSSION

Our results demonstrated that an HFD stimulates vascular remodeling mediated by the upregulation of inflammatory cytokines. It has been understood that obesity-induced low-grade inflammation as a result of the enlargement of adipocytes that leads to hypoxia and reactive oxygen species (ROS) development.[17,18] The circulating inflammatory mediators contribute to the development of cardiac inflammation in a time-dependent manner (Fig. 1). Abdominal white adipocyte tissue inflammation has also

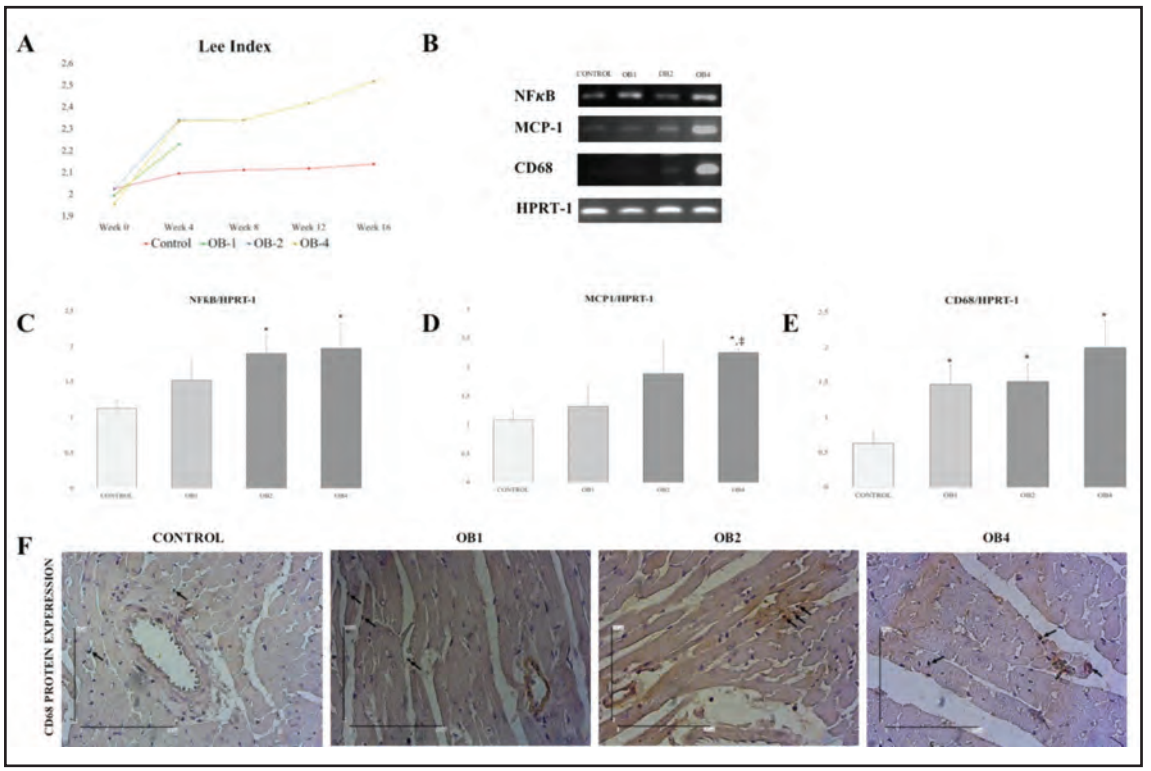


**Fig. 1:** Obesity associated with an increase in the level of inflammatory mediators. A. Bar chart of Lee Index. B. Representative figures of inflammatory mediators. C–E. Quantification of NF$\kappa$B, MCP-1, and CD68 mRNA expression. F. CD68 protein expression.

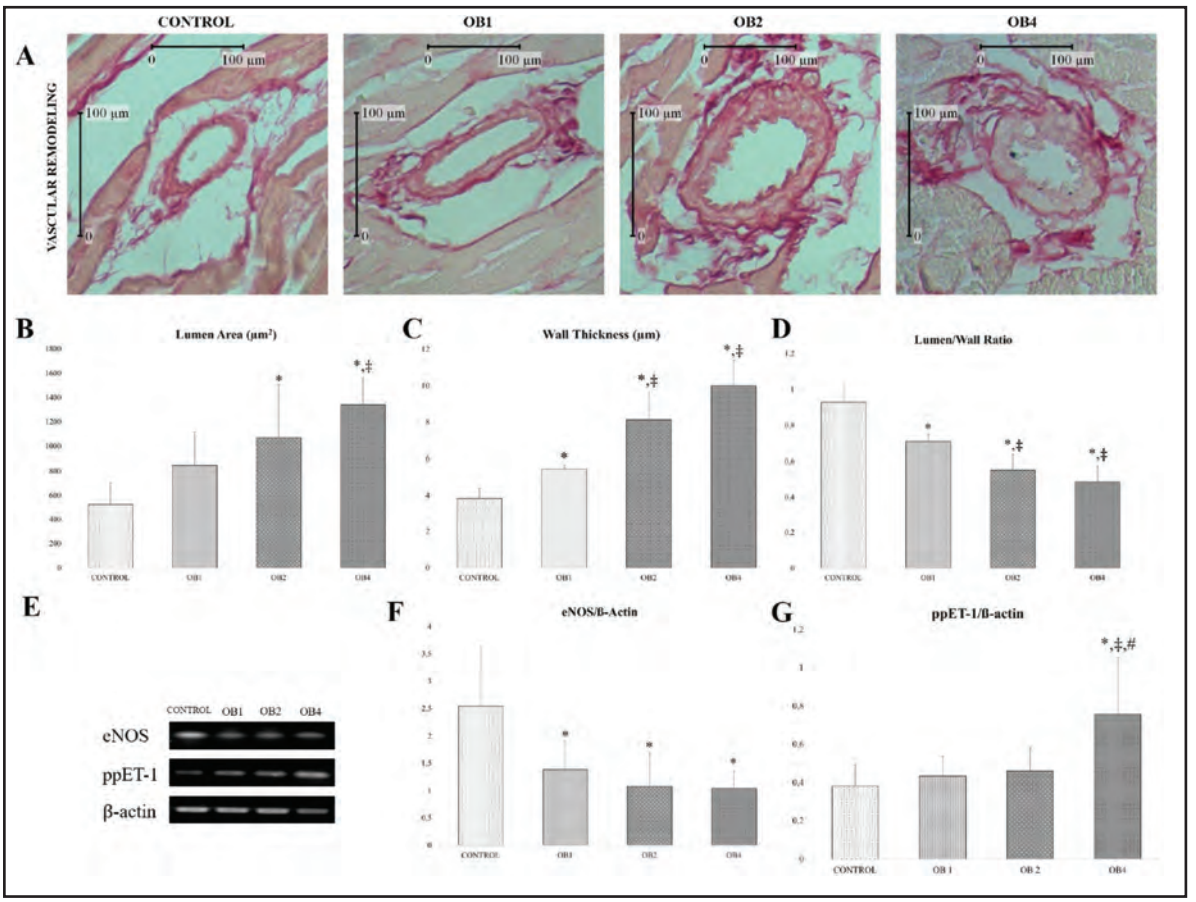


**Fig. 2:** Obesity-induced vascular remodeling. A. Representative figures of vascular remodeling by Sirius Red staining. B. Quantification of the lumen area. C. Quantification of the wall thickness. D. Quantification of the lumen/wall ratio. E. Representative figures of the eNOS and ppET mRNA expression. F. Quantification of the eNOS mRNA expression. G. Quantification of the eNOS mRNA expression.

been correlated with the development of cardiovascular diseases, such as atherosclerosis and diabetes mellitus type 2. The nuclear factor κB (NFκB) luciferase reporter assay activity in an HFD showed markedly higher activity when compared to that in the abdominal region of low-fat-diet-fed male mice.[19] In obese db/db mice, the blockade of NFκB alleviated the oxidative stress and improved the cardiac function.[18] The FFA secreted by adipocyte tissues are recognized by Toll-like receptor 2/4 (TLR2/4), which is highly expressed in the cardiac tissues of HFD-induced mice model. Downstream of TLR, the activation of MyD88, an adaptor protein, plays a crucial role in inducing pro-inflammatory cytokine through the phosphorylation of IκB, which eventually leads to nuclear translocation of NFκB and the production of pro-inflammatory genes expression, including MCP-1.[20–22] Hypoxia and oxidative stress promote cardiac inflammation through HIF-1α. HFD feeding enhances the upregulation of mRNA and protein HIF-1α in adipocyte tissues in a time-dependent manner.[23] The crosstalk between NFκB and HIF-1α has been well-documented. The proximal promotor of HIF-1α contained a NFκB binding-site at -197/-188 bp under hypoxia. This condition promotes an increase in the NFκB activity and enhanced inflammatory response.[24,25]

Saturated fatty acid *per se* induces the NLRP3 inflammasome activation, which leads to IL-1β release in macrophage cell culture in a dose-dependent manner.[26] Macrophage and adipocyte cells expressed an abundance of CCR2 under metabolic disorders. This increment is in accordance with the elevation of CCL2 or MCP1 in the adipose tissues of obese rodents.[27–29] The circulating MCP-1 extricated by adipose tissues were doubled in obese diabetic (db/db) mice model when compared to that in lean mice as a control. An elevation of circulating MCP-1 level was positively correlated with an elevation of BMI, waist circumference, IL-6, and HOMA and negatively correlated with the HDL-cholesterol level.[30] Our result suggests that an elevation of MCP-1 was demonstrated in HFD feeding for 4 months (Fig. 1). Higher MCP-1 level has been strongly correlated to the development of atherosclerosis.[30,31] It promotes the recruitments of monocytes into the subendothelial layers. After entering the subendothelial layers, monocytes differentiate into macrophages that produce foam cells of the fatty streak. The deletion of CCR2 has been correlated with the attenuation of macrophage accumulation in the atherosclerotic lesion in apoE null mice with HFD feeding.[32,33]

CD68 is a glycosylated type-1 transmembrane glycoprotein that is mostly expressed by macrophages, other mononuclear phagocytes, and endothelial cells mainly located in the endosomal compartment. Upon responding to the inflammatory stimuli, CD68 was upregulated, and it is demonstrated an ability to bind modified LDL, phosphatidylserine, and apoptotic cells.[34] Long-term inflammation accelerates an increase in M1 macrophage polarization, which in turn markedly increases the CD68 expression. Injection of inflammatory proteins, TNFα, demonstrated a significantly higher intimal plaque formation in accordance with an increase in the CD68 protein expression.[35] In addition, an excess of oxidative stress, DNA damage, high glucose, and ceramides present in a chronic state of obesity, can develop cellular senescence, which produces senescence-associated secretory phenotype (SASP). This SASP can stimulate further inflammation by secreting more IL- 6, TNF-α, MCP1, and other cytokines. With other cellular senescence effects such as extracellular matrix dysfunction and pathological angiogenesis, the accumulation of cells undergoing cellular senescence may lead to aging or age-related disorders, including heart failure and atherosclerotic diseases.[36]

Obesity is commonly accompanied by insulin resistance, the elevation of leptin, and an alteration of the renin-angiotensin system that may cause sodium retention resulting in increased fluid volume which increases markedly by dilatation of the artery. Although the vein is known as the capacitance vessel, increased fluid volume causes dilation of the arterial lumen under the condition of increased body fluid volume. The disturbing vasodilation effect of chronic inflammation may occur in a longer obesity period, which ideally occurs in 6 months, although the longest obesity period in this study was only 4 months.[37–39] However, our results demonstrated thickening of the vascular wall in the obese groups (Fig. 2). In response to the higher lumen area, the wall thickens to compensate for luminal enlargement in order to normalize the stress on the wall.[40] Other than compensating for the wall stress, an increased level of insulin may increase the arterial wall thickness through the direct trophic effect of smooth muscle cells, the generation of ROS, protein kinase C, and activation of NFκB, which stimulates the growth and proliferation of vascular smooth muscle cells.[41] Metabolic changes due to obesity enhances the downregulation of the PI3K/Akt pathway, resulting in a decrease eNOS mRNA expression in 2 months obese-prone Sprague–Dawley rats fed on a HFD.[42]

Deposition of foam cells may increase the vascular wall thickness, which then stimulates the proliferation of vascular smooth muscle cells. The proliferation of vascular smooth muscle is often associated with an imbalance of vasoconstrictor and vasodilator agents represented by the upregulation of ppET-1 mRNA expression and the downregulation of eNOS mRNA expression in long-term obesity. Long-term obesity shown by the OB4 group involved enhanced upregulation of the ppET-1 mRNA expression caused by increased oxidative stress and chronic inflammation.[43,44]

## CONCLUSION

Obesity associated with inflammation and vascular remodeling in the heart.


## ACKNOWLEDGMENTS

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# Gastric perforation in a 5-day-old infant: A case report

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## SUMMARY

**Gastric perforation in a new-born signifies a surgical emergency requiring immediate attention. Therefore, early diagnosis is expected to lead to a better prognosis. Any infant presenting with sudden and severe abdominal distention should be suspected of gastric perforation. Diagnosis is confirmed by pneumoperitoneum in the plain abdominal X-ray, including anteroposterior and lateral views. In this study, we report a case of a 5-day-old neonate, who was diagnosed with gastric perforation and accordingly proceeded with primary surgical repair.**

## INTRODUCTION

Gastric perforation in neonates is a serious and life-threatening condition associated with high morbidity and mortality rates. Spontaneous gastric perforation has been reported in approximately 1 in 2900 live births and accounts for 10-15% of all gastrointestinal perforation cases in neonates and children.[1,2]

Siebold in 1926 was the first to describe spontaneous gastrointestinal perforation. Stern, in his research, reported surgical repair, while Leger, in 1950, described the first successful repair of gastric perforation in neonates.[3,4,7] The causes of neonatal gastric perforation can be categorized into two groups: spontaneous/idiopathic or traumatic/iatrogenic. Spontaneous or idiopathic gastric perforation refers to that caused by unidentified underlying diseases and accounts for most cases. Meanwhile, iatrogenic gastric perforation case is usually caused by gastric tube insertion or intubation attempts.[2,9]

## CASE REPORT

A 5-day-old female infant presented with sudden abdominal distention since a day prior to her admission. Further history was taken from her parents, and we identified that her entire abdomen was distended and improved after the insertion of orogastric tube at the previous hospital. The frequency of her bowel movement found to be normal, ranging from 3 to 4 times a day with soft consistency. She had fever since abdominal distention and her abdominal skin appeared reddish on examination. The patient was referred from Walisongo General Hospital in Gresik and was treated with intravenous D10 (300 cc/24-h), cefo-sulbactam (150mg /12-h), and santagesik (30 mg/8-h).

Antenatal history revealed that the patient was born a second pregnancy that was routinely checked by a midwife. Antenatal ultrasonography (USG) was performed once during pregnancy by an obstetrician-gynecologist to present normal finding. Due to placenta previa, she was delivered by cesarean section at 38 – 39th week of gestation, with a birth weight of 3000 g and an APGAR score of 7 – 8.

The patient appeared lethargic and icteric (zone 2 on Kramer's scale) during her physical examination. The abdomen was found distended with reddish abdominal skin (Fig. 1A). Her bowel sounds decreased on auscultation, while the dullness of liver diminished on percussion. From rectal touché examination, we found that the tonus of sphincter ani collapse and mucosal surface was normal. Faeces, without blood, was found on the post-examination gloves. Urine output was 18 cc/2-h during the observation period at the emergency department (equal to 3 cc/kg/h).

The complete blood count test, result: hemoglobin 12.5 g/dL, WBC 3,980/uL, and platelets 139,000/uL. Her serum electrolytes levels were as follows: sodium 131 mEq/L, potassium 6.7 mEq/L, and chlorides 96 mEq/L. Her bleeding time was within normal limits. We performed plain thoracoabdominal X-ray or babygram as well as left lateral decubitus (LLD) imaging to reveal pneumoperitoneum, and the tip of the orogastric tube that appeared outside of the gastric contours, extending to the pelvic cavity. The LLD view revealed free intraperitoneal air (Fig. 1B).

Based on the results of her clinical examination and the subsequent workups, we suspected perforation of a holloworgan, and thus an emergency surgery was accordingly performed via explorative laparotomy for the primary repair of gastric perforation.

Prior to incision, 90 mg of intravenous cefazolin was administered as the surgery was considered susceptible to contamination. The surgical field was then disinfected, and the patient was draped with sterile surgical drapes. Upon transversal supraumbilical incision, air and faecal matters burst out when we reached the peritoneal cavity. Gastric perforation with a diameter of approximately 5 cm was identified at the posterior aspect of the gastric fundus near the greater curvature. Several minor tissues surrounding the stomach was detected in the slough. We also observed grade II-III intestinal adhesion. The peritoneal cavity lavage was accordingly performed. The minor tissues of the stomach were excised, and primary suturing of the perforated region was performed using 4.0 silk thread via simple interrupted and overhecting sutures. Further evaluation of the intestines including jejunum, ileum, and colon revealed no

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**Fig. 1:** A. The patient's abdomen appeared distended and reddish with dilated veins. B. Babygram revealed pneumoperitoneum and the tip of the orogastric tube appeared outside of gastric contours and the LLD view showed free intraperitoneal air.

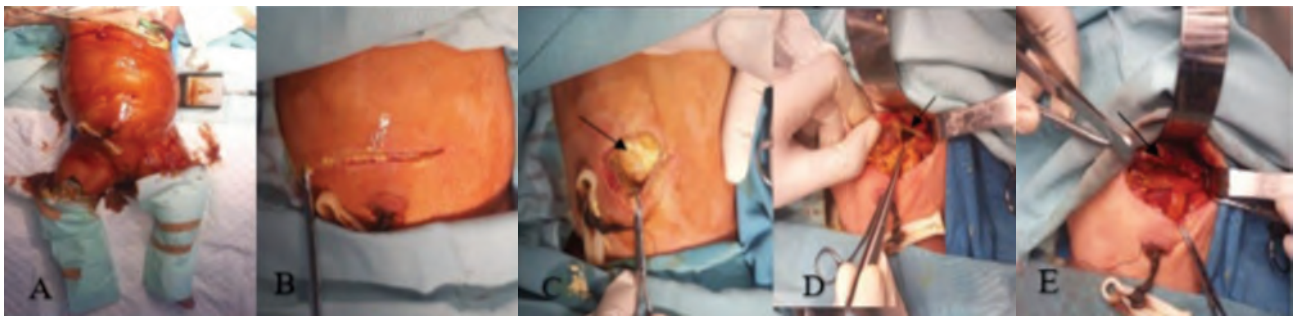


**Fig. 2:** A. Disinfecting the surgical field and draping the patient. B. Transversal supraumbilical incision. C. Air and fecal matters burst out when the peritoneal cavity was reached (arrow). D. Gastric perforation with a diameter of approximately 5 cm detected at the posterior aspect of gastric fundus near the greater curvature (arrow). E. Primary suturing using a 4.0 silk thread simple interrupted overhecting sutures (arrow).

abnormalities. Another peritoneal lavage was then performed, and the surgical incisions were closed. We did not perform any histopathological investigation.

The patient was admitted to the Neonatal Intensive Care Unit (NICU) for her post-operative care. Her respiration status deteriorated 8 h post-op, requiring mechanical ventilation as follows: PEEP 2, FiO2 35%, and the oxygen saturation was maintained >90%. She also went into a hypotensive state, but her hemodynamic status improved after resuscitation using intravenous saline, blood transfusion, and dopamine. Her abdomen was soft on palpation, showing no distention and discoloration. Total parenteral nutrition was administered via central vein catheter.

On the second day post-op, the patient was hemodynamically stable, and the ventilator parameters were reduced to the following: PEEP 3, FiO2 25%, and oxygen saturation > 90%. She was weaned-off the ventilator on the fifth day post-op when her condition had clearly improved. She was able to breath spontaneously supported by a nasal cannula. On the tenth day post-op, she resumed breastfeeding with no evidence of vomiting or bowel obstruction. She was finally discharged after 25 days of hospitalization, and we found that she even gained weight.

## DISCUSSION

Neonatal gastric perforation is classified as either spontaneous (idiopathic) or traumatic. However, in most cases, the aetiology is most likely multifactorial.[3,5] Sudden and unexplained abdominal over-distention that leads to organ perforation is regarded as a spontaneous one. Most cases presented in the first 7 days of life with various clinical manifestation.[1,6]

Chieh-Mo Lin et al. outlined that gastric acidity in a newborn is exceptionally high during the first week of life. While this does not indicate potential causality, it can possibly contribute to gastric perforation.[8] Another study by Chen et al. analyzed that preterm neonate with gastric perforations is at 4.21-times higher risk of mortality than full-term neonates.[4]

Infants with gastric perforation may present with respiratory distress, hemodynamic instability, and even symptoms suggestive of shock such as hypothermia, cyanosis, poor peripheral perfusion, and diminished urine output. The abdomen may also appear distended in a short amount of time, which may be a sign of peritoneal irritation.[4] In our case report, the patient was a 5-day-old infant who presented with sudden abdominal distention since 1 day before admission. We also recorded a history of fever that started at

the same time has the onset of distention. On clinical examination, marked abdominal distention, reddish abdominal skin, with dilated veins were observed. Bowel sounds were decreased on auscultation, and no liver dullness was found on percussion. Urine output upon observation at emergency department was 18 cc/2-h (equal to 3 cc/kg/h).

Laboratory studies are performed to aid diagnosis in gastric perforation cases, such as blood cultures, leukocytes count, haemoglobin, haematocrit, platelet count, electrolytes profile, and blood gas analysis.[1,5] In this patient, we observed workup results as follows: haemoglobin 12.5 g/dL, WBC3,980/uL, and platelet 139,000/uL. Serum electrolytes level were as follows: sodium 131 mEq/L, potassium 6.7 mEq/L, and chlorides 96 mEq/L.

Plain abdominal X-ray was also performed to aid diagnosis, which showed massive pneumoperitoneum indicated by the presence of air under diaphragm that extended laterally as well as subcutaneous emphysema, pneumoscrotum, ascites, or even the tip of oro- or nasogastric tube that extends beyond the gastric contours.[2,3,6] The plain thoracoabdominal X-ray or babygram as well as LLD view X-ray of this patient showed pneumoperitoneum and the tip of orogastric tube that appeared outside of the gastric contours, extending to pelvic cavity.

The initial management included hemodynamic stabilization by administering bolus intravenous fluid or blood transfusion, the administration of broad-spectrum antibiotic in case of infection, and primary peritoneal drainage to achieve better clinical outcome.[1,2,7,8] We performed early resuscitation on our patient but no primary peritoneal drainage, since emergency surgery could be arranged immediately, and the patient was placed in an optimal condition prior the surgery.

The main therapy for gastric perforation is surgical exploration, which is started via a transversal supraumbilical incision and evacuation of the peritoneal fluid, followed by exploration of the perforation site. When gastric perforation is not detected, careful exploration of the gastroesophageal junction, duodenum, small intestines, and large intestines must be performed. If gastric perforation is detected, which is usually near the greater curvature, then it must be closed in one or two layers and can be strengthened using an omental patch. After repairing the perforation, the peritoneal cavity lavage is usually performed using a warm saline solution.[5,7,8] In this patient, we performed explorative laparotomy and primary repair on the site of gastric perforation. No histopathological investigation was performed in this study and hence we could not identify the cause of the perforation.

Supportive care and resuscitation measures were continued post-op, broad-spectrum antibiotic administration, gastric acid suppression therapy, total parenteral nutrition, as well as gastric decompression. Enteral nutrition was started after the patient's condition had stabilized. Several of surgeons perform contrast studies prior to initiation of enteral nutrition.[7,8,16] The present patient was admitted to the (NICU) for post-operative care. Her respiration status deteriorated 8 h post-op and required mechanical ventilation. She also went into a hypotensive state, hence we performed resuscitation using intravenous saline, blood transfusion, and dopamine. Total parenteral nutrition was administered via the central vein catheter. The next day, the patient's condition had improved, and she was weaned-off the ventilator on her fifth day post-op. On the tenth day post-op, she resumed breastfeeding and was finally discharged after 25 days of hospitalization.

The clinical factor associated with poor prognostic outcome included sepsis, metabolic acidosis, and hyponatremia. Early diagnosis and identification of perforation and appropriate supportive treatment was deemed essential for the best possible clinical outcome.[1,4,9]

## CONCLUSION

Gastric perforation in newborns is a serious and life-threatening condition. Newborns, especially those of premature or low birth weight, who present with progressive abdominal distention with or without pneumoperitoneum should be suspected of gastric perforation.

Initial management of gastric perforation includes early diagnosis, resuscitation, stabilization, and surgical exploration. Early resuscitation can serve as a primary management for neonates to immediately stabilize their general condition prior to the main surgery, in order to achieve the best possible clinical outcome.

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# Increased CD4/CD8 T-cell ratio : A risk factor for mortality in patients with coronavirus disease 2019

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**ABSTRACT**

**Introduction: Although CD4 and CD8 T-cells are the main subset of T-lymphocytes, their roles in COVID-19 infection and severity remain unclear. This study aimed to determine the role of increased CD4/CD8 T-cells ratio as a risk factor for cases of 28-days in-hospital mortality in COVID-19 patients.**

**Materials and Methods: This study employed a prospective cohort design. Inclusion criteria were confirmed COVID-19 cases with a positive polymerase chain reaction report. CD4 and CD8 T-cells absolute counts were measured by flow cytometry. The CD4/CD8 ratio was calculated by dividing the absolute count of CD4 by that of CD8 T-cells.**

**Results: A total of 85 subjects were followed for 28 days. The mean age of the subjects was 52.64 years, and majority of them were females (51.8%). Twenty-eight (32.9%) subjects died within 28 days of follow-up. Receiver operating characteristics analysis obtained an area under curve of 0.68 with the cut-off value 1.26 with $p$ = 0.005. Kaplan–Meier's analysis obtained Hazard Ratio 2.91 (95%CI 1.377–6.161; $p$ = 0.0052).**

**Conclusion: Subjects with an increase in CD4/CD8 T-cells ratio >1.26 had a 2.91-times risk of 28 days in-hospital mortality.**

**KEYWORDS:**
*CD4/CD8 T cells ratio, T cell lymphocyte, COVID-19, mortality risk, laboratory testing, SARS COV-2*

## INTRODUCTION

The pathophysiological process of SARS COV-2 infection is not as yet clearly understood, including the underlying death mechanism in COVID-19 patients, which is suspected to be due to the occurrence of a cytokine storm that has a direct impact on cardiac cell damage.[1] Several cytokines whose level increases under the condition include INF-γ, IL- 1β, IL-6, IL-8, IL-12, and TNF-α, which were known to cause acute respiratory distress syndrome (ARDS).[2] Another hypothesis suggests the occurrence of bacterial sepsis as a secondary infection.[3] Several studies have shown traces of SARS-CoV-2 virus in cases of death occurring in several hematological laboratory parameters such as neutrophil to lymphocyte ratio, relative lymphocytopenia, and platelet to lymphocyte ratio through a cytokine storm mechanism.[2,4,5]

Lymphocytopenia was a common finding in COVID-19 patients that may be a critical factor associated with disease severity and mortality.[6] The mechanism of lymphopenia could be explained by several possible theories, including (i) direct viral infection of lymphocytes, (ii) viruses infecting the lymphatic organs, (iii) apoptosis of lymphocytes due to a cytokine storm, and (iv) inhibition of lymphocyte metabolism that suppresses their proliferation.[5] The development mechanism of this laboratory finding remains unclear. The examination of the CD4/CD8 T-cell ratio will provide an overview of the response of the T-lymphocyte subset. This ratio is often employed in monitoring patients infected with the human immunodeficiency virus (HIV). A CD4/CD8 T-cell ratio <1 indicates a weakened immune system as the number of CD4 T-cells decreases and their proportion is replaced by those of CD8 T-cells. The decrease in this ratio illustrates the weak level of patient immunity resulting in an increased risk of infection.[7] On the other hand, an increase in the ratio of CD4/CD8 T-cells indicates an increase in the rapid immune response against viruses.

This study aimed to determine the role of CD4/CD8 T-cell ratio increment as a risk factor for the occurrence of death in COVID-19 patients.

## MATERIALS AND METHODS

This study employed a prospective cohort design. The inclusion criteria were confirmed adult COVID-19 patients (based on the results of the COVID-19 polymerase chain reaction [PCR] examination) and having received treatment at Dr. Sardjito Hospital (SH), both as an inpatient or an outpatient. Patients who were pregnant and who had history of impaired immune system were excluded. Subjects were recruited from December 2020 to March 2021, on the first day of confirmed COVID-19 based on their PCR report. Patients agreed to participate in the study by signing an informed consent form either by themselves or their entitled family member. This research received ethical approval from the ethics committee of the Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada with the approval number KE/FK/0398/EC/2021.

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**Table I: Subject characteristics**

| Parameter | n (%) |
|---|---|
| Age (year), Median (range) | 57 (18–83) |
| Male | 41 (48.2) |
| Female | 44 (51.8) |
| Severity | |
| Mild | 9 (10.6) |
| Moderate | 28 (32.9) |
| Severe | 47 (55.3) |
| Critical | 1 (1.2) |
| Length of stay (days), median (range) | 12 (2–67) |

**Table II: Initial laboratory parameters at hospital admission**

| Parameter | Mean (± SD) / Median (range) |
|---|---|
| Hb (g/dL) | 11.33 (± 2.51) |
| Leukocyte count (x$10^3$ cell/µL) | 8.54 (2.11–31.37) |
| Lymphocyte count (x$10^3$ cell/µL) | 1.26 (± 0.65) |
| Lymphocyte percentage (%) | 13 (1.6–37.6) |
| CD4 cell count (cell/µL) | 254 (13–1066) |
| CD4 cell percentage (%) | 30.66 (3.61–68.96) |
| CD8 cell count (cell/µL) | 222 (21–1049) |
| CD8 cell percentage (%) | 23.96 (2.65–52.76) |
| CD4/CD8 ratio | 1.27 (0.26–5.72) |

**Table III: Clinical outcome and laboratory examination results**

| Parameter | Survivor N = 57 | Dead N = 28 | *p* |
|---|---|---|---|
| Age, median (min–max) | 51 (18–72) | 63 (35–83) | <0.0001 |
| Male, n (%) | 22 (53.7) | 19 (46.3) | 0.0117 |
| Female, n (%) | 35 (79.5) | 9 (20.5) | |
| Severity | | | <0.001 |
| Mild, n (%) | 7 (77.8) | 2 (22.2) | |
| Moderate, n (%) | 28 (100) | 0 (0) | |
| Severe, n (%) | 22 (53.2) | 25 (46.8) | |
| Critical, n (%) | 0 (0) | 1 (100) | |
| Length of treatment (days), median (min–max) | 13 (10–67) | 10 (2–27) | 0.0241 |
| Laboratory parameter | | | |
| Hb (g/dL), mean ± SD | 11.42 (± 2.63) | 11.24 (± 2.45) | 0.8234 |
| Leukocyte count (x$10^3$ cell/µL), median (min–max) | 8.03 (2.11–31.37) | 10.15 (3.69–30.03) | 0.0787 |
| Lymphocyte count (x$10^3$ cell/µL), mean ± SD | 1.41 (± 0.69) | 0.99 (± 0.47) | 0.0075 |
| Lymphocyte percentage (%), median (min–max) | 15.2 (4.5–37.6) | 7.3 (1.6–32.9) | 0.0010 |
| CD4 cell count (cell/µL), median (min–max) | 340 (39–1066) | 219 (13–869) | 0.0033 |
| CD4 percentage (%), median (min–max) | 30.32 (9.26–68.96) | 31.45 (3.61–43.98) | 0.8959 |
| CD8 cell count (cell/µL), median (min–max) | 255 (62–902) | 109 (21–1049) | 0.0016 |
| CD8 percentage (%), median (min–max) | 26.87 (7.81–51.39) | 18.65 (2.65–52.76) | 0.0021 |
| CD4/CD8 ratio, median (min–max) | 1.18 (0.35–5.72) | 1.51 (0.26–3.95) | 0.0136 |

Blood samples were obtained from the selected subjects (3 mL of the blood in an EDTA tube) within 24 hours of confirmed COVID-19 tests. Complete blood count analysis was performed with Sysmex XN-1000. Blood samples were then examined by flow cytometry using FACS Canto II with the lyse wash principle. The samples were incubated with CD3 FITC, CD4 PE, and CD8 FITC reagents for 15 min in a dark room and read within 2 hours of incubation. The parameters measured included complete blood count and CD4 and CD8 cells count.

The recruited subjects underwent treatment according to the standard of care at SH. The outcome measured in this study was death within 28 days of hospitalization (28 days in-hospital mortality). Follow-up was conducted for subjects for 28 days after being confirmed positive for COVID-19 and the outcome of death was recorded. The outcomes were categorized as survivors or dead. The survivors were subjects who were declared recovered and discharged from SH or being treated within 28 days. Meanwhile, the subjects who died within 28 days of treatment were categorized as dead.

Categorical data was presented in the form of frequency and proportion. Data with normal distribution are presented in the form of mean ± standard deviation (SD). Data with abnormal distribution are presented in the form of the

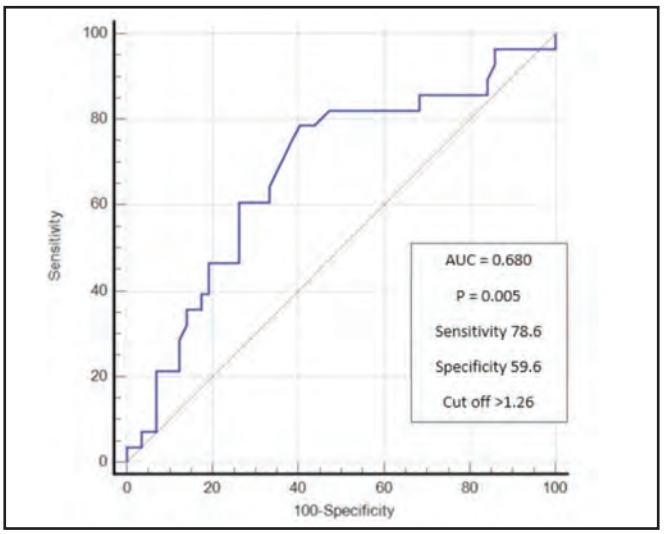


**Fig. 1:** ROC analysis of CD4/CD8 ratio toward mortality outcome in 28 days of treatment.

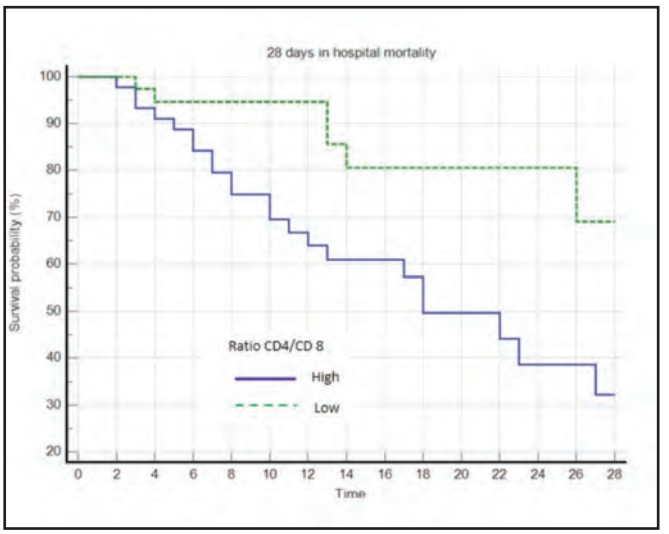


**Fig. 2:** Kaplan–Meier curve analysis of survival with CD4/CD8 ratio toward mortality outcome in 28 days of treatment.

median (min-max). Statistical analysis was performed by the Receiver Operating Characteristics (ROC) curve to obtain the cutoff value, and Kaplan–Meier analysis was performed to assess the prognosis of death. All statistical analyzes were performed using the Medcalc software, and $p < 0.05$ was considered to indicate statistical significance.

## RESULTS

A total of 85 subjects were included in this study. The mean age of the subjects was 52.64 years and majority of the subjects were women (51.8%). A total of 47 subjects (55.3%) were admitted to the hospital with severe conditions. The length of stay at the hospital varied between 2 and 67 days (Table I).

Fifty subjects (65.8%) had a lymphocyte count of <18% with an overall median of 13%. The median percentage of CD4 T-cells was 30.66%, while that of CD8 T-cells was 23.96%. The overall CD4/CD8 T-cell ratio ranged widely from 0.26 to 5.72 (Table II).

Twenty-eight subjects (32.9%) died within 28 days of hospitalization. The fastest length of treatment was 2 days in the dead group and 10 days in the survivor group. There was a significant difference between the groups for the ratio of CD4/CD8 T-cells, with the median in the dead group being significantly higher ($p < 0.05$) (Table III). ROC analysis for the ratio of CD4/CD8 T-cells obtained an area under curve of 0.68, with a cutoff value of 1.26 and $p = 0.005$ (Figure 1). A total of 45 subjects (52.94%) showed a CD4/CD8 T-cell ratio >1.26.

Statistical analysis: proportion difference test using Chi-squared test, difference test with normal distribution using independent t-test, difference test with abnormal distribution using Mann-Whitney test.

The analysis was continued with Kaplan–Meier, obtaining a hazard ratio of 2.91 (95% CI 1.377–6.161; $p = 0.0052$), indicating that the subjects with an increased CD4/CD8 T-cell ratio of >1.26 had a 2.91-times risk of death in 28 days of treatment (Fig. 2).

## DISCUSSION

The pathophysiology of COVID-19 in causing cytokine storms remains unclear. The involvement of T-cells in case progression also remains unclear. Preliminary evidence suggests that lymphocytopenia supports the aggravation of the disease.[8] The results of this study showed a similar finding, suggesting that the percentage of lymphocytes decreased in the dead group. These findings confirm the role of T-cells in the pathophysiology of COVID-19.

Previous studies have suggested a correlation between the proportion of a subset of T-cells and disease severity. Lower levels of CD4 and CD8 T-cells were associated with the severe and critically severe groups.[9] The results of this study showed similar findings in the dead group with significant differences.

T-cells, mainly consisting of CD4 and CD8 T-cells, play a major role in the adaptive immune system. T-cells act as a mediator of antibody responses produced by B cells.[10] Pathogenic exposure to CD4 T-cells either directly or mediated by dendritic cells trigger the differentiation of T-cells into their subsets, which then produce interleukin 12. This response ultimately triggers antibody production.[11] In terms of the response to infection with the SARS-CoV-2 virus, this exposure triggers the emergence of antibodies to the virus.[12,13] Likewise, CD8 T-cells that act as cytotoxic can eliminate cells infected with the SARS-CoV-2 virus through the abnormal recognition of MHC class 1.[14,15] The findings in this study indicate a fatigue response of CD4 and CD8 T-cells in patients with aggravation, leading to death.

Deaths that occur in COVID-19 patients are suspected to occur through 2 mechanisms, namely cytokine storm and secondary infection leading to bacterial sepsis.[3,16,17] The results

of this study demonstrated a quantitative decrease in the immune response in the dead group. An exhausted immune system is described by a decrease in the number of CD4 and CD8 T-cells. Although this study did not describe the kinetics of the two subsets of T-lymphocytes, the significant differences between the two groups could justify this point. Past studies conducted on infants of mothers confirmed positive COVID-19 showed normal levels of CD4 and CD8 T-cells in patients with negative results.[18] This finding indicates the kinetics of decreasing levels of CD4 and CD8 T-cells along with the fatigue level of the immune response.

The ratio of CD4/CD8 T-cells is a parameter that can indicate the function of adaptive immunity. This ratio is often used in monitoring patients with HIV to determine the condition of immunity and susceptibility to opportunistic infections.[7,19] In the case of COVID-19, this parameter could be used as a reference to assess the risk of secondary infection. Another study has indicated no significant difference in the CD4/CD8 T-cell ratio between different levels of severity.[9] This study reported that an increase in the ratio of CD4/CD8 T-cells >1.26 had a significantly higher risk of death. This mechanism is suspected in line with the increased risk of secondary infection.

## CONCLUSION

This study concludes that CD4/CD8 T-cell ratio >1.26 increases the risk of death of COVID-19 patients by 2.91-times. The limitations of the present study include that it did not measure the kinetics of the CD4/CD8 T-cell ratio and the examination of secondary infections in the subjects. Kinetic data is expected to assist in the analyses of the changes occurring in patients with varying clinical status as well as to confirm the current findings.


## ACKNOWLEDGMENT

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# Unusual radiological findings of pediatric jejunojejunal intussusception: A case report

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## SUMMARY

**Intussusception is a common cause of intestinal obstruction in children, especially in those of age <5 years. The typical signs and symptoms of this condition is colicky abdominal pain, bloody mucous stool, and palpated abdominal mass, with a classic target sign finding on abdominal ultrasound. In older children, the symptoms may vary, which necessitates investigation of the cause of intussusception, as it is often caused by a pathologic lead point. We report here the case of a 14-year-old girl with total bowel obstruction, hematochezia, a very dilated reverse C-shaped bowel loop, and intestinal pneumatosis on abdominal X-ray. During laparotomy, we detected jejunojejunal intussusception caused by jejunal polyp. After bowel resection and anastomosis, the patient recovered well and had no other events during follow-up.**

## INTRODUCTION

Intussusception is a condition in which one segment of the intestine telescope inside of another to cause obstruction, and it most commonly recorded in children aged 6 months to 3 years. Although it is mostly idiopathic, 4% of all cases may be caused by a pathological lead point (PLP) that is usually found in children of age >5 years or in adolescents.[1] Intestinal pneumatosis (IP) is described as an abnormal intramural gas of the digestive tract. The presentation of PI may suggest the occurrence of life-threatening conditions, such as gastrointestinal perforation, ischemic bowel, and bowel necrosis.[2] We report here the case of a 14-year-old girl with bowel obstruction and a very dilated reverse C-shaped bowel loop and IP on abdominal X-ray caused by jejunojejunal intussusception.

## CASE REPORT

A 14-year-old girl visited the Emergency Department (ED) of Soetomo General Hospital, Surabaya, Indonesia with intermittent colicky pain for the last 6 days, especially at the epigastric and left abdominal areas. The pain lasted for 5–10 min at each episode. Nausea and vomiting also occurred, eventually progressing to bilious vomiting. The patient did not have any bowel movements for the past 6 days and admitted to enable her to pass gas. At the ED, she had hematochezia with no mucous. Previously, she was admitted to a secondary hospital and then sent to our hospital.

She was weak and somnolent at the time of admission, tachycardic (172 beats per min) with raised body temperature to 38.3°C. The nasogastric tube production was bilious, 300 mL within 12 hours. There was no abdominal distention, bowel contour, or bowel movement. The bowel sound was decreased with muscular guarding. Digital rectal examination revealed collapsed ampulla, no mass, with fresh colored blood on the gloves. Laboratory findings were normal, but her C-Reactive Protein (CRP) level was raised to 27.2 mg/L (normal value: <6 mg/L). Central venous catheter insertion, fluid resuscitation, and broad-spectrum antibiotic administration were accordingly performed. A plain abdominal X-ray showed a reverse C-shaped dilated bowel with massive IP (Fig. 1). Further abdominal CT scan study revealed bowel obstruction due to jejunojejunal intussusception.

We performed exploratory laparotomy and found bloody peritoneal fluid and jejunojejunal intussusception with necrotic intussusception of the bowel, which could only be partially released. The intussusceptum was also necrotic with multiple perforations. We resected 48 cm of the jejunal segment from 10-cm distal to the Treitz ligament and performed end to end jejunojejunal anastomosis. After resecting, the bowel was opened, and we detected a jejunal polyp. We palpated the rest of the bowel and found no other polyp.

Histopathological examination revealed ulcerated jejunum with a wide ischemic and bleeding site, most of which were filled with lymphocytes, histiocytes, erythrocytes extravasation, and infarcted polyp. Multiple trapped air bubbles were found in the submucosa (Fig. 2). The polyp could not be evaluated further because it was probably already necrotic for quite some time, albeit there was no malignant cell.

After surgery, the patient has put nil per month for 2 days due to postoperative paralytic ileus, and oral feeding was started on the 3rd postoperative day. She received 3rd generation of cephalosporin and analgesics. The patient recovered uneventfully after 5 days of recovery time. She recovered well and, at 2 weeks of follow-up, the wound healed nicely. She had no gastrointestinal symptoms at 6 months and 2 years follow-up.

## DISCUSSION

Intussusceptions are a common cause of acute abdomen in

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**Table I: Conditions associated with pneumatosis intestinalis**

| | | |
|---|---|---|
| **Pulmonary**<br>Asthma<br>COPD<br>Emphysema<br>Bronchitis<br>Cystic Fibrosis | **Drug Induced**<br>Corticosteroids<br>Chemotherapy agents<br>Lactulose<br>Sorbitol<br>Chloral hydrate | **Organ transplantation**<br>Bone marrow e.g. for leukemia<br>Kidney, Lung, Liver<br>Graft versus host |
| **Gastrointestinal**<br>IBD<br>Diverticulitis<br>Colitis<br>Necrotizing enterocolitis<br>Enteritis<br>Toxic Megacolon<br>Appendicitis<br>Intestinal Obstruction<br>Bowel stenosis<br>Adynamic Ileus<br>Malignancy<br>Peptic Ulcer<br>Celiac sprue | **Infectious**<br>HIV and AIDS<br>Virus (CMV, rota-, adeno-, varicella- zoster virus)<br>Candida albicans<br>Mycobacterium tuberculosis | **Autoimmune and systemic**<br>Lupus variants<br>Polymyositis<br>Dermatomyositis<br>Polyarteritis nodosa<br>Scleroderma<br>Sacroidosis |
| **Vascular**<br>Mesenteric vascular disease Intestinal infarction and ischemia | **Iatrogenic**<br>Blunt abdominal trauma Endoscopy<br>Postsurgical intestinal anastomosis<br>Jejunoileal bypass<br>Barium enema<br>Enteric tube placement PEEP ventilation | **Idiopathic (primary)** |

**Fig. 1:** (left) abdominal X-ray showed reverse C shaped dilated (yellow arrow) bowel and multiple IP ;(right) abdominal CT scan revealed jejunojejunal intussusception (red arrow) with dilated proximal duodenojejunum.

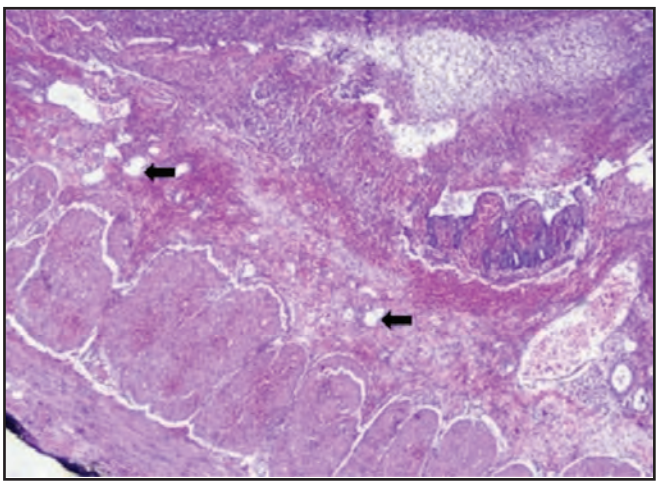

**Fig. 2:** Air bubbles (black arrow) trapped in the submucosal area (HE staining).

children. In our case, the patient presented with acute abdominal pain, a sign of bowel obstruction, and rectal bleeding, which should have raised a high index of suspicion for a life-threatening condition due to bowel strangulation. After rapid resuscitation, subsequent acute abdomen imaging studies were performed without delaying the definitive surgical treatment.

The abdominal X-ray revealed large reverse C-shaped bowel consistent with the dilated third portion of the duodenum, duodenojejunal junction, and proximal jejunum. The fixed retroperitoneal located distal duodenum, traction from the Treitz ligament, and narrow proximal jejunal mesentery made this reverse C-appearance possible. At the end of the reverse C-shaped bowel, we detected soft tissue mass opacity indicating intussuscepted bowel. There were also multiple linear and cystic-shaped lucency on the wall of proximal dilated part of the duodenum and jejunum, and the also in the walls of the jejunal intussusception, which was consistent with IP.

Computerized Tomography (CT) imaging is a common examination modality performed to detect IP as well as other signs of digestive pathology. The absence of bowel enhancement is a specific finding associated with ischemia that occurs during the late stage. IP with the associated absence of bowel enhancement, bowel wall thickening, mesenteric fat stranding, and ascites are prominent findings that indicate surgical situation.[2] In this case, the abdominal CT scan confirmed the X-ray findings, showed dilatation of the stomach, duodenum, and proximal jejunum. The intussuscepted bowel mass was also clearly depicted at the distal portion of the reverse C-shaped bowel. The heterogenous hypodensity at the proximal and medial portion of the C-shaped bowel may constitute the overlapping intraluminal gas, bowel wall edema, and multiple IP.

A massive intramural gas detected in this patient was considered as a rare finding in intussusception. It may appear in the intramural layer in a cystic, bubbly, curvilinear, or linear shape. In general, IP is classified into 2 types, primary and secondary IP3. Primary IP, found in approximately 15% of all IP cases, is a benign condition that is usually asymptomatic and caused by respiratory diseases, systemic diseases, after organ transplants, pathological process, endoscopic procedures, immunological imbalance, mucosal disruption, and other intraabdominal pathology. The management of primary IP depends on the cause and, not every case requires surgical management.[1,4] On the other hand, secondary IP is described as an air collection, forming a linear pattern that reflects on a pathological condition. Pear, in 1998, divided the cause of IP into 4 big groups: bowel necrosis, mucosal disruption, increased mucosal permeability, and pulmonary disease; while St. Peter divided IP based on pathology that reflected the origin of the gas: intraluminal gastrointestinal gas, bacterial gas, and pulmonary gas.[5] Various conditions associated with IP are described in Table I.

In our case, the IP was secondary, probably due to bowel mucosal disruption, caused by both leaked intraluminal gastrointestinal gas and gas-forming bacteria due to bacterial translocation after a long period of bowel obstruction that leads to distention and ischemia.

Intussusception in an older child is usually caused by PLP. It is different from the idiopathic intussusceptions that usually occur at ileocolon, the predilection of intussusceptions with PLP are at the caecocolic (2.5%) and jejunojejunal (2.5%).[1] In this patient, we detected a jejunojejunal intussusception caused by a jejunal polyp. Although rare, the jejunal polyp is often recorded as an intussusception caused in various ages, arising only from an obstruction to atresia.[6–8]

Some past studies have reported solitary jejunal polyp in association with Peutz Jegher syndrome, but, in this case, we detected no signs of this syndrome such as dark skin freckling at the mouth, eye, nostril, or anus, and other polyps along the gastrointestinal tract.[6,7] Other causes included juvenile polyp, adenocarcinomatous polyp, and inflammatory fibrous polyp.[9,10] In this patient, due to an old ischemic cell, the pathologist only described an infarcted polyp, but could not differentiate the type of the polyp.

Treatment for IP is mostly performed conservatively unless signs of peritonitis or strangulation are detected. Cause of IP that can be managed medically: autoimmune disease, vascular disease, or drug-induced IP. In case of doubt, surgery decision making should depend on several points; concomitant critical CT findings (mesenteric ischemia, bowel obstruction, bowel perforation, portal venous gas), critical laboratory findings (Leukocyte/inflammatory marker increase, lactic acid increase, acidosis), conspicuous physical examination (muscle guarding, peritonitis, and bowel sounds), and past medical history and its medications. The decision to perform surgery should be tailored to the clinical conditions of patients and supported by meticulous examination.[5]

The ideal follow-up should be based on the probable diagnosis of the polyp, but the limitation, in our case, is that the histopathology of the polyp was inconclusive due to old infarction and bleeding. As it might have been an isolated juvenile polyp, we considered it as a benign polyp that did not necessarily need further evaluation.

## CONCLUSION

IP can be a sign of life-threatening conditions in children. Although it is most commonly seen in neonates with necrotizing enterocolitis, other pathology such as intussusception, gastrointestinal perforation, bowel ischemia, and necrosis must be considered seriously in older children.

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# Risk factors for failure of hydrostatic reduction in children with intussusception in Hasan Sadikin General Hospital

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## ABSTRACT

**Introduction: Intussusception is a medical emergency caused by proximal insertion of the intestinal segment to its lumen, which results in ischemia, necrosis, and sepsis-associated mortality in pediatric patients. Intussusception is managed mainly by surgical reduction; hydrostatic reduction is a noninvasive alternative with lower risk of complications. The study was aimed to analyze the risk factors for the failure of hydrostatic reduction in children with intussusception at the Hasan Sadikin General Hospital. Materials and Methods: The medical records of children diagnosed with intussusception and treated with hydrostatic reduction during January 2010 and September 2019 were included. Variables of the study included age, sex, onset of symptoms, and outcome. Logistic regression analyses were performed to determine the significance and strength of correlation on the included characteristics with outcomes of hydrostatic reduction in the population. The $p < 0.05$ was deemed significant.**

**Results: There were a total of 56 children with intussusception who were treated with hydrostatic therapy during the study period. The failure rate of hydrostatic therapy was 83.9%. Age, sex, onset of symptoms, and location of intussusception were not significantly associated with the failure of hydrostatic reduction ($p > 0.05$). Dehydration was the only symptom significantly associated with the failure of hydrostatic reduction (OR 16.80; $p = 0.001$).**

**Conclusion: Dehydration is significantly associated with the failure of hydrostatic reduction in children with intussusception.**

**KEYWORDS:**
*intussusception, dehydration, risk factor, pediatric*

## INTRODUCTION

Intussusception is a potentially life-threatening medical emergency caused by the insertion of the proximal portion of the intestine into the distal lumen of the intestine, which causes obstruction, followed by ischemia and subsequent intestinal necrosis in pediatric patients. Morbidity and mortality may occur due to sepsis related to intestinal necrosis; this condition is associated with higher morbidity and mortality in cases of delayed diagnosis and/or treatment.[1–6] Fatalities associated with delayed diagnosis and/or treatment in children with intussusception has been reported to reach up to 20% in Indonesia.[7]

Intussusception requires immediate surgical reduction to treat; less-invasive alternative that may be utilized in treating intussusception in children is through hydrostatic reduction.[8,9] Management using hydrostatic reduction was associated with lower complication rates when compared to conventional surgical reduction.[9,10] The cure rates for hydrostatic reduction in previous studies were noted to be similar to that of conventional surgical reduction.[10] This study was aimed to analyze the variables correlated with the failure rate of hydrostatic reduction of pediatric patients with intussusception treatment in Hasan Sadikin General Hospital (HSGH).

## MATERIAL AND METHODS

An analytical study with retrospective data was performed in the HSGH. Data was collected from medical records and consisted of pediatric intussusception cases treated with hydrostatic reduction during January 2010 and September 2019. The inclusion criteria for the study were as follows: patients aged <18 years, diagnosed with intussusception, and treated with hydrostatic reduction at the HSGH. The patients were excluded from the study if they had incomplete medical record or had refused treatment. Consecutive sampling was performed in this study.

Dependent and independent variables in this study were collected from the medical records for children with intussusception who were treated at the HSGH between January 2010 and September 2019. The dependent variable of this study was the outcome of hydrostatic reduction. Independent variables of this study included age, sex, onset of symptoms (until management with hydrostatic reduction), location of intussusception, symptoms, and outcomes. The patients were grouped into 3 age groups: 0–12 months; 13 months–3 years; and >3 years. Onset to management was defined as time (in hours) from the first reported symptoms to receiving treatment for intussusception. Locations of intussusception were defined into ileoileal, ileocolic, ileocolocolic, ileocecal, and colocolic. The symptoms were defined as the chief complaint found during the initial presentation in the emergency department. The assessment of the outcome was defined postoperatively; unsatisfactory

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**Table I: Baseline characteristics**

| Variables | N = 56, count (%) |
|---|---|
| Body weight (kg), mean (± SD) | 8.20 ± 2.60 |
| Age | |
| 0–12 months | 50(89.3%) |
| 13 months–3 years | 3(5.4%) |
| >3 years | 3(5.4%) |
| Sex | |
| Male | 35(62.5%) |
| Female | 21(37.5%) |
| Onset of symptoms | |
| <24 hours | 31(55.4%) |
| > 24 hours | 25(44.6%) |
| Location | |
| Ileoileal | 2(3.6%) |
| Ileocolic | 40(71.4%) |
| Ileocolocolic | 5(8.9%) |
| Colocolic | 6(10.7%) |
| Ileocecal | 3(5.4%) |
| Bloody stool | |
| Yes | 53(94.6%) |
| No | 3(5.4%) |
| Vomiting | |
| Yes | 51(91.1%) |
| No | 5(8.9%) |
| Bloated stomach | |
| Yes | 44(78.6%) |
| No | 12(21.4%) |
| Dehydration | |
| Yes | 45(80.4%) |
| No | 11(19.6%) |
| Obstipation | |
| Yes | 1(1.8%) |
| No | 55(98.2%) |
| Diarrhea | |
| Yes | 34(60.7%) |
| No | 22(39.3%) |
| Hydrostatic reduction outcome | |
| Successful | 9(16.1%) |
| Failed | 47(83.9%) |

hydrostatic reduction (assessed by Doppler ultrasonography) was continued with the conventional intestinal reduction.

Descriptive statistics were employed to describe the frequency and percentage of categorical variables. Mean with standard deviation and median with range (min–max) were used to describe numerical variables. Chi-square test (with the alternative of Fisher's exact test) was applied to compare the differences between both the outcomes of hydrostatic reduction. The strength of correlation of a variable with the outcome of hydrostatic reduction was analyzed by logistic regression, with the values described in odds ratio (OR). The $p < 0.05$ were deemed to be significant.

## RESULTS

During the study period, there were a total of 180 children who were treated for intussusception. Of these, 56 children had received hydrostatic reduction for the treatment of intussusception. Mean body weight of the patients was 8.2 kg. The majority of the patients were aged 0–12 months. Most patients were males. Most the patients received treatment in <24 h since the first onset of symptoms; 25 patients had received delayed treatment (>24 h after the first onset of symptoms). Ileocolic intussusception was the most prevalent location of intussusception. Bloody stool was the most commonly detected symptoms in patients with intussusception, followed by vomiting, dehydration, bloated stomach, diarrhea, and obstipation. Hydrostatic reduction success rate was low; only 9 out of 56 cases (16.1%) were successfully treated with hydrostatic reduction and 47 out 56 cases failed (83.9%). The remaining cases require conventional surgery after failure of hydrostatic reduction.

The success rate of hydrostatic reduction in treating intussusception was low in 0–12-month-old patients and in patients >3 years. No significant difference in terms of the outcomes between male and female patients ($p = 0.231$) were noted. The onset of symptoms ($p = 0.475$) was not significantly correlated with the hydrostatic reduction outcomes. Dehydration is the only symptom that was significantly correlated with worse outcome of hydrostatic reduction in this study (OR 16.80, $p = 0.001$). Other symptoms included in this study were not significantly correlated with the outcome of hydrostatic reduction in treating intussusception.

**Table II: Patients' characteristics correlated with successful outcome**

| Variables | N | % | OR | *p* |
|---|---|---|---|---|
| Age | | | N/A | |
| 0–12 months | 8 | 16 | | |
| 13–3 years | 0 | 0 | | |
| >3 years | 1 | 33.3 | | |
| Sex | | | 2.4 | 0.231 |
| Male | 4 | 11.4 | | |
| Females | 5 | 23.8 | | |
| Onset of symptoms | | | 1.70 | 0.475 |
| <24 hours | 4 | 12.9 | | |
| >24 hours | 3 | 20.0 | | |
| Location | | | N/A | |
| Ileoileal | 2 | 100 | | |
| Ileocolic | 3 | 7.5 | | |
| Ileocolocolic | 1 | 20 | | |
| Colocolic | 2 | 33.3 | | |
| Ileocecal | 1 | 33.3 | | |
| Blood stool | | | 2.81 | 0.420 |
| Yes | 8 | 15.1 | | |
| No | 1 | 33.3 | | |
| Vomiting | | | 4.19 | 0.152 |
| Yes | 7 | 13.7 | | |
| No | 2 | 40 | | |
| Bloated stomach | | | 2.11 | 0.349 |
| Yes | 6 | 13.6 | | |
| No | 3 | 25.0 | | |
| Dehydration | | | 16.80 | 0.001*** |
| Yes | 0 | 0 | | |
| No | 9 | 54.5 | | |
| Obstipation | | | N/A | |
| Yes | 0 | 0 | | |
| No | 9 | 16.4 | | |
| Diarrhea | | | 3.88 | 0.079 |
| Yes | 3 | 8.8 | | |
| No | 6 | 27.3 | | |

OR=odds ratio

## DISCUSSION

In this study, hydrostatic reduction showed higher success rate in children aged <3 years. Previous study had hypothesized the correlation between the diameter of small intestines and the outcomes of hydrostatic reduction. In this case, younger children had smaller lumen diameter of small intestines. The smaller diameter may have caused difficulties in reducing the portion of the intestine that were strangulated on the distal part.[11]

Successful reduction in higher rates in female patients relative to that in male patients were noted in this study, although the difference was not significant. Physiological differences between both the sexes may have contributed to the difference in the outcomes of hydrostatic reduction for the management of intussusception. In females, food digestion rate was slower, and the colon was longer relative to those in males. Weaker peristaltic forces associated with the physiological difference mentioned above may have contributed to the higher success rate of hydrostatic reduction in female patients than in male patients.[12]

The onset of symptoms was not significantly correlated with the success of hydrostatic reduction; this study had found that delayed presentation and treatment of intussusception (as defined as >24 hours had elapsed from the first onset of the symptoms to receiving treatment) was more common when compared to early treatment of intussusception (<24 hours). Khorana et al. noted the onset of symptoms to treatment was not correlated with the outcomes of hydrostatic therapy (with presentation ranging from 1 hour to the maximum of 120 hours). Hydrostatic reduction was indicated as an alternative to conventional surgery in treating intussusception; it may be performed even in individuals with delayed presentation of intussusception and still garner similar outcomes.[10,13]

In this study, intussusception of the ileocolic region was the most prevalent location of intussusception. The ileocolic region had numerous free-hanging structures of the abdominal region. Anatomical variations of the cecum and ileus may have contributed to the development of ileocolic intussusception, such as decreased cecum rigidity due to the absence of a secondary cecum and taenia coli; papillary structure of the ileocecal valve; and longitudinal muscle fibers of the colon around the valve. Decreased rigidity by anatomical variation may have caused intussusception due to the prolapse of the ileocolic segment.[14,15]

Several symptoms are associated with higher risk of failure in hydrostatic reduction. In this study, patients with dehydration showed significantly higher risk of failure in treatment using hydrostatic reduction; other symptoms, such as bloody stool and other gastrointestinal symptoms were not associated with the failure of hydrostatic reduction; however, patients with bloody stools were present in relatively higher

rate when compared to those with other symptoms listed. Dehydration may be related to the hardening of the feces; reduction of intestinal content requires higher pressure to treat through hydrostatic reduction; as such, it was associated with higher risk of failure of hydrostatic reduction in patients with dehydration.[11,16] In a previous study by Ekenze et al., patients with bloody stool were at higher risk for gastrointestinal complication and intestinal resection, while patients with bloody stool often presented with a higher prevalence of intestinal angulation, strangulation, and/or compression. Bloody stool was associated with a reduction of the venous blood flow of the intestine.[17] The study showed different findings related to the risk factors associated with the failure of hydrostatic reduction by He et al.; in the referenced study, bloody stool was significantly associated with the failure of hydrostatic reduction (OR 9.27; $p < 0.05$).[18] Conversely, in this study, bloody stool, despite the higher prevalence-failed treatment group, was not significantly associated with the failure of hydrostatic reduction. Katz et al. had noted that dehydration (mild, moderate, and severe) was significantly associated with the failure of hydrostatic reduction;[19] this study had found the similar results that patients with dehydration is significantly associated with failure of hydrostatic reduction in children with intussusception.

There were several limitations for this study. The disease is relatively rare and performed only at a single institution (with the consideration that the said institution is a tertiary referral hospital); as such, the sample may not be wholly representative of the characteristics of the patients in West Java, Indonesia. In this study, there were several patients who had experienced delays in treatment for >24 hours; there were some cases in which the patients had only sought treatment after experiencing the symptoms up to a week's period. In cases of extreme delays, there was a probable confounding factor in the evolution of symptoms (which may explain the current study to have several differences with previous studies performed outside Indonesia). Despite these limitations, this is one of the first studies to discuss the risk factors associated with the failure of hydrostatic reduction in treating children with intussusception, particularly in West Java, Indonesia.

## CONCLUSION

Dehydration in pediatric intussusception cases is significantly associated with the failure of hydrostatic reduction in children with intussusception. Age, sex, and other gastrointestinal symptoms were not significantly associated with the failure of hydrostatic reduction.


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# Mature Jejunal Teratoma in adolescents: A case report

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**SUMMARY**

**Jejunal teratoma (JT) is a rare type of extragonadal teratoma. To date, the subject of mature jejunal teratomas has not yet been discussed in the literature. This type of teratoma contains cystic, solid, and calcified components. JT may be suspected on a computed tomography (CT) scan, whichcan describe the various features of the germinal layer components, followed by normal laboratory results of alpha feto protein (AFP) and β-human chorionic gonadotropin (β-hCG). This case report describes that of a teenager with a mature JT whose chief complaints were recurrent general weakness due to anemia and an abdominal mass. The patient was initially treated with non-operative management; however, his symptoms remained unresolved, and he accepted surgical intervention. No additional chemotherapy or radiotherapy was required after complete tumor excision. The patient no longer complained of general weakness following surgery.**

## INTRODUCTION

Jejunal teratomas (JT) are very rare neoplasms, and no publication discussing this type of tumor is yet available. JT contain cystic, solid, and calcified components. This type of tumor may be suspected on a computed tomography (CT) scan, which can describe the various features of the germinal layer components, followed by normal laboratory results of alpha feto protein (AFP) and β-human chorionic gonadotropin (β-hCG). In this report, we discuss the case of a teenager with a mature jejunal teratoma.

## CASE REPORT

A 15-year-old male arrived at Dr. Soetomo Hospital (SH), Surabaya, Indonesiawith a chief complaint recurrent general weakness due to anemia. This problem was solved temporarily by repeated hospitalization within the last 1 year for recurrent transfusion. The patient's appetite and sleeping quality was good, his daily activities were within normal limits. Our patient had no motor or sensory complaints. Every time he felt weak, his hands and face turned pale and then he was admitted to the peripheral hospital. The patient denied other complaints such as headache, fever, bloody stool, blood in urine, history of trauma, or spontaneous bleeding from other places whenever he felt weak. The patient didn't have medical record about the laboratory value from peripheral hospital. When his anemia had been resolved, he felt fit and was discharged from the hospital. His last transfusion was 2 months before this admission. When arrive in SH, the patient was found to be anemic, which required transfusion until his condition improved and acceptable for operation of his lump. He had a painless lump in his lower left abdomen which was recognized by his mother since he was a 3-days-old neonate and increased in size as the patient grew older. The patient reported no other lumps grew elsewhere on his body. The patient had a good appetite, never complained of abdominal distension, no blood in his feces, had normal bowel habits, and never had other digestive problems. His urine was clear and yellow, and no complaints regarding his urination were made. The patient's weight was stable in the last 1 year, and he had no history of other diseases or high blood pressure. No peripheral blood smear examination nor fecal occult blood test (FOBT) was previously performed.

The patient had no family history of recurrent anemia, tumor, cancer, or similar complaints. The patient's mother was 24 years old when the patient was conceived. Routine checks by the midwife and an ultrasound examination of the womb by an obstetrician revealed that the fetus was normal. On physical examination, the patient's weight was 54 kg, and his vital signs, heart, and lungs were normal. His conjunctiva was anemic. In abdominal examination, we obtained the impression of a mass lump in the left lower quadrant with the skin surface was normal as the surrounding mass, normal bowel sounds, tympanic percussion, palpation of a single solid mass, flat surface, with firm impression boundaries, fixed at the base, painless, mass size 11 × 8 × 6 cm, does not cross the midline. No palpable enlarged lymph nodes were found in the head, neck, axilla, or inguinal regions. Liver and spleen impressions were within normal limits. Both testes were palpable within normal limits. The rectal digital examination, we found that the ampulla recti did not collapse, and no palpable impression of an intraluminal or extraluminal mass was noted, hand gloves was visible feces without blood. Motor and sensory functions were within normal limits. Laboratory testing showed normal tumor markers of αFP < 1.3 and βhCG< 2.0, blood count show Hb = 8.0 g/dL. Other laboratory values were within the normal range.

The results of physical examination revealed an intra-abdominal mass with anemia; thus, further radiological examination was recommended. Abdominal ultrasound on 1 month before operation revealed a donut-shaped intraluminal mass in the left lower quadrant of the abdomen; this mass appeared to lead to intussusception, but no symptoms of intussusception that matched the patient's clinical condition. A Computed Tomography (CT) Scan with contrast of the abdomen was then performed for further investigation.

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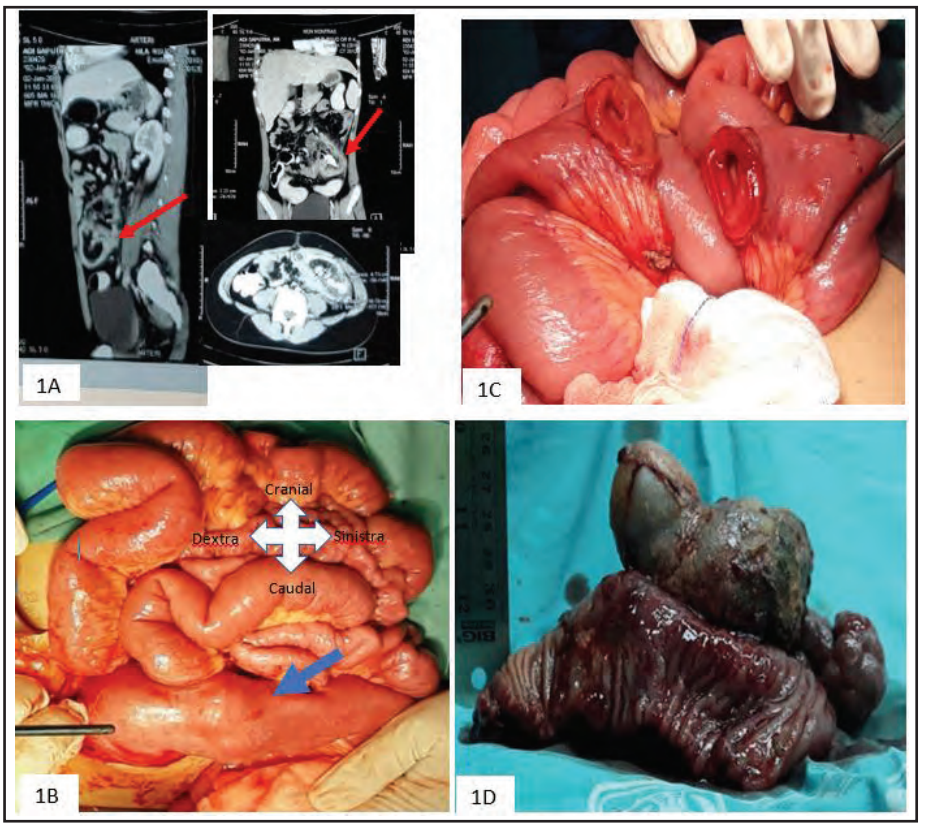


**Fig. 1:** (A) Abdominal CT scan with contrast showing a mass (red arrow). (B) Intraoperative mass attached to interintestine (blue arrow). (C) Intraoperative jejunum after tumor resection. (D) Cut section of tumor specimen.

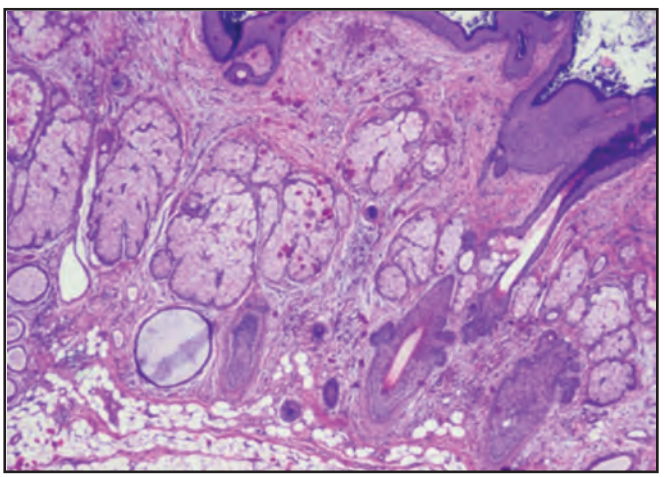

**Fig. 2:** Histopathology of the teratoma (magnification, 10×).

CT scan with contrast of the abdomen on 1 week after ultrasonography showed a lesion with solid components, fat, and calcification. The lesion measured 10.7 × 7.72 × 4.71 cm and was located in the left parailiac region. Contrast administration showed enhancement of the edges, septa, and solid parts of the lesion. Figure (1A) shows the lesion in the lower left quadrant of the patient's abdomen.

From the laparotomy, we found clear peritoneal fluid. The tumor was located in the intraluminal jejunum and thi sjejunum attached to the left white line, sigmoid, and terminal ileum, which was pulled to the left. Release of intestinal adhesion followed by tumor evaluation was then performed (Fig 1B). The intraluminal tumor in the jejunum was observed approximately 40 – 52 cm (about 12 cm long) distal to the ligament of Treitz. The tumor was freed from the surrounding tissue and then total tumor was resected along with the surrounding jejunum for about 20 cm (Fig 1C, 1D).After opening the resected jejunum, there was single heterogenous (solid and cystic) oval mass tightly attached with mesenteries side of jejunum, with size 11cm × 10 cm × 5 cm, no ulceration, no hemorrhage as shown in Figure (1D). On further exploration, we obtained liver had sharp edges, a flat surface, and a normal red color. No mesenteric lymph node enlargement was noted. After the intact resection of the jejunum with tumor, end-to-end jejunum anastomosis were performed.

Macroscopic examination of the anatomical pathology of the tumor revealed that the preparation contained portions of jejunum tissue weighing 275 g, 3.5 cm in proximal jejunum diameter, and 3 cm in distal jejunum diameter. The outer surface was partly smooth and partly covered with fat. On the slices, the mass was observed as sebaceous tissue, bone, teeth, and hair, measuring 10 cm × 9.5 cm × 4.5 cm. Some cysts measuring 1.2 cm in diameter with a yellowish-gray color and dense, chewy consistency were noted.

Microscopic findings showed sections of intestinal tissue with tumor growth consisting of skin epidermis, sebaceous glands, hair follicles, skin adnexa, glandular epithelium, hard bone tissue, fatty tissue, squamae, and calcifications, as shown in

Figure 2. The tumor had grown to the serous layer. No glial tissue and neuroepithelial components were seen. The ends of the resection were tumor free. No tumor was seen in the surrounding seven lymph nodes.

## DISCUSSION

The most common prevalence for gastrointestinal teratoma is gastric teratoma, but gastric teratoma alone accounts for less than 1% from total of teratomas.[1,2] The most frequent teratoma is sacrococcygeal teratoma (45% of all teratoma or 1 out of every 40.000 humans). To the best of our knowledge, there is no publication describing mature JT has yet been published. Small intestinal teratomas are believed to occur as a result of incomplete totipotential cell division in the subserosa of the jejunum in early life. However, the exact mechanism of this type of tumor remains unknown because of its rarity.[3]

This disease is usually diagnosed based on the patient's history, physical examination of the intra-abdominal mass, laboratory results revealed normal AFP and β-hCG values, and abdominal contrast CT scan. In our study, the results of the CT scan showed a lesion with solid components, fat, and calcifications. The lesion was a little bit bigger in width by operation 1 month after the CT scan was taken.

Teratoma is a common form of germ cell tumor (GCT). This tumor can develop congenitally or during childhood.[4,5,6] Because the tumor in our case was observed as early as when the patient was 3 days old, it may have developed congenitally. Mature teratomas show insignificant tumor growth. Our patient showed no clinical complaints or signs of distant spread, which often appears in immature or malignant tumors. Surgical removal of the tumor was performed only after the patient's repeated complaints of anemia as a teenager. His family was also afraid of operating option explained why he had just operated the lump when he was 15 years old. The patient's repeated anemia may be due to microscopic bloody stool, unfortunately we didn't perform FOBT to confirm this supposition. The type of anemia could not be identified because peripheral blood smear test was not conducted. While this type of tumor is not likely to metastasize to the bone marrow, this belief could not be confirmed because no peripheral blood smear test was performed. Surgery revealed a tumor located approximately 40 cm distal to the ligament of Treitz, which is still part of the jejunum. The jejunal with intraluminal tumor attach to the surrounding tissue confirmed that the tumor had been growing over a long period, but no signs of mesenteric or locoregional spread were noted, as supported by the patient's good liver and good mesenteric conditions. Thus, the tumor could be removed completely.

Macroscopic and microscopic pathological examination of the tumor showed sebaceous tissue, bone, teeth, hair, fatty tissue, squamous, and skin. These findings explained that tumor contains parts of endoderm, ectoderm, and mesoderm where each part can differentiate into many different tissues.5,6 Different types of tissue from these three germ layers lead to a teratoma. From the end of the tumorresection, histopathologic examination revealed no glial cells or neuroepithelial components. This finding indicates the absence of malignancy (i.e., a mature teratoma).

Chemotherapy is recommended for patients with grades 2 and 3 congenital immature teratomas after surgical resection.[3,7] Our type of JT was mature, no additional chemotherapy or radiotherapy was required after complete tumor excision. The variation of serum AFP level could be an indicator of tumor recurrence and of the need of further chemotherapy for patient with teratoma, especially in immature teratoma.[3] In our case,three months follow up after operation of this patients show normal AFP and β-hCG values, and 1.5 years after operation also no sign recurrent general weakness, no sign of recurrent abdominal mass.

## CONCLUSION

Teratomas are a type of GCT that can occur in various tissues, including the jejunum. Mature JT are a very rare type of tumor. No case report of a mature jejunal teratoma has previously been published. Our patient underwent surgery at the age of 15 years, which, in theory, could increase the risk of tumor malignancy. However, the patient showed good clinical and laboratory results before and during surgery, and no tumor spread was noted. Anatomical pathology also indicated a good prognosis. Histopathological results suggested a mature teratoma. Workup for the tumor markers αFP and β-hCG 3 months after the operation show normal values. Our patient had recurrent anemia prior to surgery, which could have been caused by occult blood from the mass. Unfortunately, we did not perform FOBT or peripheral blood smear to confirm the true cause of anemia. Approximately 1.5 years after surgery, no complaint of abdominal mass or general weakness due to anemia was made. The prognosis of this patient was good.

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# Coping with Coronavirus disease 2019: current state review and SWOT analysis to improve the urological services in Southeast Asia

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**ABSTRACT**

**Introduction: We investigated the impact of Coronavirus Disease 2019 (COVID-19) pandemic on urological services by analyzing current attitudes and practices of urologists in the Southeast Asian (SEA) countries and create ways for improvement.**

**Materials and methods: Quantitative data were used as critical indicators of workload of urological services from each country in SEA. Qualitative data analysis was done to describe the current state of attitudes of urologists against COVID-19 in the region. A strengths, weaknesses, opportunities, and threats (SWOT) analysis was performed to formulate strategic action plans.**

**Results: A total of seven urologists from six SEA countries completed the survey. Approximately 21–40% reduction in elective surgeries and outpatient visits, as stated by 42.9% and 57.1% of respondents, respectively was noted. Collectively, most respondents (71.4%) experienced <20% reduction in emergency visits. Various strategies were utilized as reaction to the pandemic. These include utilization of virtual communication platforms, pre-surgical COVID-19 screening, and limited number of accepted outpatient appointments and surgeries. Face to face patient consultations were still considered needed by many urologists although most countries had prohibited direct patient contact. The national response of countries such as Malaysia, Singapore, Thailand, and Vietnam were successful in controlling the pandemic. However, Indonesia and Philippines struggled because of the limited testing and tracing capabilities. Through the SWOT analysis, strategies were identified which can help overcome COVID-19 and any other future pandemics: (1) restarting the urological services in a safe and sustainable manner; (2) optimizing financial and infrastructural capacities; and (3) regional collaboration to strengthen the health systems.**

**Conclusion: COVID-19 negatively impacted many health aspects, especially the delivery of urological services in SEA. Therefore, to ensure sustainability of urological services during the pandemic crisis, health care system should focus on safe, resilient, and adaptive approach with regional collaboration.**

**KEYWORDS:**
*Southeast Asia; Urological Services; Urologist; Global Urology; Developing Countries; High-Income Countries; Low and Middle-Income Countries*

## INTRODUCTION

All countries worldwide are in the state of turmoil shortly after the emergence of a novel coronavirus disease 2019 (COVID-19) and its rapid global spread. Southeast Asia (SEA) was hit the earliest outside its origin in China. The first spread was reported in Thailand on January 13, 2020.[1] Furthermore, the 11 SEA countries including Brunei, Myanmar, Cambodia, Timor-Leste, Indonesia, Laos, Malaysia, Philippines, Singapore, Thailand, and Vietnam were greatly affected by the pandemic. As of July 15, 2021, the cumulative number of confirmed cases in 11 SEA countries reached over 19,000,000 patients. In order to combat the outbreaks, regional authorities have implemented numerous non-pharmaceutical interventions (NPIs) and preventative strategies like mandated quarantine and case isolation, restriction of all mass gatherings or public events, suspension of schools and other educational facilities, and large-scale social restriction including local and national lockdowns.[2]

Pandemic readiness and preparedness vary among SEA countries and there are several countries that are particularly vulnerable to this destructive outbreak. During this challenging time, urologists, as part of subspecialized healthcare resources may take an important leadership role: endeavoring to maintain patient care virtually, prioritizing patient safety, and protecting the larger community from potential coronavirus exposure. Generally surveying the impacts of COVID-19 on urological services, the rapid increase in the number of cases is overwhelming, as confirmed by many studies. However, less is known about the personal and daily experience of the practicing urologists in developing countries. Furthermore, a better understanding of potential factors may be helpful for urologists during this

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pandemic, such as collegial supports and coping strategies. The primary objective was to examine how the current COVID-19 pandemic affected the urological services in SEA countries, represented by the authors who are urologists in Indonesia, Malaysia, Philippines, Singapore, Thailand, and Vietnam. The secondary objective was to create strategies that will help improve the delivery of urological services.

## MATERIAL AND METHODS

A survey based on the hybrid-methods design was used. Quantitative and qualitative data from each participating urologists across SEA countries were collected to describe the primary objective.

*Descriptive Quantitative Data and Qualitative Data Analysis*

The survey consisted of a 20-point questionnaire which was answered by each respective certified urologist. Convenience sampling was employed to include several urologists in the SEA. The questionnaire was based on standard urological clinical pathway per region's hospitals and their perception to several adaptive models of relevant urological services during COVID-19 pandemic. These urologists were asked to fill-out a quantitative survey and describe the daily urological practices in their respective hospitals through qualitative questions. Each country was classified based on gross national income per capita from the World Bank Country's status, to illustrate the possible impact of socio-economic welfare on national policy towards pandemic. The qualitative response was coded by the first and second authors. They assigned a code to each qualitative response after reading through all responses. Answers could be given to multiple coding categories. No statistical analyses were performed based on qualitative responses.

*Strengths, Weaknesses, Opportunities, and Threats Analysis*

Strengths, weaknesses, opportunities, and threats (SWOT) analysis refers to assessing and evaluating the four elements of the acronym that influence a specific topic.[3] It is effective situation analysis techniques which comprehensively, systematically, and accurately assess the scenario of the case. This method helps formulate corresponding strategies, plans, and countermeasures in clinical decision-making and complicated systems analysis. Internal factors such as financial resources, healthcare resources and accessibility were identified as either strengths or weaknesses. In contrast, external factors such as policy trends, demographics, political and economic regulations were identified as opportunities or threats. Therefore, SWOT analysis was performed to develop prospective solutions meant to improve the collaborative development of urological services in SEA during the COVID-19 crisis.

## RESULTS

The questionnaires were sent to ten urologists, three of them refused to join the study and were classified as non-responder. Demographic data of the consultant urologists are displayed in Table I. All respondents were males, majority practicing in an academic institution and focusing primarily on stones and oncology subspecialties.

Regarding the epidemiological burdens, four out of seven respondents disclosed that they were working in COVID-19 managing hospitals, and two out of all participants were working in a hospital with a total of >30 COVID-19 cases. One respondent reported with cases of severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) among colleagues in their department (Fig. 1).

Regarding preventive potential disruption in urological services, four urologists stated that their urological association in their country did not develop or apply specific COVID-19 guidelines. It was disclosed by five out of seven surgeons that their institution provides healthcare personnel training on COVID-19. Three out of seven respondents experienced COVID-19 testing with nasopharyngeal or oropharyngeal swab tests. Surgical masks were reported as the personal protective equipment (PPE) that was commonly provided in their hospitals (Fig. 1).

Numerical data to describe the current situation each country is summarized in Table II. With the relatively rapid action and effective measures designated to control the spread of the pandemic by each government, several countries such as Singapore, Thailand, Malaysia, and Vietnam had achieved impressive results compared with Indonesia and Philippines. It appears that the low and middle-income countries (LMICs) status did not necessarily affect the current pandemic status of the region.

The current situation and personal perspective of urologists from each country are described below.

*Indonesia*

Indonesia was deeply affected by COVID-19, same as other developing countries. Despite being ranked as the largest economy in the region, Indonesia is struggling to cope with COVID-19 crisis.[4]

By mid July 2021, the proportions of death-related to COVID-19 reached to 69,210 cases, making it one of the highest number of cases in the world. The government gradually established the decentralized lockdowns but never reached the same rigidity level compared to other countries.[5] The implementation of polymerase chain reaction (PCR) testing across countries was low during that time.[6]

Danarto (An-Nur Surgery Specialty Hospital, Yogyakarta) noted that the spread of coronavirus in Indonesia had significantly impacted the urological services in general, shown by a drastic reduction during the earlier phase of the pandemic. However, this only lasted for eight weeks after the government decided to adjust the PSBB rules in many regions by early June 2020, resulting in a bounce-back rate of outpatient visits, elective surgeries, and ED visits. Due to the ongoing crisis, the Indonesian Urological Association took immediate action by releasing specific guidelines for urological services during COVID-19 pandemic to help local urologists make a clinical decision. With the current situation, Indonesia still has a long way to overcome the pandemic; thus, sustaining the urological services is going to be challenging with extra precautions, and utilization of PPE is mandatory.

*Malaysia*

In Malaysia, the first COVID-19 case was diagnosed on January 25, 2020. They had the largest cumulative number of COVID-19 cases among SEA countries by March 21, 2020. The active cases had reached from <30 cases at the earlier March 2020 to 2,766 cases by the end of March 2020. Nevertheless, the outbreak was considered under control by early June 2020. Currently, Malaysia has entered its recovery from COVID-19 pandemic.[7] It took not less than eight months after the government announced the first case, and the country was recognized to successfully managed the pandemic by the World Health Organization (WHO).[8] One of the contributing factors was because to the Ministry of Health of Malaysia constructed prompt and responsive action for COVID-19, promulgated the Movement Control Order early March 2020, and strictly enforced mass testing and rapid contact tracing.[9]

Teng Aik Ong (University of Malaya, Kuala Lumpur) reported that urological services in his center had been back to almost normal. However, during the earlier phase COVID-19, there was a massive cut down in outpatient visits and the number of elective surgeries. In terms of current challenges, issues that need to be addressed include concerns over clearing the backlog of cases. Zoom platform is a new thing learned to have meetings and ward rounds. For the latest development, there was a resurgent of COVID-19 cases in Malaysia in the early part of 2021. However, the health care system had learned to cope with such surge. The clinical service was adjusted to the appropriate level to mitigate the spread of the disease, while maintaining adequate service to urgent and semi-urgent cases, such as uro-oncology. Such balancing act will be the strategy in the new normal.

*Philippines*

As the second most populous country in the region, Philippines was affected by the pandemic quite early and currently is still struggling to defend against COVID-19.10 At first, the Philippine government declared a state of a national public health emergency that engendered the formation of an Inter-Agency Task Force, enforcing multiple forms of home quarantines and lockdowns in regions with high number of COVID-19 cases.[11] These measures have continued in changing levels of severity until recently and the government is focusing now on escalating the number of mass testing to reproduce the success of better-performing countries such as Singapore and South Korea.[12]

Abraham and Zialcita of the National Kidney and Transplant Institute in Manila reported the Philippines' perspectives. Abraham stated that the pandemic had really caused a significant reduction in the delivery of urological services nationwide. This was mainly because of the prioritization of the pandemic related cases by the health care system. A concerted effort was made by the urological department to prioritize cases according to their severity, consistent with the guidelines set forth by the EAU. This prioritization has led to a reduction of cases being catered per day. The decline in case load was aggravated by fear of patients to seek medical help during the pandemic, thereby causing a drop in outpatient consultations. In order to overcome the risks of contracting the disease, several practitioners applied control measures such as the widespread utilization of virtual consultations and specialized PPE. Many hospitals were challenged but specialized centers in the Philippines such as the National Kidney and Transplant Institute and St. Luke's Medical Center had resumed all elective surgeries and were able to cope with the pandemic. In fact, percutaneous nephrolithotomy, laparoscopy and robotic-assisted surgeries were offered to patients who needed them. The expedient preoperative evaluation of patients for COVID-19 infection was made possible with the GeneXpert® testing which allowed the prompt release of results within hours. Ziacilta confirmed the importance of utilization of virtual consultations, such as Viber® and WhatsApp® platforms, in order to avoid face-to-face consultations. Dr Abraham also expressed the importance of preoperative evaluation with oropharyngeal and nasopharyngeal swab reverse transcription-polymerase chain reaction (RT-PCR) among patients and inclusive of their family members. In terms of organizational reactions to the pandemic, the Philippine Urological Association responded by arranging webinars to disseminate guidelines on the practice of urology during the pandemic, with the goals of optimizing the delivery of care while protecting the practicing physician from contracting the coronavirus disease.

*Singapore*

No government was adequately prepared for this new pandemic, including Singapore as the most developed country in the region. However, since the first confirmed cases were reported in the country on January 23, 2020, Singapore has eventually achieved impressive results to surmount the spread of COVID-19 by enacting public health measures based on advice of experts to learn from the 2003 SARS pandemic.[13] This included a combination of social and workplace distancing, aggressive contact tracing, strict quarantine measures of infected and the closed contacts individuals, travel advisories, and entry restriction, which was then supported by high public compliance.[14] As of July 15, 2021, there were 62,804 cases with 36 deaths in Singapore.

Allen Sim (Department of Urology, Singapore General Hospital) expressed his gratitude that most of the positive cases are contained in dormitories with few positive community cases. Thus, the healthcare system is not overwhelmed. He explained that the urological services had not been much affected other than the decrease in volume during the peak of the pandemic. One of the critical issues now is that almost all meetings are conducted online, with many webinars readily available for all range of urological topics. Despite of the measures created, he expressed the craving for the international conferences and human physical interactions, which he believed many urologists are experiencing webinar fatigue and shared the same sentiment.

*Thailand*

Being the first country to encounter the coronavirus in the region, Thailand is considered by many experts to have successfully handled the pandemic.[15] In total, 372,215 cases were detected, and 3,032 deaths by the mid of July 2021.

**Table I: Demographic data**

| Variables | N = 7, count |
|---|---|
| Gender | |
| Male | 7 |
| Female | 0 |
| Years of practice | |
| 1–5 | 1 |
| 6–10 | 1 |
| >10 | 5 |
| Country origin | |
| Indonesia | 1 |
| Malaysia | 1 |
| Philippines | 2 |
| Singapore | 1 |
| Thailand | 1 |
| Vietnam | 1 |
| Types of hospital/institution | |
| Academic hospital | 4 |
| Non-academic public hospital | 1 |
| Private practice | 1 |
| Mixture of public and private practices | 1 |
| Subspecialty field of urology | |
| General urology | 5 |
| Stones | 6 |
| BPH | 4 |
| Uro-oncology | 6 |
| Renal transplantation | 3 |

**Table II: Summary of actual status of COVID-19 pandemic's burden across Southeast Asian region**

| Country | Classification* | Population (in millions)† | Date first reported confirmed cases | No. confirmed cases** | No. of test performed** | CFR (%) | IFR (%) |
|---|---|---|---|---|---|---|---|
| Indonesia | Upper-middle income | 267.0 | March 2, 2020 | 2.670.046 | 22.373.873 | 2.6 | 2.6 |
| Malaysia | Upper-middle income | 32.7 | January 25, 2020 | 867.567 | 15.866.357 | 0.7 | 0.7 |
| Philippines | Lower-middle income | 109.2 | January 30, 2020 | 1.485.457 | 15.662.056 | 1.7 | 1.8 |
| Singapore | High-income | 6.2 | January 23, 2020 | 62.804 | 14.751.144 | <0.1 | 0.1 |
| Thailand | Upper-middle income | 69.0 | January 13, 2020 | 372.215 | 8.129.670 | 0.8 | 0.8 |
| Vietnam | Lower-middle income | 98.7 | January 23, 2020 | 38.239 | 8.434.266 | 0.4 | 0.4 |

Abbreviations: COVID-19, coronavirus disease 2019; CFR, case fatality rate; IFR, infection fatality rate
*Classified according to the World Bank country income status.30 According to official statistics of World Bank for 2021 fiscal year, calculated using the World Bank Atlas method, low-income economies are defined as those with a gross national income (GNI) per capita of $1,035 or less in 2019; lower middle-income economies are those with a GNI per capita between $1,036 and $4,045; upper middle-income economies are those with a GNI per capita between $4,046 and $12,535; high-income economies are those with a GNI per capita of $12,536 or more
†Data from U.S. and World Population Clock by United States Census Bureau,31 as of July 15, 2020
**Based on data reported to the registries as of July 15, 2020

Three months after the government established a state of emergency in late March 2020, most daily activities returned to normal in Bangkok and other royal territories. The early response was probably in disarray, but with improved coordination and communication, the government managed to respond better to the pandemic.[16]

It is suggested that robust central control and a well-developed health care system, complemented by near-universal health coverage, had been the key to such accomplishment. This allowed the successful implementation of active case finding, contact tracing, and other confinement protocols through the extensive and well-distributed network of healthcare providers. The country has been listed among the Global Health Security Index's top tier as the most prepared country for national health security.[17] Thailand has also become the highest adopter in compliance with mask-wearing in public places, which appears to have helped the slow spread of the virus.[18]

Supachai Sathidmangkang (Faculty of the Medicine Siriraj Hospital, Mahidol University, Bangkok) admitted that COVID-19 era has transformed healthcare activity into the new normal since the first confirmed cases on January 13, 2020. The number of COVID-19 cases had risen rapidly, overfilled hospital beds, medical personnel, and wearing of PPE keep patients and healthcare personnel safe. Regarding the impact on the urological services in his daily practices, he has been asked to limit his work in outpatient and surgical procedures. This resulted in a three-month backlog of cases of the non-urgent surgeries delayed because of the postponement. The preoperative COVID-19 screening protocol is compulsory for patients who needed to undergo surgery. He noted that the most crucial matter to consider is patient care based on prioritization and consequently the tiered surgery system is the key.

**Table III: SWOT analysis**

| Influencing Factors | Influencing Factors | Weaknesses |
|---|---|---|
| | 1. Risk-stratify the elective surgery according to latest evidence[32]** <br> 2. Specific guidelines for COVID-19 launched by several urological societies and groups32* <br> 3. Growing recognition of urology as an essential part of a robust health system33* <br> 4. Widely available resources[34]* | 1. Possibility to operate on large volume diseases caused by delay due to pandemic, lead to longer operating room time and more positive surgical margins* <br> 2. Geographical maldistributions of urological workforces in low and middle-income countries (LMIC)[35]* <br> 3. Poor health insurance coverage in some countries[36]* <br> 4. Large patient volumes[34]*** |
| | **Strategies** | |
| **Opportunities** | **⇧ S → ⇧ O** | **W → ⇧ O** |
| 1. Surgery may be the safer option among various treatment modalities which requires multiple visits, e.g. chemotherapy and radiotherapy[22]**** <br> 2. Incorporation of technology in bridging the distance, e.g. telemedicine & video conferencing23* <br> 3. Task-sharing/hospital co-management and task-shifting[29]* | 1. Perform surgery based on risk stratification and wear the proper PPE[32]** <br> 2. Enhance technical skills and appropriate knowledge among resident and non-urologist through additional training, webinars, podcast, etc.[29]** <br> 3. Offers rewards or grants for HCW such as job promotion or recognition-of-services* | 1. Allocate additional/extended work after hours or during weekend to cope the backlog[37]** <br> 2. Expand the telemedicine usages, optimization of the OR schedules and augment with ambulatory surgery center, if necessary[23]* <br> 3. Revitalize the advanced HCW e.g. the physician assistants, nurse practitioners, and advanced registered nurse[29]* |
| **Threats** | ⇧ S → T | |
| 1. Despite its benefits, the surgery risk stratification approach could potentially lead to worse disease progression[22,32]**** <br> 2. The long waiting list could create anxiety and deprived overall quality of life among patients[38]*** <br> 3. Shortage of PPE due to surging global demand & depleted stockpiles[28] * <br> 4. Healthcare personnel duty-hour restriction[39]** <br> 5. Political inconsistencies among countries are unequivocally rising from religious, cultural, economic and government systems diversity* <br> 6. Catastrophic expenditure on surgery26* <br> 7. Economic changes[25]* <br> 8. Ethical obligations[40]** | 1. Perform critical analysis of the literature on surgical delay as a guide timing of treatment to minimize risk to patients and hospital resources[32]** <br> 2. Perform a multi-disciplinary approach to consider all the aspects of each case, including the type and stage of disease, the age, the physical status, the psychological issues, and the availability of alternative treatments[38]** <br> 3. Keep patients well-informed through website's posts, emails or texts[23]* <br> 4. Relationship building among staffs[23]** | 1. Optimization in resource allocation according to benchmark for PPE required in urological procedures[23]* <br> 2. Use effective shift duration and staff placement according to the routine tasks[39]* <br> 3. Optimize workforce schedules[39]* <br> 4. Ramping up telehealth to deliver services to keep staff on the job[23]* <br> 5. Consider patient's perspective as a victim and vector of disease, while still putting forward the basic ethical principles (autonomy, non-maleficence, beneficence and justice) when encountered difficult judgment[40]** |

S: Strengths, W: Weaknesses, O: Opportunities, T: Threats, ⇧: Optmizing, : Eliminate
*Administrative factors; **Healthcare worker related factors;
***Patient related factors; ****Disease related factors

*Vietnam*

Vietnam is among the most successful countries to halt the COVID-19 and earned much international praise for its effective pandemic response.19 Since its first confirmed cases on January 23, 2021 to July 31, 2021, Vietnam had detected 38,239 cases with only 138 deaths, compared to 5,829,724 detected cases with more than 100,000 deaths in France during the same timeline. One contributing factor was the ability of the government to fully implement strict enforcement of large-scale lockdown protocols and obligatory quarantine with very high public compliance, utilizing its state apparatus of a centralized system of the government.[20]

In terms of the specific impact on urological services, Dong Nguyen (Bình Dân Hospital, Ho Chi Minh City) reported no significant changes during the pandemic. This was probably due to COVID-19 situation being relatively under control in the country. The Vietnam Urological Association did not develop specific guidelines, and Mr. Dong did not apply specific guidelines for his daily practices.

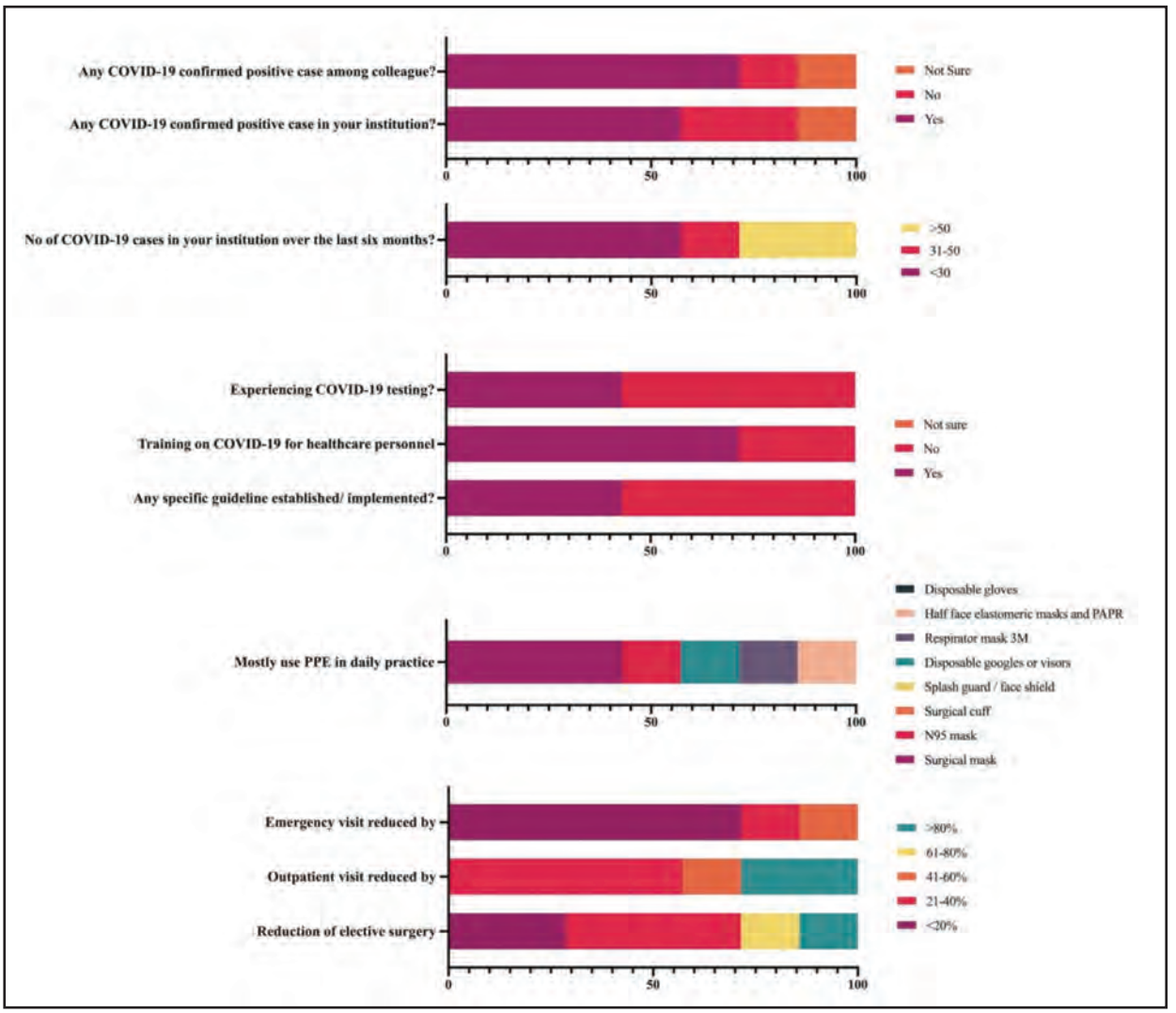


**Fig. 1:** Overview of the individual consultant urologist's statements on the questionnaire. For the full question texts, please refer to the questionnaire in the supplemental data.

## DISCUSSION

Through the SWOT analysis (See Table III), several strategies can be developed: (1) reboot the urological services safely and sustainably; (2) optimize financial and infrastructural capacities; and (3) regional collaboration to strengthen the health systems. Although these cannot cover all possible problems, these strategies aimed at urologists at SEA countries, and we tried to cover a wide range of situations encountered during the pandemic.

*Reboot the urological services in a safe and sustainable manner*

Resumption, followed by a consistent delivery of specialized services such as urology during a pandemic crisis is essential. A delay can potentially lead to serious adverse events, which can result to an extensive procedure, and may complicate by worsening the disease condition and progression. However, the safety of both healthcare providers and patients is paramount. Proper strategy is effective to maintain the balance between the resumption of urological routine services while strengthening the efforts to curb the spread of COVID-19 through the following principles: (1) Determine if the pandemic curve, the outbreak is within the region.[21] This is intended to engage relevant stakeholders and authorities to take necessary actions. Urological services, especially elective surgeries, should ideally be started when the curves flatten out, no vast surges or sustained spikes, and the community spreads is considerably under control. It is essential to note from the previous Spanish flu pandemic experienced, a second wave which was more lethal than the previous one. (2) Perform a reliable COVID-19 screening.[22] The gold standard is the molecular testing of upper or lower respiratory tract samples through RT-PCR. The rapid serology tests are less reliable due to antibodies' uncertain serodiagnostic power against SARS-COV-2. Nevertheless, this can be done as an alternative, by combining with chest computerized tomography as being adopted in some hospitals in Beijing. (3) Carry out a graded approach based on risk stratification for urological services based on the guidelines published by several groups and societies, and the provided objective perspectives on prioritizing patients during the pandemic. (4) Develop a well-planned non-surgical program for urological patients. Management of urological

patients often require non-surgical programs such as routine consultations and rehabilitation. This urges incorporation of technology in bridging the gap, such as telemedicine.[23] (5) Ensure sustainable efforts.[24] Without a dynamic system and continuity, the whole process will not be completed. The key strategy here is to maintain a steady but gradual services capacity and flexibility to adapt to the ever-changing situation. Start with small capacity of services, then adjust the number based on the epidemic curve in the SEA region. Maximize the procedure that can be done without hospitalization. Keep updated on the current development of the pandemic and adjust the health practices appropriately.

*Optimizing financial and infrastructural capacities*
There are several potential problems with the infrastructural and financial aspects of the government such as reduced access to urological facilities due to lockdown orders, reduced funds for most surgical cares due to ratcheting or reallocation of the state budget,[25] and most surgical cares have financially enormous spending.[26] These can be a hinderance to our efforts towards managing the pandemic.

The role of related stakeholders is essential, but a urologist may still contribute in several ways. First, by embracing adoption of technology to help patients with access challenges. Although still new and relatively low uptake, telemedicine is still the primary choice. Secondly, build a solid supply chain by promoting and empowering locally made PPEs.[27] The pandemic demands us to be creative, and utilization of the locally made PPE can be the perfect alternative while maintaining the high standards of production and quality control procedures. Thirdly, drastically reduce the expenditure in surgical cases & PPE using supply chain resilient concepts.[28] This is important to cope with the risk of enormous spending from surgical diseases and can be done through sustainability practices. Fourthly, divide the PPE stockpiles into emergency and routine care purposes.[27] The goal is to be prepared when pandemic-related emergency cases occur while maintaining routine urological care sustainability.

*Regional collaboration to strengthen the health systems*
The discrepancy of urologist distribution highlights an area for further improvement. The geographic proximity and close economic and structural ties of SEA countries call for multi-regional collaborations. This can be by information sharing from the experts to provide joint statements through additional training, webinars, podcast, etc. and may optimize patient care during the COVID-19 crisis.[29]

On a larger scale, the second phase of this and in any future pandemic, which many countries have not experienced, may need a regional capacity sharing system.[29] This allows us to share essential resources such as regional vaccines and PPEs. This will enable less developed countries to employ healthcare workforces from nearby countries through mutual recognition arrangements.

From this point forward, health security, in general, is a pivotal part of regional and national security and is best managed by a united SEA. Urologists should be prepared to contribute to further development by taking an important leadership role during this unprecedented time.

The limitations of the study include the small sample size, lack of diversity, and the recruitment methods with limited number of respondents. Larger studies are needed to expand the data from another developing country, to increase generalizability, and to compare unique experiences among urologists during the COVID-19 crisis.

## CONCLUSIONS

Several challenges and opportunities in SEA countries regarding continuing urological services during the COVID-19 pandemic were identified in this study. Each country has a unique approach and progress towards COVID-19 management. These findings helped us recognize potential areas for improvement and strategies to emphasize the safety for both patients and urologists. Thus, strengthening future collaboration by joint efforts should be implemented by recognizing the need for multi-stakeholder initiatives and involving all SEA members in an approach to ensure' sustainability of urological services during a pandemic. Finally, the focus of urological services recovery should be based on resilient, adaptive, and sustainable approach.

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# Predictive accuracy of the APACHE IV scores on mortality and prolonged stay in the intensive care unit of Dr Sardjito Hospital

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## ABSTRACT

**Introduction: Acute Physiology and Chronic Health Evaluation (APACHE) is the most widely used scoring system in the intensive care unit (ICU). The APACHE IV showed a good level of discrimination and calibration on predicting mortality and prolonged stay (PLOS) in some countries. This study is aimed to determine the predictive accuracy of the APACHE IV score on mortality and PLOS at the ICU of Dr Sardjito General Hospital (SGH).**

**Materials and Methods: This study involved all adult patients at the ICU of SGH during 2018 that met the inclusion criteria. The discrimination of APACHE IV scores on mortality and PLOS was analyzed with Receiver Operating Characteristic Curve, and the optimal cut-off point was assessed with the Youden Index. The calibration of the APACHE IV score was assessed with the Hosmer-Lemeshow goodness-of-fit test, and a *p*-value of >0.05 is considered a good calibration.**

**Results: From the data of 742 patients, only 476 were included. The overall mortality and PLOS rate was 25.4 % and 15.1 %, respectively. The mean of APACHE IV score was 66.27±27.7. The area under the receiving curve with a 95% confidence interval for mortality is 0.99(0.97–1.00) and for PLOS was 0.68(0.62–0.74). The optimal cut-off point of the APACHE IV score for mortality was 78.9, with a sensitivity of 0.96 and a specificity of 0.96. The optimal cut-off point of the APACHE IV score for PLOS is 62.5 (in the 6th percentiles), with a sensitivity of 0.72 and a specificity of 0.61. The calibration is good for mortality prediction (*p*=0.98) but is poor for PLOS prediction (*p*=0.01).**

**Conclusion: APACHE IV score has excellent accuracy for mortality prediction but is poor for PLOS prediction in patients in the ICU of SGH.**

**KEYWORDS:**
*APACHE IV score, ICU, Mortality, PLOS, Prediction*

## INTRODUCTION

The scoring system to predict the outcome of critically ill patients is needed for consideration of decision making, resources allocation, benchmarking, and stratification for clinical trials.[1] The accuracy of the risk prediction models are measured by their good calibration, discrimination, and generalizability (good reproducibility of the score and transportability across geographic, time, and methodology). Some scores have excellent performance in the population they were originally developed. Still, some lose the accuracy when applied to different populations because of the differences in the population characteristic from where the score was initially created.[2] Population characteristics, the severity of disease, and health policy differ within and between countries from time to time; therefore, the risk prediction models require regular validation and refinement (for example, by recalibration).[3]

The intensive care unit (ICU) scoring system for intensive care has been developed since 1980 in response to demands for the evaluation and monitoring of health services. The system allows for a comparative audit and evaluation of intensive service research. Several assessment systems have been developed for critically ill patients to predict the likelihood of patients surviving in the hospital. Mortality and prolonged stay (PLOS) prediction in critically ill can benefit health services for quality improvement.[1]

The Acute Physiology and Chronic Health Evaluation (APACHE) and Simplified Acute Physiology Score (SAPS) are the most widely used scoring systems in the ICU.[4] The APACHE IV was introduced in 2006. The development of the APACHE IV system is based on 104 ICUs and 131,618 patients from the United States of America (USA).[5]

The APACHE IV score has a good level of discrimination and calibration with a broadly validated sample size and receiver operating characteristics (ROC) of 0.88.[5] The APACHE IV score can be used as an ICU benchmark by using the standardized number of mortality ratio to evaluate groups of patients.[6]

This study is aimed to assess the predictive accuracy of the APACHE IV score on mortality and PLOS of patients in a single tertiary hospital.

## MATERIAL AND METHODS

This retrospective study was based on the data of patients who were admitted to the ICU in SGH from January 1 to December 31, 2018. The study was conducted after the

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**Table I: Demographic Data of Research Participants**

| Variable | TOTAL n(%) | Dead (n=119) n(%) | PLOS(70) n(%) |
|---|---|---|---|
| Gender | | | |
| Female | 256(53.4) | 45(37.8) | 30(42.9) |
| Male | 220(46.6) | 74(62.2) | 40(57.1) |
| Age (years) | | | |
| < 20 | 12(2.5) | 3(2.5) | 2(2.9) |
| 21-40 | 144(30.3) | 25(20.0) | 17(24.3) |
| 41-60 | 210(44.1) | 32(26.9) | 32(45.8) |
| 60-80 | 100(21.0) | 52(43.7) | 16(22.9) |
| >80 | 10(2.1) | 7(5.9) | 3(4.3) |
| Diagnosis at Admission | | | |
| Medical | 108(22.7) | 63 (52.9) | 38(54.3) |
| Post Neurosurgery | 172(36.1) | 16 (13.4) | 18(25.7) |
| Post Orthopedic surgery | 31(6.5) | 1 (0.8) | 0(0) |
| Post Urologic surgery | 17(3.6) | 1 (0.8) | 0(0) |
| Post Digestive surgery | 43(9.0) | 23 (19.3) | 5(7.1) |
| Post Oncologic surgery | 10(2.1) | 1 (0.8) | 1(1.4) |
| Post-Obstetric-gynecologic surgery | 47(9.9) | 7 (5.9) | 5(7.1) |
| Post-Thorax/Vascular surgery | 48(10.1) | 7 (5.9) | 3(4.3) |

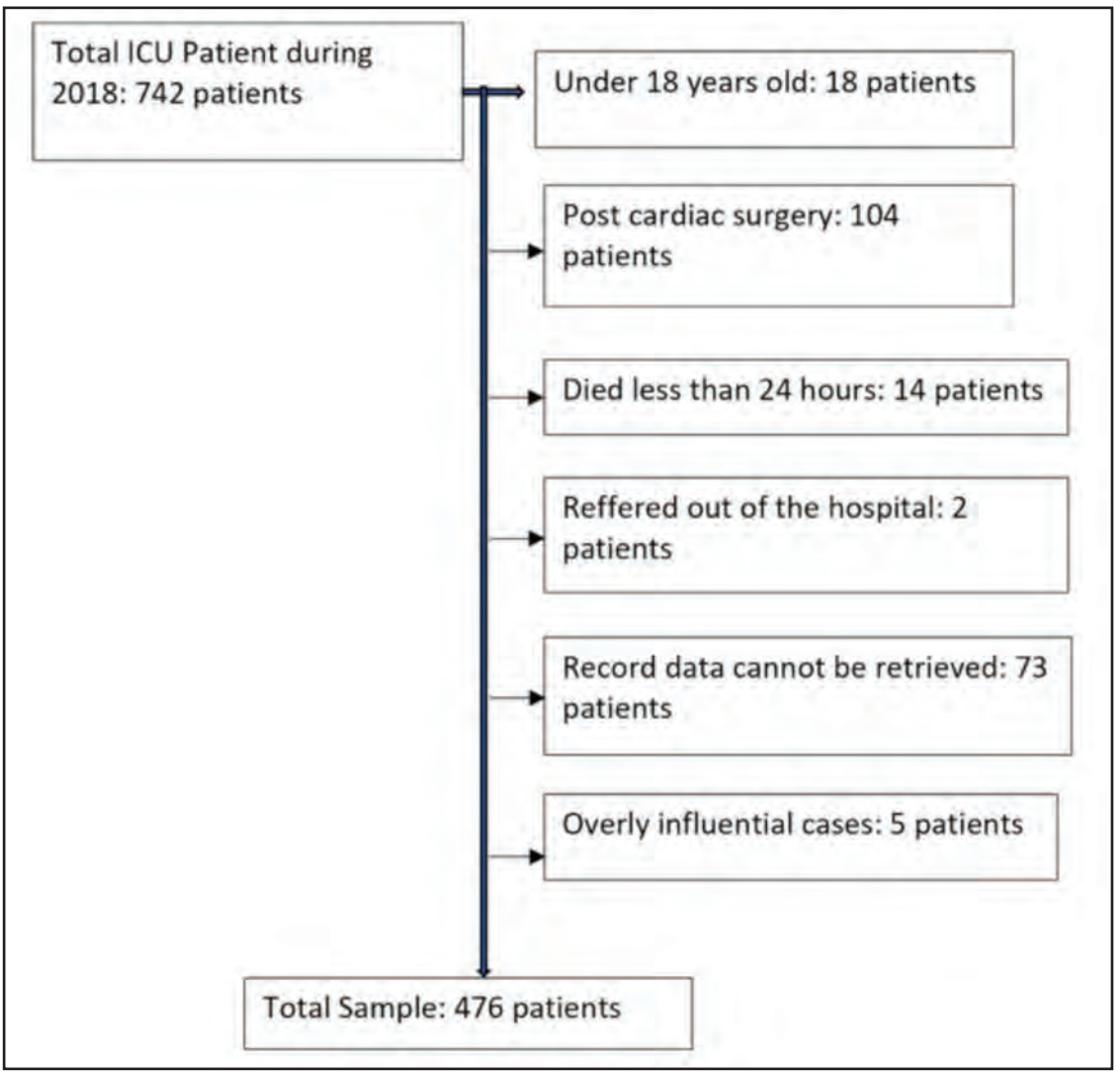


**Fig. 1:** The study sampling

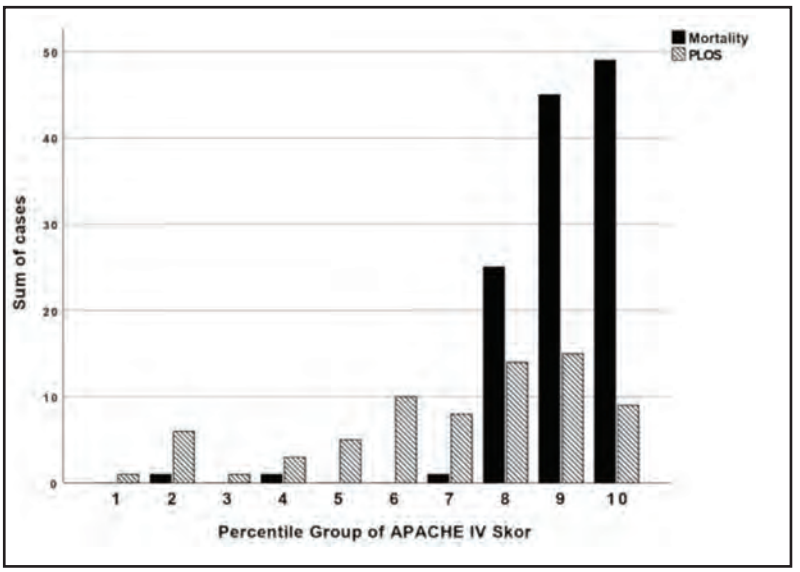


**Fig. 2:** The outcome based on the deciles of the acute physiology and chronic health evaluation (APACHE) IV.

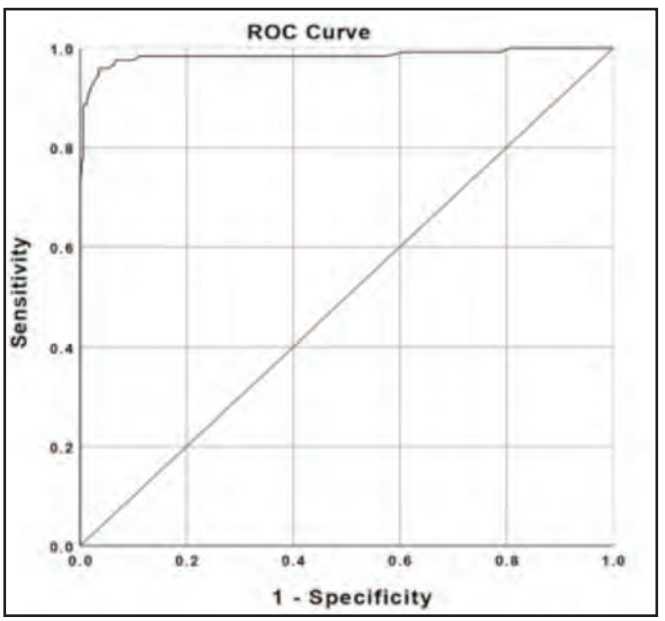


**Fig. 3a:** The discrimination of the acute physiology and chronic health evaluation (APACHE)IV score for mortality, the area under the curve (AUC) of Receiver Operating Characteristics (ROC) with 95% confidence interval (CI) for mortality: 0.99(0.97–1.00).

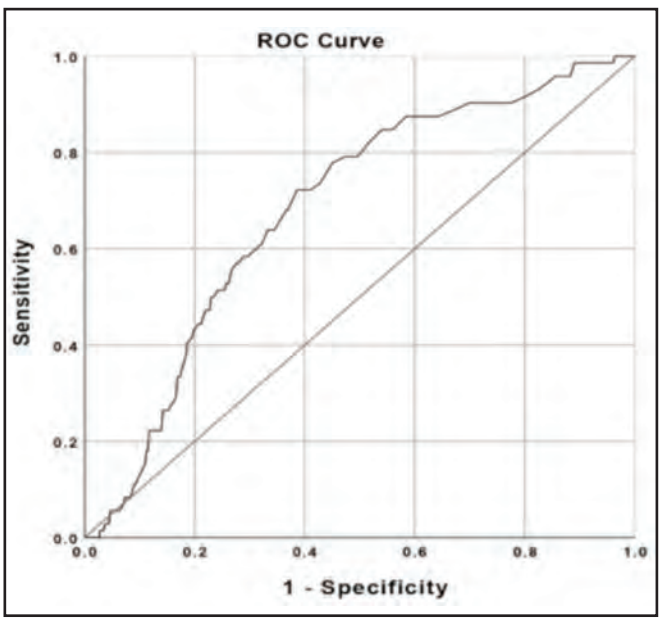


**Fig. 3b:** The discrimination of the acute physiology and chronic health evaluation (APACHE) IV score for PLOS, the area under the curve (AUC) of Receiver Operating Characteristics (ROC) with 95% confidence interval (CI) for a prolonged length of stay: 0.68 (0.62–0.74).

approval from the Ethical Committee No KE/FK/0684/EC/2019. The inclusion criteria in this study were all adult (≥18years old) patients admitted to the ICU of SGH. The exclusion criteria include the following: post-cardiac surgery, treatment <24 h, referred out of the hospital, and patients whose medical record data could not be collected during the sampling period. The dependent variable is mortality,defined as ICU mortality and PLOS as ICU stay >7 days. The independent variable in this study is the APACHE IV score.

The APACHE IV score was calculated according to the website (http://www.mecriticalcare.net/icu_scores/apacheIV.php), based on the patient's data in the first 24 h. The ROC producing an area under the curve (AUC) with 95% confidence intervals (CIs) was used to assess discrimination power. The optimal cut-off points of sensitivity and specificity were calculated using Youden Index. The calibration of the APACHE IV score was assessed with the Hosmer-Lemeshow goodness-of-fit test, and a p-value of >0.05 is considered a good calibration.

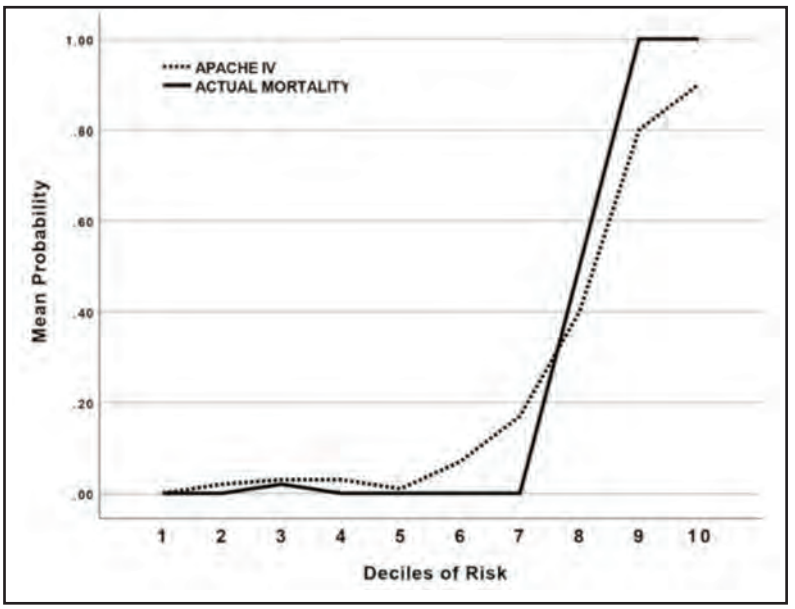


**Fig. 4:** The Observed versus Predicted Mortality by the acute physiology and chronic health evaluation (APACHE) IV score.

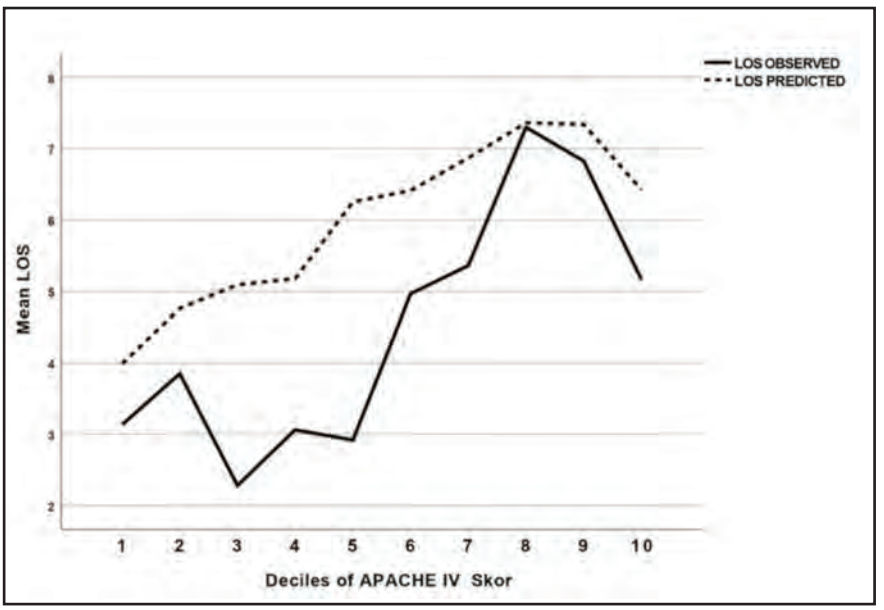


**Fig. 5:** The Observed versus Predicted prolonged length of stay by the acute physiology and chronic health evaluation (APACHE) IV score.

The ROC and Hosmer-Lemeshow tests were calculated using the Statistical Package for the Social Sciences (version 27.0 SPSS Inc., Chicago, IL, USA). Descriptive statistics were calculated with continuous data presented as mean ±SD and categorical data presented as percentages. Overly influential variables were removed from the data.

## RESULTS

The study was conducted on the medical records of 742 patients, but only 476 patients were included in the study sample (Fig. 1).

The baseline characteristics of the study group are presented in Table I. Of 476 patients, there were 220 males (46.6%), with an average age of 48±16 years. The mortality rate was 25.4%, the PLOS rate was 15.1%, and the APACHE IV score was underestimated at 23.4% and 6%, respectively. Male patients have a higher incidence of mortality (62.3%) and PLOS (58.3%). Patients aged 61–80 years had the highest mortality rate (44.1%), whereas 41–60 mostly experienced PLOS (45.8%).

Patients admitted to the ICU were divided into two large groups, namely, 108(22.7%) medical cases and 368 (77.3%) surgical cases, and most patients who died had a medical

admission diagnosis (52.9%) followed by digestive surgery 23(19.3%). Most patients with PLOS were diagnosed with a medical condition (52.8%), followed by a neurosurgery procedure 18 (25.7%) (Table 1).

Fig. 2 shows the outcome distribution of APACHE IV scores into deciles, which led that patients who died mainly in the APACHE score range 81–100 and patients who got PLOS especially were 61–80. The percentage of patients living was 100% in patients with APACHE IV scores of <40, whereas 100% of patients were dead in scores of>100,which reveal the fact that the greater the APACHE IV score, the higher the mortality rate, but not the same for PLOS.

The accuracy of the APACHE scores to discriminate the patients who died was excellent with AUC with a 95% CI for mortality: 0.99(0.97–1.00) (Fig. 3a). The AUC (95%CI) for PLOS was 0.68 (0.62–0.74), which is considered poor (Fig.3b). The optimal cut-off point of the APACHE IV score for mortality was 78.9 (in the 8th percentiles), with a sensitivity of 0.96 and specificity of 0.96. The optimal cut-off point for PLOS prediction was 62.5 (in the 6th percentiles), with a sensitivity of 0.72 and specificity of 0.61.

The calibration of the APACHE IV score for mortality was good with a p-value of 0.98, but poor for PLOS with a p-value=0.01. The APACHE score slightly overestimated the mortality in the low risk and underestimated the high risk (Fig.4). For PLOS prediction, the APACHE IV score tends to overestimate the length of stay (Fig. 5).

## DISCUSSION

This study revealed that the APACHE IV score consistently gives good discrimination in predicting mortality. The accuracy of predicting mortality was higher than the accuracy of predicting PLOS. This finding is consistent with another study.[5-14] The discrimination of the APACHE IV score for mortality prediction in our research is as good as in the original population, despite the different characteristics of a population. In our study, the age of ICU patients was younger, and the mean of the APACHE score was higher (66.27 vs 46.43).[5] The number of patients who died with the APACHE IV score of >100 was 59% in our study, whereas the original APACHE IV score was only 47%, and the remaining 39.7% had the APACHE IV score of 80–100.

The APACHE IV score is a physiology-based classification system that measures disease severity in a group of patients with a critical illness. The accuracy of the APACHE IV score plays a role in improving patient services. Its ability to provide mortality prognosis has been tested in USA and parts of Europe and Asia.[5,7–9]

The calibration of the APACHE IV score in predicting mortality is good. A study by Choi et al. also revealed the superiority of the APACHE IV calibration ($p$=0.905) compared to the APACHE II score ($p$=0.805).[9] Costa et al. (2011) prospectively examined APACHE IV, SAPS III, and MPMIII scores in critical patients with acute renal failure and showed good discrimination (AUROC 0.74) and calibration ($p$-value:0.574).[14] The calibration of the APACHE IV on mortality, especially in high-risk patients, is underestimated. However, the overall calibration on mortality is still good. However, some studies showed that the mortality prediction of the APACHE IV was not accurate. The differences in patient characteristics, clinical practice, assurance, quality, and services of health care systems may make different outcomes. The study by Chan et al.[15] reported that the accuracy of the APACHE IV score for mortality prediction was poor. They conducted a retrospective study of a group of postoperative patients with surgical abdominal sepsis and showed that the APACHE IV score poorly predicted mortality. The other study also reported that the APACHE IV score did not accurately predict mortality in patients with trauma.[16]

Recently, the accuracy of the APACHE IV score was compared to the APACHE II and Sequential Organ Failure Assessment (SOFA) score for mortality in patients with Coronavirus disease in the ICU and showed that all scores had bad discrimination on overall population (the APACHE IV AUROC 0.67 vs 0.63 of APACHE II vs 0.53 of SOFA score). On the other hand, the APACHE IV had the best discriminative power of the three scoring systems in the subgroup of patients with low molecular weight heparin (APACHE IV AUROC 0.82 vs 0.7 of APACHE II vs 0.49 of SOFA score).[17]

The APACHE IV score lacked both discrimination and calibration in predicting PLOS and tends to overestimate prediction. Patients with PLOS in our population mainly were patients with a medical diagnosis, sepsis, and more than one organ failure. Thus, they tended to have a shorter length of stay because they died earlier than patients with a lower score. Another factor determining the inaccuracy of the APACHE IV score in predicting PLOS is the differences in time units to calculate the length of treatment from the time patients enter until discharge. The original study of APACHE scores used hours, whereas our study used days.[6] The APACHE IV scores have poor performance in patients with severe sepsis[6] and have poor accuracy in the length of stay prediction, especially in severe sepsis and trauma cases.[11,18]

The difference in hospital policy related to the patient's end-of-life status, case-mix differences, insurance policies, step-down policy, and differences in clinical practice between USA and Indonesia affected the accuracy in predicting PLOS. This study has several limitations. Firstly, 9.8% of medical records cannot be taken and secondly only data for1-year was taken, and more extensive data may give a different result.

## CONCLUSION

APACHE IV score has excellent accuracy for mortality prediction but poor accuracy for PLOS prediction in patients admitted in the ICU of SGH.


## ACKNOWLEDGMENT

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## CONFLICT OF INTEREST

None to declare.

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# Cholelithiasis in children: A characteristic study

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## ABSTRACT

**Introduction: Pediatric cholelithiasis (PC) is relatively rare when compared to adult cholelithiasis. This study is aimed to describe the clinical characteristics of pediatric cholelithiasis treated at Hasan Sadikin General Hospital (HSGH), Bandung, Indonesia.**

**Materials and Methods: This is a descriptive study of children aged 0–18 years who were diagnosed with and treated for cholelithiasis at the HSGH over 4–5 years. Variables collected during this study were sex, age, chief complaint, previous medical history, diagnostic test, definitive management, and clinical outcomes.**

**Results: There were 12 cases of pediatric cholelithiasis during the study period, including those of 5 boys (41.7%) and 7 girls (58.3%). The mean and median age of the patients was 10.75 years and 12 years, respectively. The most prevalent complaint of the patients was abdominal pain (75%), followed by jaundice (16.6%) and abdominal distension (8.4%). Thalassemia was the most frequently associated disorder among the patients (25%). Ultrasonography was diagnostic imaging used on 66.6% of patients. Fifty-eight percent of patients have performed the surgery. The most frequently used surgical technique to manage the patients included laparoscopic cholecystectomy (33.3%), followed by laparotomic cholecystectomy (16.7%).**

**Conclusion: PC is an uncommon disorder, but easier to diagnose reasonably with the development of imaging study. Minimally invasive procedures using laparoscopic cholecystectomy were the most frequently performed surgical treatment in this study.**

**KEYWORDS:**
*cholelithiasis, characteristics, pediatric surgery*

## INTRODUCTION

Cholelithiasis is a gallbladder disease commonly reported in adults. The incidence of cholelithiasis in children is relatively rare. An increasing trend of cholelithiasis in children has been noted.[1-3] Ultrasonography (USG) had been recommended for the diagnosis of cholelithiasis under clinical settings.[4] The worldwide prevalence of cholelithiasis in children was approximately 0.15%–0.22%. Ganesh et al. had reported a lower prevalence of PC in Kanchi Kamakoti Child Trust Hospital; from 13,675 children treated at the institute, 43 (0.3%) were diagnosed with pediatric cholelithiasis. The gallbladder stones were of size <5 mm, and 56% of the cases involved solitary stones. Only 2 out of 43 children (5.7%) diagnosed with cholelithiasis presented with symptoms.[5] The publications for the epidemiology of PC remains unknown. This is partly because of the lack of symptoms in most cases. Thus, the present study was aimed to describe the characteristics of pediatric patients diagnosed with cholelithiasis.

## MATERIAL AND METHODS

This is a descriptive retrospective study of pediatric patients diagnosed and treated for cholelithiasis at the Pediatric Department of HSGH during January 1, 2016 to July 30, 2020. Secondary data was collected from medical records. Relevant data were collected for this study, which includeed sex, age, symptoms, medical history, diagnostic tests, and definitive management of patients.

## RESULTS

A total of 12 patients with cholelithiasis were treated during this period. The subjects included 5 boys (41.7%) and 7 girls (58.3%). The mean age of the patients was 10.75 years (0–18 years). Nine patients (75%) reported upper abdominal pain as the chief complaint. There were 2 patients (16.6%) who were admitted to the hospital for jaundice and 1 patient (8.4%) presented with abdominal distension.

According to their past medical history, 3 patients had thalassemia (25%), 2 had a past history of abdominal tuberculosis (16.5%), 1 had a past history of cholangitis (8.4%), 1 was obese (8.4%), and 1 had chronic bronchitis (8.4%). A total of 4 patients (33.3%) had no medical history.

Patients diagnosed with cholelithiasis underwent further supporting examination. Complete blood count and abdominal USG were performed in 8 patients (66.6%), and complete blood count without abdominal USG and computed tomography (CT) was performed in 3 patients (25%). One patient (8.4%) underwent an abdominal CT scan and complete blood count examination.

Varying definitive management was noted in the study. There wasa total of 7 patients (58.4%) who had received surgical treatment, 5 patients (41.7%) had received laparoscopic cholecystectomy, and 2 patients (16.7%) had received open cholecystectomy. There wasa total of 5 patients (41.6%) who refused to undertake any definitive treatment.

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**Table I: Characteristic Demography Patients**

| Parameter | n (%) | Mean | Median (Min-Max) |
|---|---|---|---|
| Gender | | | |
| Boy | 5 (41.7%) | | |
| Girl | 7 (58.3%) | | |
| Age | | 10.75 | 12 (0-18) |

**Table II:Chief complaint and previous medical history**

| Parameter | Proportion % |
|---|---|
| Chief complaint | |
| Abdominal pain | 75% |
| Jaundice | 16.6% |
| Abdominal distention | 8.4% |
| Previous medical history | |
| No past medical history | 33.3% |
| Thalassemia | 25% |
| TBC abdominal | 16.5% |
| Cholangitis | 8.4% |
| Obesity | 8.4% |
| Chronic bronchitis | 8.4% |

**Table III: Characteristic Diagnostic Examination**

| Parameter | Proportion % |
|---|---|
| complete blood count, USG | 66.6% |
| complete blood count, USG + CT Scan | 25% |
| complete blood count + CT Scan | 8.4% |

**Table IV: Management**

| Parameter | Proportion % |
|---|---|
| Surgical | 58.4% |
| Laparoscopic cholecystectomy | 33.3% |
| Open cholecystectomy | 16.7% |
| Conservative treatment | 41.6% |

## DISCUSSION

In all there were a total of 12 patients who were diagnosed with cholelithiasis, predominantly girls. The data in the present study was different from those of previous study by Gunawan et al.6 the latter study recorded a higher ratio of boys to girls (2.3:1) in cases of PC. The mean age of the patients was 10.75 years (0–18 years). The age demographics of this study were similar to those of a previous study by Bhasin et al.7who reported a mean age of pediatric patients with cholelithiasis as 10.1 years.

Upper right abdominal pain was the most commonly reported clinical symptom in our study. Bhasin et al.[7] recorded several symptoms associated with pediatric cholelithiasis: the recurrent upper right abdominal pain with or without nausea was recorded in 75% of the study population with the remainingsubjects showing varying symptoms, such as jaundice and failure to thrive. Thalassemia and anemia were commonly recorded in patients with PC, accounting for 25% of all patients.[7] Tannuri et al.[8] noted that 62.3% of the pediatric patients with cholelithiasis were previously diagnosed with a hemolytic disorder such as sickle cell anemia or thalassemia.

The most commonly used diagnostic tool to confirm cholelithiasis in our study was USG, with 83.2% of the patients diagnosed with it. Real-time USG examination is highly accurate, reaching 96% in diagnostic accuracy, for the diagnosis of gallbladder and liver disorders. Cholelithiasis is readily diagnosed with a relatively high degree of accuracy by USG.[4]

Surgical treatment was the mainstay of management for cases of PCin this study. In our study, 58.4% of the patients received cholecystectomy, while 33.3% received laparoscopic cholecystectomy. Over the last decade, laparoscopic cholecystectomy has attained the status of the standard for definitive management of cholelithiasis.[4] Lugo-Vicente et al. reported that 71% of all infants and children with cholelithiasis received laparoscopic cholecystectomy, while the remaining received conventional or open cholecystectomy. There are several advantages in opting for laparoscopic intervention for the treatment of cholelithiasis, including the minimally invasive nature of this procedure that reduces the risk of postoperative pain, reduces the length of stay, and creates only small surgical wounds.[9,10]

## CONCLUSION

Cholelithiasis is often asymptomatic; occasionally, some cases may present with visible symptoms that require surgical treatment. Most cases can be diagnosed with USG. Minimally, invasive procedures using laparoscopic cholecystectomy demonstrate fewer morbidities when compared with the conventional approach.


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# Circulating levels of Interferon-Gamma in patients with neovascular age-related macular degeneration in Yogyakarta

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## ABSTRACT

**Introduction:Neovascular age-related macular degeneration (nAMD) is a major factor contributing to blindness and impaired visual acuity in elderly people. The pathophysiology of nAMD involves excessive inflammation events in the macula. Thus, it is crucial to study the dynamics of an important pro-inflammatory cytokine, interferon-gamma (IFN-γ).**

**Materials and Methods: This research is aimed to investigate plasma IFN-Ɣ profiles of patients with nAMD. In this cross-sectional study, blood plasma samples of 16 patients with AMD and 23 age-matched controls were collected. Samples were examined for two inflammatory cytokines (IFN-Ɣ) using a commercially available enzyme-linked immunosorbent assay.Acquired data were log transformed to normalize any outliers before conducting student'st-test using the SPSS software.**

**Results: IFN-Ɣ levels were higher in the control group, without statistically significant difference between the two groups.**

**Conclusion: IFN-Ɣ levels were not significantly different between patients with AMD and controls.**

**KEYWORDS:**
*Age-related macular degeneration, inflammation, inflammatory cytokines, IFN-Ɣ, ELISA*

## INTRODUCTION

Neovascular age-related macular degeneration (nAMD) is a major risk factor for impaired central visual acuity and irreversible blindness.[1,2] Genetics plays a key role in the onset of nAMD. Epidemiological study conducted in Indonesia showed a strong association of rs11200638 High temperature requirement factor A1 (HTRA1),[3] rs10490924 HTRA1/Age-related maculopathy susceptibility 2 (ARMS2), del443ins54 ARMS2, and rs10490924 Complement factor H (CFH) polymorphisms with the onset of nAMD.[4] However, other studies have failed to show a correlation of interleukin-1beta (IL-1β)[5] and HTRA1[6] protein levels with nAMD pathogenesis.

The aetiology of nAMD is characterized by retinal swelling, which is caused by abnormal vascularization, leading to leaky blood vessel formation in the macula. Genetic and epidemiological evidence indicate the key role of inflammation in nAMD. An important pro-inflammatory cytokine, interferon-gamma (IFN-γ), is considered a vital factor in nAMD onset. Recent studies indicate an emerging relationship between IFN-γ and mechanisms underlying AMD pathogenesis. Alone or along with other pro-inflammatory factors such as IL-1β and tumour necrosis factor-alpha, IFN-γ appears to trigger inflammatory pathways[7] and its associated biomarkers, including the complement cascade,as well as recruit immune cells such as macrophages, microglia, natural killer, and Tcells.[8–11] Reportedly, in the affected eyes, cytokines were observed in the outer retina and drusen.[12–14] Excessive cytokines could compromise photoreceptors,[14,15] leading to central vision impairment or blindness. The pathways activated by IFN-γ are interconnected in a complicated manner and are not fully understood. Furthermore, clinical data related to IFN-γ and AMD therapy are limited. The possibility of using IFN-γ as target for AMD therapy remains debatable. Accordingly, the present research aimed to investigate IFN-γ levels in patients with nAMD and age-matched controls.

## MATERIAL AND METHODS

This cross-sectional case control study was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health and Nursing (FK-KMK) UGM, Universitas Gadjah Mada (approval no.: KE-FK-0215-EC-2021). Following screening, 38 patients with AMD and 16 age-matched control were included. Recruitment was conducted from January until August 2019. All participants understood and signed informed consent form before undergoing ophthalmic testing and blood collection. Only patients without other retinal or systemic diseases were included to rule out the effect of other disease. All patients underwent standard eye examination, including visual acuity assessment, fundus imaging, and optical coherence tomography to diagnose nAMD or verify the control eye group. A structured questionnaire was used to collect baseline data regarding lifestyle, including smoking status (active or

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**Table I: Characteristics of patients with AMD and age-matched control**

| Variables | nAMD (n:16; %) | Age-matched control (n:23/%) | *p*-value |
|---|---|---|---|
| Sex | | | |
| Male | 58 | 52 | 0.301 |
| Female | 42 | 46 | |
| Age (years) | | | |
| <59 | 23 | 8 | 0.235 |
| 60–69 | 35 | 52 | |
| 70–79 | 35 | 30 | |
| >80 | 5 | 8 | |
| Smoking status | | | |
| Yes | 23 | 30.5% | 0,23 |
| No | 76 | 69.5% | |
| Blood pressure (mmHg) | | | |
| Hypertension | 35.2% | 21.7% | 0,4 |
| Normal | 64.8% | 78.3% | |
| Cytokines (mean (±SD)) | | | |
| IFN-γ (pg/mL) | 14.88 (± 6.96) | 18.70 (± 17.93) | 0.087 |

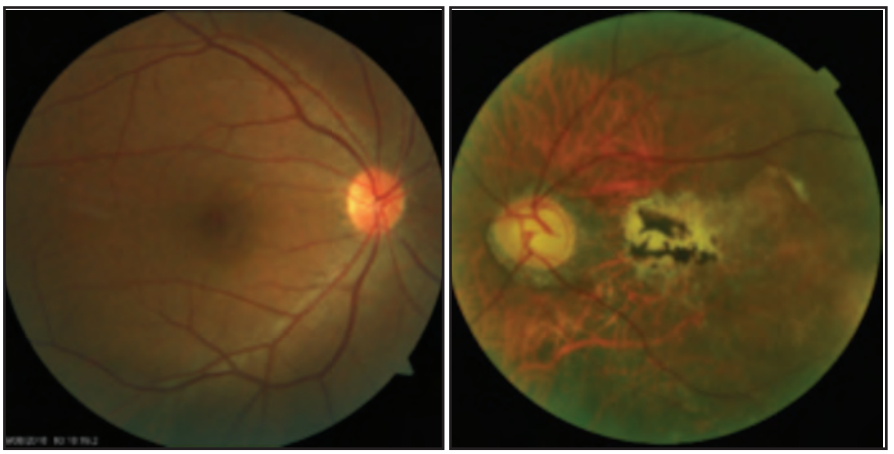

**Fig. 1:** Funduscopic image of the affected eye (left) and clear normal eye (right). Hemorrhage and neovascularization were observed in the affected eye.

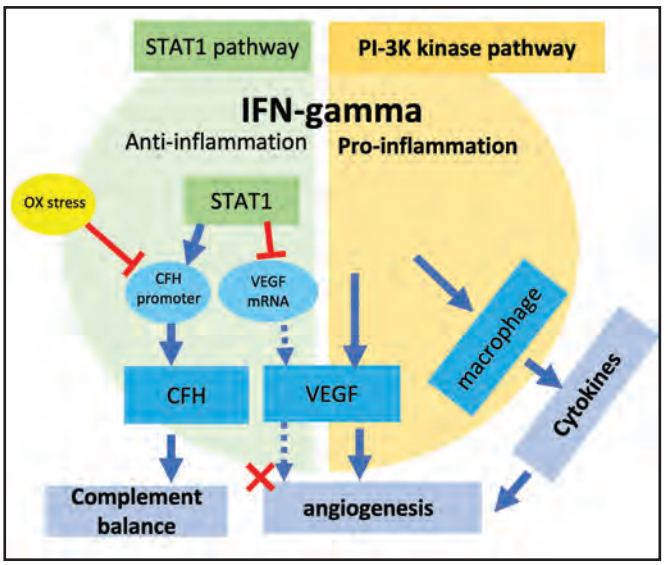


**Fig. 2:** Pro- and anti-inflammatory roles of IFN-γ in nAMD pathogenesis

passive) and indoor/outdoor working activity of AMD and control groups. The study was conducted at Dr. Sardjito Central General Hospital, Dr. S. Hardjolukito Air Force Main Hospital, and Dr. Yap Eye Hospital, Yogyakarta. ELISA was conducted at the Integrated Research Laboratory, FK-KMK, UGM.

Whole blood was centrifuged (1,000 ×g for 15 min), and the upper layer was collected to retrieve plasma. ELISA was performed in accordance with the manufacturer's protocol (Finetest®, Wuhan Fine Biotech, Wuhan, China). First, 100 µL of the plasma sample and the standard solution each was placed into 96-well plates, followed by incubation for 37°C for 90 min. After discarding the mixture and standard,the empty plate was washed twice, and 100 µLof Biotin was added to each well before incubating the plate at 37°C for 60 min. Next, the mixture was discarded, and the plate was washed three times. Subsequently, streptavidin conjugate buffer was added before incubating at 37°C for 30 min. Finally, the mixture was discarded, and the plate was washed five times. Then, 90 µLof TMB substrate buffer was added before incubating the plate at 37°C for 10–20 min (depending on the change in color) in the dark. Immediately after the color accurately changed according to the standard chart, 50 µLof

stop solution was added. Then,using a microplate reader,the optical density at 450 nm obtained, which was interpolated into nanogram per milliliter using the CurveExpert 1.4 software.

All acquired data were analyzed using the Mann–Whitney Test (SPSS) for unevenly distributed data. The obtained results were expressed as means and standard deviations with 95% confidence interval (CI) to determineany association between IFN- γ level and the incidence of AMD.

## RESULTS

All patients with AMD or age-matched controls were diagnosed by an ophthalmologist (Fig. 1). For statistical analysis, the data from 16 patients with AMD and 23 age-matched controls were used.

In the nAMD and control groups, the mean value of circulating IFN-y was 14.88 ± 6.96pg/mLand 18.70 ± 17.93pg/mL, respectively. There was no positive association between cytokine level and AMD ($p$> 0.05, 95% CI:12.650 – 21.606) (Table I).

Baseline parameterswere similar in the case and control groups. The majority of subjects were non-smokers (76% in nAMD group and 69.5% in control group) and had normal blood pressure (64.8 mmHg in nAMD group and 78.3 mmHg in control group).

## DISCUSSION

To our knowledge, this is the first study reporting the association between circulating IFN-γ level and neovascular AMD in an Indonesian population. The results suggest that IFN-γ levels were not associated with AMD. Patients with AMD had lower IFN-γ cytokine level than controls. However, there was no association between IFN-γ cytokine level and nAMD onset (p>0.05, 95% CI).

In exudative nAMD, it is not clear what the role of IFN-gamma is, since IFN-γ can either maintain or inhibit inflammation in different ways (Figure 2). Several reports have demonstrated the role of IFN-γ in initiating immunomodulatory and protective functions. However, IFN-γ was reported as a pro-inflammatory factor in nAMD.[8,16] In addition, IFN-γ inhibits the angiogenic activity of VEGF via the activation of the STAT1 pathway in human endothelial cells1[7], while also down-regulating Vascular endothelial growth factor (VEGF) mRNA in a dose-dependent manner18. This process might be of potential in AMD therapy. Therefore, IFN-γ-associated STAT1 activation may be beneficial.

Interestingly, another study has suggested that IFN-γ can mediate VEGF expression in Retinal pigment epithelium (RPE) cells through the PI-3K/Akt/mTOR/p70 S6 kinase pathway and is independent of STAT1.[19] Another piece of evidence comes from the study demonstrating IFN-γ up-regulating CFH expression in RPE cells.[20] CFH can keep the complement cascade in check and prevent tissue injury from excessive complement activation.[21] CFH is transcriptionally upregulated by STAT1; however, oxidative stress, one of the most important risk factors for AMD, can disrupt this process by acetylating FOXO3, which competes with STAT1 for binding to the CFH promoter.[22,23] Reportedly, STAT1-deficient mice were highly susceptible to autoimmune disorders[24], and considering this response pattern, AMD may be considered an autoimmune disease.[25,26]

One limitation of this study was that only patients with nAMD who were only in the most severe stage of the disease were included. At this stage, IFN-γ may no longer play an important role in inducing or maintaining AMD condition. Further studies should recruit patients in the early and intermediate stages of AMD to compare cytokines levels. Our findings may also be limited due to the lack of samples in the study groups. Future studies with larger number of samples are required to validate and better understand the association between IFN-γconcentration and AMD incidence.

## CONCLUSION

Although insignificant, higher concentrations of IFN-γ were observed in the control group than in the AMD group.


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# Network pharmacology for deciphering molecular mechanism of mahogany in dyslipidemia treatment of menopausal conditions

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**ABSTRACT**

**Introduction: Estrogen deficiency during menopause is associated with pathological menopausal syndromes and metabolic disorders, including dysregulated lipid metabolism (dyslipidemia). Mahogany is a promising material for use in an alternative treatment for preventing dyslipidemia during menopause. This study investigated the potency of mahogany compounds and their molecular mechanism as an alternative treatment for preventing dyslipidemia during menopause.**

**Method and Materials: The determination of the potential of the compounds of interest was performed by machine learning using KNIME software to identify the potential compounds for HMG-CoA Reductase (HMGCR) inhibitor. Target genes for a potential mahogany compound were obtained from Swiss Target Prediction. Analysis of the KEGG-Pathways, Gene Ontology profiling, and protein–protein interaction networks of the target gene were analyzed.**

**Results: We identified five compounds: β-sitosterol, swietemacrophyllanin, 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chroman-4-on (7-HMC), scopoletin, and stigmasterol as candidates for HMGCR inhibitors. The target prediction results mined 294 genes. The top 20 hub genes with the highest degree included MAPK1/3, PI3K/AKT, ER1, and mTOR, which played a role in lipid metabolism.**

**Conclusion: The possible molecular mechanisms mainly involved direct inhibitory pathway of HMGCR and an indirect inhibitory pathway of HMGCR. The tidal inhibitory pathway was Indirectly mediated via the PI3K/AKT, MAPK1/3, MTOR, ER1, and SREBP1/2 signaling pathways. Further investigation is warranted to validate the results of the present study.**

**KEYWORDS:**
*Mahogany, Dyslipidemia, Menopause, HMG-CoA Reductase, and Bioinformatics*

## INTRODUCTION

Menopause is a condition of the cessation of the menstrual cycle in women as a result of aging of the reproductive organs.[1] A new hormonal pattern is established at menopause, which is characterized by low estrogen level.[2] Estrogen is a steroid hormone produced primarily in the ovaries that contributes to the regulation of some physiological mechanisms. Estrogen deficiency during menopause is associated with pathological menopausal syndromes, psychiatric disorders, cardiovascular diseases (CVDs), cancer, and metabolic disorders, including dysregulated lipid metabolism or dyslipidemia.[3] Hormone replacement therapy (HRT) using estrogen synthesis has been the primary treatment for preventing menopausal symptoms, CVD, and dyslipidemia.[4] However, clinical studies have demonstrated that HRT increases the risks of endometrial and breast cancers and hence cannot be used as an idealized therapy.[5] Seeking alternative dietary estrogenic compounds is believed to be crucial for the prevention of menopause-associated metabolic syndromes.[6]

Decreasing level of estrogen during menopause causes dyslipidemia through multiple mechanisms.[7] Decreased level of estrogen increases the hepatic HMG-CoA reductase (HMGCR) gene expression and activation, leading to the elevation of the serum cholesterol levels.[8] HMGCR is a rate-limiting enzyme that plays a pivotal role in cholesterol biosynthesis. Dysregulation of lipid metabolism during menopause can alter the level of various lipids circulating in the blood, such as lipoproteins, apolipoproteins, low-density lipoproteins (LDLs) high-density lipoproteins (HDL), and triacylglycerol (TG).[9]

Mahogany *(Swietenia macrophylla and Swietenia mahogany)* has the potential resource of phytoestrogen[10] Mahogany has been used in the preparation of traditional medicine in Asia to treat hypertension, diabetes, and pain.[11] Chemical compounds in mahogany include flavonoids, saponins, alkaloids, steroids, and tannins. Recent studies have demonstrated that mahogany seeds have an estrogenic activity that can increase the uterine weight and bone density, prolong the estrus phase, and increase the breast gland proliferation in ovariectomized rats.[12] Another study also showed that compound contained in the mahogany extract could decrease the cholesterol, triglyceride, low-density lipoprotein, increased high-density lipoprotein, and reduced atherogenic indexes.[13-15] According to the study performed by Kalpana and Pugalendi, the *in vivo* study of

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ethanolic extract of mahogany seeds on rats proved reduction in the level of total cholesterol, triglyceride, LDL, and VLDL and increase in the level of HDL.[15] This finding suggests that mahogany offers potential efficacy for preventing menopause syndrome and hyperlipidemia that often occur during menopause.

Mahogany is a promising plant for use in preventing dyslipidemia during menopause. However, the detailed therapeutic targets and signaling mechanisms behind the benefits of mahogany remain unknown.[13] Cholesterol in our body can arise from *de novo* synthesis in our cells or obtained through food consumption.[16] In the process of cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (HMGCR) catalyzes HMG-CoA conversion to mevalonate. It is known as a rate-limiting enzyme in cholesterol synthesis, signifying its important role. The multivalent system regulates HMGCR through various mechanisms.[17]

This study was conducted to investigate the potency of mahogany compound's molecular targets and signaling mechanisms as an alternative treatment for preventing dyslipidemia during menopause by focusing on the indirect effects. In this study, bioinformatics analysis was performed to investigate the potential compound of mahogany that can inhibit HMGCR, an enzyme that plays an essential role in lipid metabolism. The use of bioinformatics analysis can simplify and organize bioinformatics data such that it allows researchers to access existing information and submit new entries as they are produced and make new conclusions and understand new possibilities. In conclusion, we identified and developed potential compound candidates and predicted their molecular target and molecular signaling role in lipid metabolism.

## MATERIAL AND METHODS

*Prediction inhibition activity prediction using KNIME*

The prediction model for the inhibition of mahogany compounds against HMGCR was developed using the dataset inhibitor compound activity against HMGCR obtained from ChEMBL (https://www.ebi.ac.uk/chembl), which were entered in CHEMBL ID and analyzed by the KNIME version 4.3.1 with the pipeline/workflow created. Mahogany metabolite profiles were analyzed to predict the inhibitory activity on HMGCR based on the prediction model prepared. The outputs produced were the "ROC curve" and "Prediction value," which provided method validation and prediction value of mahogany's metabolites. Furthermore, the potential compounds in mahogany's metabolites were selected by identifying the compounds that predicted the inhibitory activity based on the machine learning (ML) prediction outcomes.[18]

*Target prediction and collection*

The potential compounds of mahogany metabolites were analyzed for their predictive target genes using online databases based on the SMILES code. The database used included Swiss Target Prediction (http://www.swisstargetprediction.ch) with the default settings. The total target genes were collected, combined, and then sliced using the InteractiVenn (http://www.interactivenn.net) to ensure no duplicity in the target.[19]

*Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis*

KEGG pathways and GO enrichment analysis was conducted to explore the potential functions of the essential protein targets of mahogany metabolites with the Database for Annotation, Visualization, and Integrated Discovery (DAVID). GO analysis involved three categories: biological process (BP), cellular component (CC), and molecular function (MF). $P < 0.05$ was set as the thresholds for significant enrichment analysis.[20]

*Protein–protein interaction (PPI) network of predictive biomarkers and hub gene construction*

The PPI data were obtained from the Search Tool for the Retrieval of Interacting Genes (STRING) database (https://string-db.org), which can predict interactions among proteins. The prediction method of this database was derived from experiments, databases, and text mining of the neighborhood, gene fusion, co-occurrence, and co-expression. In the present study, PPIs with combined scores >0.4 were selected for further research. The Cytoscape software (http://www.cytoscape.org) was used to establish a PPI network. In addition, the Cytohubba was applied to analyze the PPI network to obtain the top 20 nodes that were considered hub genes according to the degree value.[21]

## RESULTS

*Predicted compounds of mahogany showed inhibitory activity against HMG-CoA reductase*

The study of mahogany metabolites on predicting HMGCR inhibition was performed using the KNIME software by predicting the inhibitory activity of one or more compounds at once against the targeted protein (mahogany-stated inhibition of HMGCR). The prediction model was constructed with the KNIME software by adapting the Teach-Open CADD pipeline.[18] The dataset for constructing the prediction model was a dataset of HMGCR inhibitory activity, which is provided in ChEMBL with ID dataset CHEMBL407. Based on the MACCS fingerprint of the inhibitor compounds from the dataset, ML had mapped all compound fingerprints to prepare a predictive model of the inhibitory activity from the targeted protein.[22] The HMGCR inhibitory compound data set was split into active and inactive compounds and used to train ML classifiers based on two ML algorithms: Random Forest (RF) and Artificial Neural Network (ANN). Validation of the prediction models from two different MLs suggested that all prediction models had an overall accuracy value >90% (RF 92.07% and ANN 90.24%) and ROC curve p scores >0.75 (RF 0.932 dan ANN 0.879). These results indicated that the prediction model was valid and could predict the inhibitory activity of compounds in mahogany.[23]

The results of the inhibitory activity prediction revealed that potential compounds of mahogany metabolites inhibited HMGCR. Based on the prediction model under ML algorithms, ANN showed 5 compounds, namely, β-sitosterol, swietemacrophyllanin, 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chroman-4-on (7HMC), scopoletin, and stigmasterol, which were predicted to possess inhibitory

activity against HMGCR based on the number of predictive values being 1.00 and 0.99. Analyzing the RF algorithm presented the same result as that with ANN, with the highest predictive value of 0.9 for scopoletin (Table I). These inhibitory activities were predicted based on the likeness of structure and inhibitory activity with those of known inhibitor compounds processed by the RF and ANN algorithms.

*Targeted genes prediction of mahogany potential compounds*
Predictive target genes of each potential mahogany metabolites using databases SwissTargetPrediction obtained from the default settings. We obtained 100 targets for each compound (Fig. 1). A Venn diagram was used to determine all compounds' total targeted genes to ensure no duplication of the targets. A total of 294 genes were known predicted as the target of the 5 potential compounds (Table II and Table III).

*KEGG pathway and GO enrichment analysis*
A total of 294 common targets were subjected to the DAVID v6.8 database for gene enrichments. GO and KEGG gene enrichment results were put in order according to the percentage of genes. The top 5 GO results in the KEGG pathway, biological process, cellular component, and molecular function were recorded.

KEGG pathway enrichment analysis revealed that the genes were regulated in the cancer signaling pathways, PI3K-Akt, neuroactive ligand-receptor interaction, proteoglycan in cancer, and viral carcinogenesis pathway (Table IV). The results for GO analysis were evaluated through the related term option of the DAVID database. According to biological process results, protein phosphorylation, signal transduction, response to drug, oxidation-reduction process, and positive regulation of transcription from RNA polymerase II promoter showed higher targeted numbers in the count. Molecular function results with higher targeted numbers included protein binding, ATP binding, protein kinase activity, protein serine/threonine kinase activity, and zinc ion binding, which were found to be related to at least one of the KEGG pathways such as the cancer signaling pathways, PI3K-Akt, neuroactive ligand-receptor interaction, proteoglycan in cancer, and viral carcinogenesis pathway. Moreover, based on the analysis, most of the genes were present in the cellular components of the plasma membrane, nucleus, cytoplasm, and integral component of membrane.

*PPI network construction and hub gene selection*
Construction of the PPIs network among the targeted genes of mahogany compounds and identification of the most significant modules using the online tool STRING with a cutoff score of ≥0.4. A total of 294 genes were constructed to the PPI network complex containing 294 nodes and 3150 edges, with an average node degree of 22.2, an average local clustering coefficient of 0.465, and a PPI enrichment p-value of <1.0e–16 (Fig. 2a). Using the cytoHubba plugin Cytoscape, we detected the top 20 targeted genes with the uppermost degree score, including AKT1, MAPK3, EGFR, SRC, MAPK1, VEGFA, SKP90AA1, ESR1, PTGS2, MTOR, PIK3CA, APP, MMP9, MAPK14, AR, ERBB2, MDM2, KDR, BCL2L1, and STAT1 (Fig 2b, Table V).

## DISCUSSION

The present study investigated the potency of mahogany compounds and their molecular targets and signaling mechanism as an alternative treatment for preventing dyslipidemia during menopause through bioinformatics analyses. This study was conducted to determine which compounds were responsible for the anti-dyslipidemic effect of mahogany. Moreover, the predictive targeted genes of the compounds showed inhibit activity against HMGCR under hyperlipidemia conditions. It uses data on HMGCR inhibitor compounds that have been studied, which is then used to perform ML on the fingerprint pattern of the inhibitor compounds for the prediction of compounds in mahogany with inhibitory activity against HMGCR.[18] Past analysis suggests β-sitosterol, swietemachropyllanin, 7-HMC, scopoletin, and stigmasterol as compounds that can be suspected to be responsible for the anti-dyslipidemia effect of mahogany through the inhibition of HMGCR (Table I). Targeted genes' exploration predicted 294 genes is the target of the 5 mahogany potential metabolites. Twenty potential hub genes including MAPK, AKT1, PI3K, MTOR, and ER1, and others were screened as effective targets of mahogany compounds (Fig. 2, Table V).

Past studies have shown that the ethanolic extract of the seeds, leaves, and bark of mahogany tree possesses hypolipidemic activity and can reduce the HMGCR activity.[24] HMGCR is a rate-limiting enzyme of cholesterol biosynthesis. HMGCR inhibitors (ex, statins) are currently the mainstay of treatment for dyslipidemia. Nevertheless, during the menopause condition, treatment with only a single statin cannot alleviate the other menopausal syndromes.[25] Under physiological conditions, HMGCR activity is regulated by multiple mechanisms. At the transcriptional level, the expression of the *HMGCR* gene is regulated by the sterol regulatory element-binding proteins (SREBP).[26] SREBPs can be mainly categorized as SREBP1 and SREBP2, where every subunit plays an essential role in lipid and cholesterol biosynthesis. SREBP1 activates mostly those genes that are related to fatty acid biosynthesis or carbohydrate metabolism, while SREBP2 primarily activates cholesterol synthesis-related genes.[27] SREBP2 was also predicted as a

**Table I: The predicted value of HMG-CoA reductase inhibitory activity of mahogany metabolites**

| No | Compound | Prediction value (1.0) | |
|---|---|---|---|
| | | ANN | RF |
| 1 | β-sitosterol | 0.99 | 0.84 |
| 2 | Swietemacrophyllanin | 1.00 | 0.79 |
| 3 | (7HMC) | 1.00 | 0.86 |
| 4 | Scopoletin | 1.00 | 0.9 |
| 5 | Stigmasterol | 1.00 | 0.83 |

**Table II: Prediction 100 protein target of a potential mahogany compound through Swiss Target Prediction**

| No | B-sitosterol | Swietemacrophyllanin | 7HMC | Stigmasterol | Scopoletin |
|---|---|---|---|---|---|
| 1 | AR | PGF | CYP1B1 | AR | CA7 |
| 2 | HMGCR | VEGFA | CYP19A1 | NPC1L1 | CA12 |
| 3 | CYP51A1 | MMP2 | TAS2R31 | HMGCR | CA9 |
| 4 | NPC1L1 | MMP9 | CA7 | CYP51A1 | CA13 |
| 5 | NR1H3 | SQLE | CA12 | CYP19A1 | CA1 |
| 6 | CYP19A1 | BACE1 | CA4 | NR1H3 | CA14 |
| 7 | CYP17A1 | CA5B | ADORA1 | CYP17A1 | CA4 |
| 8 | RORC | MMP13 | ADORA3 | RORC | EGFR |
| 9 | ESR1 | MMP12 | ESR1 | ESR1 | CA5A |
| 10 | ESR2 | PTGS1 | ESR2 | ESR2 | XDH |
| 11 | SREBF2 | CA2 | MAOB | SHBG | CA6 |
| 12 | SHBG | CA1 | HSD17B1 | SREBF2 | CA2 |
| 13 | SLC6A2 | DNM1 | MMP13 | ACHE | SRD5A1 |
| 14 | CYP2C19 | CA4 | ABCG2 | CYP2C19 | CBR1 |
| 15 | RORA | CA7 | ABCC1 | PTPN1 | CDK2 CCNA1 CCNA2 |
| 16 | PTPN1 | CA12 | SHBG | SLC6A2 | MAOA |
| 17 | BCHE | CA9 | CBR1 | BCHE | ESR2 |
| 18 | SERPINA6 | CYP19A1 | MMP12 | RORA | ALOX5 |
| 19 | SLC6A4 | HIF1A | PTGS1 | SERPINA6 | CCND1 CDK4 |
| 20 | CHRM2 | BCL2 | CA2 | SLC6A4 | FLT4 |
| 21 | VDR | MMP14 | CA1 | CHRM2 | INSR |
| 22 | ACHE | SYK | CA6 | G6PD | PTK2 |
| 23 | G6PD | MAPT | CA5A | VDR | PLK1 |
| 24 | NR1H2 | TERT | CA3 | NR1H2 | TEK |
| 25 | GLRA1 | PGD | APP | HSD11B1 | MAP3K8 |
| 26 | CES2 | ST3GAL3 | KLK1 | PTGER1 | HSPA1A |
| 27 | PTGER1 | FUT7 | KLK2 | PTGER2 | NUAK1 |
| 28 | PTGER2 | FUT4 | PLA2G1B | CDC25A | FGR |
| 29 | HSD11B1 | PDK1 | ACHE | GLRA1 | CA5B |
| 30 | PTGES | ABCB1 | CHRNA7 | CES2 | AKR1C1 |
| 31 | CDC25A | PIK3CA PIK3R1 | BACE1 | PTGES | DAO |
| 32 | PPARA | PIK3CD PIK3R1 | SRC | PPARA | GSK3B |
| 33 | PPARD | PIK3R1 PIK3CB | SGK1 | PPARD | KCNA3 |
| 34 | DHCR7 | MTOR | ERN1 | SQLE | PTGS2 |
| 35 | SQLE | PIK3CG | RPS6KB1 | DHCR7 | GSR |
| 36 | PTPN6 | PIK3CA | AURKA | NR1I3 | HSD17B3 |
| 37 | NR1I3 | MET | POLB | PTPN6 | SRC |
| 38 | FDFT1 | CA3 | CA9 | NR3C1 | KDR |
| 39 | SIGMAR1 | CA6 | CA13 | CDC25B | ACHE |
| 40 | NOS2 | CA5A | CA5B | NOS2 | CYP1A2 |
| 41 | NR3C1 | ALPL | GRM2 | HSD11B2 | NAT1 |
| 42 | PPARG | PLAA | PLA2G5 | TBXAS1 | MAOB |
| 43 | CDC25B | FCER2 | EDNRA | PPARG | PTPN1 |
| 44 | UGT2B7 | TYMP | MET | UGT2B7 | ESR1 |
| 45 | HSD11B2 | PRKDC | MMP2 | POLB | MB |
| 46 | POLB | MAPK3 | NOX4 | PREP | PARP1 |
| 47 | PREP | MAPK1 | PLG | SIGMAR1 | KCNMA1 |
| 48 | PTGER4 | PCNA | MMP3 | PTGER4 | ERBB2 |
| 49 | IDO1 | POLB | MMP7 | IDO1 | SQLE |
| 50 | DRD2 | FBP1 | PPARG | MDM4 | BACE1 |
| 51 | TBXAS1 | MMP7 | MMP9 | DRD2 | MET |
| 52 | ATP12A | MMP8 | SLC5A2 | MDM2 | COMT |
| 53 | MDM4 | GABRA1 GABRB2 GABRG2 | BCL2 | ATP12A | GPR35 |
| 54 | MDM2 | PLA2G2A | CDK2 CCNA1 CCNA2 | MGLL | CDK9 CCNT1 |
| 55 | PTGIR | PLA2G5 | DNM1 | PTGIR | AKR1C3 |
| 56 | DHCR7 EBP | PLA2G10 | DYRK1A | DHCR7 EBP | ALPG |
| 57 | FABP4 | GAK | RXRA | FABP4 | PLAA |
| 58 | TERT | ERN1 | BCHE | FABP3 | AKR1B1 |
| 59 | FABP3 | HDAC2 | HSD17B2 | FABP5 | AURKA |
| 60 | FABP5 | P2RX3 | CHEK1 | FABP1 | CHRM1 |
| 61 | FABP1 | CYP1B1 | CDK2 | BACE2 | AOC3 |
| 62 | ADORA3 | PIM1 | CDC7 | ADORA3 | AURKB |
| 63 | MAPK3 | PIM2 | KDR | MAPK3 | CISD1 |
| 64 | PTPN11 | PIM3 | BRAF | PTPN11 | AKT1 |
| 65 | AKR1B10 | EDNRA | ODC1 | AKR1B10 | PLEC |
| 66 | PRKCG | KLK1 | BCL2L1 | CCR1 | CSNK1A1 |
| 67 | PRKCD | KLK2 | CLK1 | GABBR1 | CSNK1D |
| 68 | PRKCB | ADAM17 | DYRK1B | BACE1 | HMGCR |
| 69 | PRKCE | NCOR2 HDAC3 | DNMT1 | PTPRF | ALPL |

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**Table II: Prediction 100 protein target of a potential mahogany compound through Swiss Target Prediction**

| No | B-sitosterol | Swietemacrophyllanin | 7HMC | Stigmasterol | Scopoletin |
|---|---|---|---|---|---|
| 70 | PRKCQ | HDAC5 | PGD | PLA2G1B | BRAF |
| 71 | PTPRF | HDAC7 | ST3GAL3 | ACP1 | ALDH5A1 |
| 72 | PLA2G1B | PIK3CB | FUT7 | SCD | ABAT |
| 73 | ACP1 | HDAC4 | FUT4 | MAPK14 | CSNK2A1 |
| 74 | IGF1R | HDAC10 | STAT1 | GRM2 | APEX1 |
| 75 | SRC | HDAC3 | SQLE | ACACB | CXCR1 |
| 76 | KDR | PLG | MMP8 | MTNR1A | PIK3CG |
| 77 | ALK | PLAU | CDK4 | MTNR1B | CLK1 |
| 78 | SCD | PLA2G7 | CES1 | TOP2A | DYRK1B |
| 79 | MGLL | CHRM1 | SERPINE1 | IGF1R | IGF1R |
| 80 | BACE1 | TOP1 | ABCB1 | SRC | PTPRC |
| 81 | CCR1 | CDK2 | RPS6KA3 | KDR | GRK6 |
| 82 | TOP2A | CDK1 | CHEK2 | ALK | TNNC1 TNNT2 TNNI3 |
| 83 | GABBR1 | HSP90AA1 | RPS6KA1 | PRKCG | EPHB4 |
| 84 | BACE2 | KCNH2 | WEE1 | PRKCD | PDGFRB |
| 85 | MAPK14 | JAK3 | BMP1 | PRKCB | PLK4 |
| 86 | F11 | JAK1 | OPRK1 | PRKCE | LYN |
| 87 | PTGFR | SCN9A | DUSP3 | PRKCQ | PNP |
| 88 | PTGER3 | CCNE2 CDK2 CCNE1 | HDAC5 | PTPN2 | TAAR1 |
| 89 | PTGDR | LCK | HDAC7 | KCNA5 | PIM3 |
| 90 | TOP1 | ITK | HDAC4 | TACR1 | HTR2B |
| 91 | HIF1A | JAK2 | PNP | PTGFR | TERT |
| 92 | ACACB | TXK | YWHAG | PTGER3 | CA3 |
| 93 | PRKCH | F11 | CES2 | PTGDR | JAK1 |
| 94 | PTPN2 | PNMT | AKR1C3 | AGTR1 | JAK2 |
| 95 | TACR1 | KDR | TYMS | CNR1 | TYK2 |
| 96 | MTNR1A | FGFR1 | ECE1 | TOP1 | F2 |
| 97 | MTNR1B | HSD17B2 | MMP14 | HIF1A | CHEK1 |
| 98 | NPY5R | HSD17B3 | GABRA1 GABRB2 GABRG2 | F11 | WEE1 |
| 99 | SLC22A6 | ESR1 | PTPN1 | HTR2B | ADRA2A |
| 100 | MAP3K14 | ESR2 | TERT | AVPR1A | ADRA2C |

**Table III: The total of 294 genes were known predicted as the target of the 5 potential compounds**

| No | Genes | No | Genes | No | Genes |
|---|---|---|---|---|---|
| 1 | *FDFT1* | 101 | *NAT1* | 201 | *CDC25A* |
| 2 | *PRKCH* | 102 | *MB* | 202 | *PPARA* |
| 3 | *NPY5R* | 103 | *PARP1* | 203 | *PPARD* |
| 4 | *SLC22A6* | 104 | *KCNMA1* | 204 | *DHCR7* |
| 5 | *MAP3K14* | 105 | *ERBB2* | 205 | *PTPN6* |
| 6 | *PGF* | 106 | *COMT* | 206 | *NR1I3* |
| 7 | *VEGFA* | 107 | *GPR35* | 207 | *SIGMAR1* |
| 8 | *SYK* | 108 | *CDK9 CCNT1* | 208 | *NOS2* |
| 9 | *MAPT* | 109 | *ALPG* | 209 | *NR3C1* |
| 10 | *PDK1* | 110 | *AKR1B1* | 210 | *CDC25B* |
| 11 | *PIK3CA PIK3R1* | 111 | *AOC3* | 211 | *UGT2B7* |
| 12 | *PIK3CD PIK3R1* | 112 | *AURKB* | 212 | *HSD11B2* |
| 13 | *PIK3R1 PIK3CB* | 113 | *CISD1* | 213 | *PREP* |
| 14 | *MTOR* | 114 | *AKT1* | 214 | *PTGER4* |
| 15 | *PIK3CA* | 115 | *PLEC* | 215 | *IDO1* |
| 16 | *FCER2* | 116 | *CSNK1A1* | 216 | *DRD2* |
| 17 | *TYMP* | 117 | *CSNK1D* | 217 | *TBXAS1* |
| 18 | *PRKDC* | 118 | *ALDH5A1* | 218 | *ATP12A* |
| 19 | *MAPK1* | 119 | *ABAT* | 219 | *MDM4* |
| 20 | *PCNA* | 120 | *CSNK2A1* | 220 | *MDM2* |
| 21 | *FBP1* | 121 | *APEX1* | 221 | *PTGIR* |
| 22 | *PLA2G2A* | 122 | *CXCR1* | 222 | *DHCR7 EBP* |
| 23 | *PLA2G10* | 123 | *PTPRC* | 223 | *FABP4* |
| 24 | *GAK* | 124 | *GRK6* | 224 | *FABP3* |
| 25 | *HDAC2* | 125 | *TNNC1 TNNT2 TNNI3* | 225 | *FABP5* |
| 26 | *P2RX3* | 126 | *EPHB4* | 226 | *FABP1* |
| 27 | *PIM1* | 127 | *PDGFRB* | 227 | *PTPN11* |
| 28 | *PIM2* | 128 | *PLK4* | 228 | *AKR1B10* |
| 29 | *ADAM17* | 129 | *LYN* | 229 | *PRKCG* |
| 30 | *NCOR2 HDAC3* | 130 | *TAAR1* | 230 | *PRKCD* |
| 31 | *PIK3CB* | 131 | *TYK2* | 231 | *PRKCB* |
| 32 | *HDAC10* | 132 | *F2* | 232 | *PRKCE* |

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**Table III: The total of 294 genes were known predicted as the target of the 5 potential compounds**

| No | Genes | No | Genes | No | Genes |
|---|---|---|---|---|---|
| 33 | *HDAC3* | 133 | *ADRA2A* | 233 | *PRKCQ* |
| 34 | *PLAU* | 134 | *ADRA2C* | 234 | *PTPRF* |
| 35 | *PLA2G7* | 135 | *KCNA5* | 235 | *ACP1* |
| 36 | *CDK1* | 136 | *AGTR1* | 236 | *ALK* |
| 37 | *HSP90AA1* | 137 | *CNR1* | 237 | *SCD* |
| 38 | *KCNH2* | 138 | *AVPR1A* | 238 | *MGLL* |
| 39 | *JAK3* | 139 | *MMP2* | 239 | *CCR1* |
| 40 | *SCN9A* | 140 | *MMP9* | 240 | *TOP2A* |
| 41 | *CCNE2 CDK2 CCNE1* | 141 | *MMP13* | 241 | *GABBR1* |
| 42 | *LCK* | 142 | *MMP12* | 242 | *BACE2* |
| 43 | *ITK* | 143 | *PTGS1* | 243 | *MAPK14* |
| 44 | *TXK* | 144 | *DNM1* | 244 | *PTGFR* |
| 45 | *PNMT* | 145 | *BCL2* | 245 | *PTGER3* |
| 46 | *FGFR1* | 146 | *MMP14* | 246 | *PTGDR* |
| 47 | *TAS2R31* | 147 | *PGD* | 247 | *ACACB* |
| 48 | *ADORA1* | 148 | *ST3GAL3* | 248 | *PTPN2* |
| 49 | *HSD17B1* | 149 | *FUT7* | 249 | *TACR1* |
| 50 | *ABCG2* | 150 | *FUT4* | 250 | *MTNR1A* |
| 51 | *ABCC1* | 151 | *ABCB1* | 251 | *MTNR1B* |
| 52 | *APP* | 152 | *MMP7* | 252 | *PIK3CG* |
| 53 | *CHRNA7* | 153 | *MMP8* | 253 | *ALPL* |
| 54 | *SGK1* | 154 | *GABRA1 GABRB2 GABRG2* | 254 | *PLAA* |
| 55 | *RPS6KB1* | 155 | *PLA2G5* | 255 | *PIM3* |
| 56 | *NOX4* | 156 | *ERN1* | 256 | *CHRM1* |
| 57 | *MMP3* | 157 | *CYP1B1* | 257 | *JAK1* |
| 58 | *SLC5A2* | 158 | *EDNRA* | 258 | *JAK2* |
| 59 | *DYRK1A* | 159 | *KLK1* | 259 | *HSD17B3* |
| 60 | *RXRA* | 160 | *KLK2* | 260 | *GRM2* |
| 61 | *CDC7* | 161 | *HDAC5* | 261 | *SHBG* |
| 62 | *ODC1* | 162 | *HDAC7* | 262 | *BCHE* |
| 63 | *BCL2L1* | 163 | *HDAC4* | 263 | *CES2* |
| 64 | *DNMT1* | 164 | *PLG* | 264 | *PPARG* |
| 65 | *STAT1* | 165 | *CDK2* | 265 | *ADORA3* |
| 66 | *CDK4* | 166 | *HSD17B2* | 266 | *PLA2G1B* |
| 67 | *CES1* | 167 | *MAOB* | 267 | *HMGCR* |
| 68 | *SERPINE1* | 168 | *CBR1* | 268 | *IGF1R* |
| 69 | *RPS6KA3* | 169 | *AURKA* | 269 | *MAPK3* |
| 70 | *CHEK2* | 170 | *CA13* | 270 | *F11* |
| 71 | *RPS6KA1* | 171 | *CDK2 CCNA1 CCNA2* | 271 | *TOP1* |
| 72 | *BMP1* | 172 | *CHEK1* | 272 | *HIF1A* |
| 73 | *OPRK1* | 173 | *BRAF* | 273 | *CYP19A1* |
| 74 | *DUSP3* | 174 | *CLK1* | 274 | *POLB* |
| 75 | *YWHAG* | 175 | *DYRK1B* | 275 | *TERT* |
| 76 | *TYMS* | 176 | *WEE1* | 276 | *CA5B* |
| 77 | *ECE1* | 177 | *PNP* | 277 | *CA2* |
| 78 | *CA14* | 178 | *AKR1C3* | 278 | *CA1* |
| 79 | *EGFR* | 179 | *HTR2B* | 279 | *CA4* |
| 80 | *XDH* | 180 | *AR* | 280 | *CA7* |
| 81 | *SRD5A1* | 181 | *CYP51A1* | 281 | *CA12* |
| 82 | *MAOA* | 182 | *NPC1L1* | 282 | *CA9* |
| 83 | *ALOX5* | 183 | *NR1H3* | 283 | *MET* |
| 84 | *CCND1 CDK4* | 184 | *CYP17A1* | 284 | *CA3* |
| 85 | *FLT4* | 185 | *RORC* | 285 | *CA6* |
| 86 | *INSR* | 186 | *SREBF2* | 286 | *CA5A* |
| 87 | *PTK2* | 187 | *SLC6A2* | 287 | *PTPN1* |
| 88 | *PLK1* | 188 | *CYP2C19* | 288 | *ACHE* |
| 89 | *TEK* | 189 | *RORA* | 289 | *SRC* |
| 90 | *MAP3K8* | 190 | *SERPINA6* | 290 | *ESR1* |
| 91 | *HSPA1A* | 191 | *SLC6A4* | 291 | *ESR2* |
| 92 | *NUAK1* | 192 | CHRM2 | 292 | *SQLE* |
| 93 | *FGR* | 193 | *VDR* | 293 | *KDR* |
| 94 | *AKR1C1* | 194 | *G6PD* | 294 | *BACE1* |
| 95 | *DAO* | 195 | *NR1H2* | | |
| 96 | *GSK3B* | 196 | *GLRA1* | | |
| 97 | *KCNA3* | 197 | *PTGER1* | | |
| 98 | *PTGS2* | 198 | *PTGER2* | | |
| 99 | *GSR* | 199 | *HSD11B1* | | |
| 100 | *CYP1A2* | 200 | *PTGES* | | |

**Table IV: Top five results of KEGG pathway dan Gene Ontology enrichment analysis of the potential targeted genes using DAVID v6.8.**

| Term | Count | P-Value | Genes |
|---|---|---|---|
| hsa05200: Pathways in cancer | 49 | 3.21162E-13 | *GSK3B, PIK3CD, PIK3CB, PIK3CG, IGF1R, EDNRA, CCND1, AKT1, JAK1, PDGFRB, PRKCG, HSP90AA1, PRKCB, MMP2, MMP9, PGF, AR, PIK3CA, CCNE2, CCNE1, AGTR1, PPARG, MET, PPARD, PTGER4, HDAC2, PTGER1, PTGER2, PTGER3, PIK3R1, PTGS2, HIF1A, EGFR, RXRA, ERBB2, MAPK1, MAPK3, NOS2, STAT1, BRAF, MTOR, PTK2, VEGFA, CDK4, CDK2, BCL2, MDM2, FGFR1, BCL2L1* |
| hsa04151:PI3K-Akt signaling pathway | 41 | 2.03355E-10 | *CHRM2, GSK3B, CHRM1, FLT4, PIK3CD, PIK3CB, PIK3R1, EGFR, PIK3CG, IGF1R, RXRA, CCND1, KDR, AKT1, MAPK1, JAK2, JAK3, YWHAG, JAK1, MAPK3, PDGFRB, HSP90AA1, SYK, INSR, MTOR, PGF, PTK2, VEGFA, PIK3CA, CCNE2, RPS6KB1, CCNE1, CDK4, CDK2, BCL2, MDM2, TEK, SGK1, MET, FGFR1, BCL2L1* |
| hsa04080: Neuroactive ligand-receptor interaction | 37 | 7.16148E-11 | *PTGER4, CHRM2, GABRB2, PTGFR, CHRM1, PTGER1, PTGER2, CHRNA7, PTGER3, HTR2B, PLG, NR3C1, GRM2, GLRA1, EDNRA, CNR1, ADORA3, ADORA1, DRD2, PTGDR, GABRA1, PTGIR, GABBR1, NPY5R, GPR35, OPRK1, TACR1, AVPR1A, ADRA2C, F2, TAAR1, GABRG2, ADRA2A, MTNR1A, P2RX3, MTNR1B, AGTR1* |
| hsa05205: Proteoglycans in cancer | 32 | 1.78808E-11 | *SRC, PIK3CD, PIK3CB, PIK3R1, HIF1A, EGFR, PIK3CG, IGF1R, CCND1, PLAU, ERBB2, KDR, AKT1, MAPK1, MAPK3, PRKCG, PRKCB, MMP2, PTPN11, BRAF, MAPK14, MMP9, ESR1, MTOR, PTK2, VEGFA, PIK3CA, RPS6KB1, MDM2, PTPN6, MET, FGFR1* |
| hsa05203: Viral carcinogenesis | 30 | 7.95804E-10 | *HDAC4, HDAC5, HDAC2, HDAC3, HDAC10, SRC, PIK3CD, PIK3CB, PIK3R1, PIK3CG, HDAC7, POLB, CCND1, CHEK1, MAPK1, JAK3, YWHAG, JAK1, MAPK3, LYN, SYK, CCNA2, CCNA1, PIK3CA, CCNE2, CCNE1, CDK4, CDK2, CDK1, MDM2* |
| **Molecular function** | | | |
| **Term** | **Count** | **P-Value** | **Genes** |
| GO:0005515~protein binding | 199 | 2.66061E-07 | *APP, HDAC10, SERPINE1, PREP, RPS6KA3, EDNRA, CHEK2, RPS6KA1, CHEK1, KDR, AKT1, EPHB4, PDK1, PTGDR, CSNK2A1, PRKCB, PRKCE, PRKCD, CSNK1D, GABRG2, AR, AGTR1, PRKCQ, SLC22A6, ABCB1, PRKDC, TXK, PIK3R1, HIF1A, NUAK1, TERT, CCR1, LYN, PLK4, INSR, PLK1, CDC7, BRAF, PTK2, AKR1B10, NOX4, TOP2A, ACHE, ITK, SLC6A2, SLC6A4, JAK2, JAK3, JAK1, PARP1, SYK, TNNC1, AVPR1A, TYK2, TACR1, F2, SREBF2, GAK, BACE1, NCOR2, FCER2, MMP14, TNNT2, KCNMA1, PPARG, MAPT, SGK1, PPARA, PPARD, PTGER4, PCNA, ODC1, KCNA5, PTGS2, EGFR, GLRA1, RXRA, ALOX5, AOC3, GABBR1, STAT1, CSNK1A1, AKR1C1, NR1H2, F11, NR1H3, VEGFA, FABP1, FABP3, FABP5, APEX1, HSPA1A, CCNT1, RORC, RORA, COMT, NR3C1, IGF1R, CCND1, PLAU, ADORA1, PIM1, MAP3K8, PIM3, PIM2, PDGFRB, KCNH2, G6PD, DYRK1A, DYRK1B, PGF, CDC25A, CDC25B, ERN1, ADAM17, MTNR1A, CCNE2, CCNE1, MTNR1B, PLAA, ABCG2, DNMT1, ACACB, NPC1L1, GRK6, DRD2, VDR, ESR1, ESR2, CDK9, CLK1, DAO, BMP1, PTPRC, CDK4, CDK2, BCL2, MDM2, CDK1, ALPL, MDM4, MAP3K14, BCL2L1, FGFR1, ALK, GSK3B, FLT4, PIK3CD, ECE1, PIK3CB, PIK3CG, POLB, GRM2, CA1, CA2, CA4, ACP1, YWHAG, HSP90AA1, MMP2, MMP3, ADRA2C, MMP9, ADRA2A, DNM1, FGR, CCNA2, CCNA1, PIK3CA, LCK, TOP1, MET, FBP1, PLEC, HDAC4, HDAC5, HDAC2, HDAC3, SRC, PLG, HMGCR, AURKB, AURKA, HDAC7, ERBB2, MAPK1, TNNI3, MAPK3, PTPN1, NOS2, OPRK1, PTPN11, MAPK14, MTOR, WEE1, RPS6KB1, PTPN6, TEK, PTPN2* |
| GO:0005524~ATP binding | 83 | 2.15845E-21 | *ALK, TOP2A, GSK3B, ITK, FLT4, PIK3CD, PIK3CB, ATP12A, PIK3CG, IGF1R, RPS6KA3, CHEK2, RPS6KA1, CHEK1, PIM1, KDR, AKT1, PIM3, MAP3K8, PIM2, JAK2, JAK3, EPHB4, JAK1, PDK1, PDGFRB, PRKCG, ABCC1, HSP90AA1, PRKCH, CSNK2A1, SYK, PRKCB, PRKCE, PRKCD, DYRK1A, DYRK1B, CSNK1D, TYK2, GAK, ERN1, FGR, PIK3CA, LCK, PRKCQ, SGK1, MET, ABCG2, ABCB1, SRC, PRKDC, TXK, ACACB, EGFR, AURKB, AURKA, NUAK1, ERBB2, GRK6, MAPK1, MAPK3, LYN, PLK4, CSNK1A1, INSR, PLK1, BRAF, CDC7, MAPK14, MTOR, PTK2, CDK9, CLK1, WEE1, P2RX3, RPS6KB1, CDK4, CDK2, CDK1, TEK, MAP3K14, FGFR1, HSPA1A* |
| GO:0004672~protein kinase activity | 51 | 1.5312E-30 | *GSK3B, PIK3CG, RPS6KA3, CCND1, CHEK2, RPS6KA1, CHEK1, AKT1, PIM3, MAP3K8, PIM2, JAK2, PDK1, PRKCG, PRKCH, CSNK2A1, SYK, PRKCB, PRKCE, PRKCD, DYRK1A, DYRK1B, CSNK1D, TYK2, GAK, PRKCQ, MET, SRC, PRKDC, TXK, EGFR, AURKB, AURKA, NUAK1, ERBB2, CSNK1A1, PLK1, BRAF, CDC7, MAPK14, MTOR, PTK2, CDK9, CLK1, WEE1, RPS6KB1, CDK4, CDK2, CDK1,* TEK, MAP3K14. |

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**Table IV: Top five results of KEGG pathway dan Gene Ontology enrichment analysis of the potential targeted genes using DAVID v6.8.**

| | | | |
|---|---|---|---|
| GO:0004674~protein serine/threonine kinase activity | 45 | 9.24148E-24 | *GSK3B, CCNT1, PRKDC, PIK3CG, AURKB, AURKA, RPS6KA3, NUAK1, CHEK2, CHEK1, RPS6KA1, PIM1, AKT1, MAPK1, PIM3, MAP3K8, PIM2, MAPK3, PLK4, PRKCH, SYK, CSNK2A1, PRKCB, CSNK1A1, PRKCE, PRKCD, PLK1, DYRK1A, DYRK1B, BRAF, CSNK1D, CDC7, MAPK14, MTOR, CDK9, GAK, ERN1, CLK1, PIK3CA, CDK4, CDK2, CDK1, PRKCQ, SGK1, MAP3K14.* |
| GO:0008270~zinc ion binding | 43 | 8.45694E-06 | *HDAC4, DNMT1, NR1I3, RORC, RORA, NR3C1, GLRA1, CA1, RXRA, CA5B, CA3, CA2, CA5A, CA4, CA7, CA6, CA9, CA13, CA12, PRKCG, PTPN1, MMP7, PARP1, PRKCB, VDR, NR1H2, MMP2, MMP3, NR1H3, MMP8, MMP9, ESR1, ESR2, MMP12, AR, MMP14, BMP1, MMP13, MDM2, PPARG, MDM4, PPARA, PPARD.* |
| **Biological process** | | | |
| **Term** | **Count** | **P-Value** | **Genes** |
| GO:0006468~protein phosphorylation | 56 | 2.74809E-30 | *APP, GSK3B, CCNT1, PIK3CD, PIK3CG, RPS6KA3, CCND1, CHEK2, RPS6KA1, PIM1, AKT1, PIM3, MAP3K8, PIM2, JAK2, JAK3, JAK1, PDK1, PRKCG, PRKCH, CSNK2A1, SYK, PRKCB, PRKCE, PRKCD, DYRK1A, DYRK1B, CSNK1D, TYK2, CDC25B, GAK, ERN1, FGR, PIK3CA, CCNE1, LCK, SGK1, TXK, PIK3R1, AURKB, AURKA, NUAK1, ERBB2, GRK6, MAPK1, MAPK3, LYN, PLK4, CSNK1A1, PLK1, BRAF, MTOR, CDK9, RPS6KB1, CDK4, FGFR1.* |
| GO:0007165~signal transduction | 51 | 3.74565E-09 | *ALK, ITK, CHRM1, PIK3CD, PIK3CB, NR3C1, IGF1R, RPS6KA3, EDNRA, PLAU, RPS6KA1, ADORA1, AKT1, JAK2, PDGFRB, HSP90AA1, PRKCH, CSNK2A1, PRKCB, PRKCE, PRKCD, CSNK1D, ADRA2A, PGF, AR, PLAA, PPARG, MET, PTGES, SRC, PLA2G1B, NR1I3, CHRNA7, PIK3R1, HIF1A, EGFR, EBP, ERBB2, GRK6, MAPK1, LYN, CSNK1A1, VDR, MAPK14, ESR1, MTOR, ESR2, P2RX3, RPS6KB1, CDK4, TEK.* |
| GO:0042493~response to drug | 40 | 1.44432E-22 | *HDAC4, HDAC5, HDAC2, ABCB1, MAOB, SRC, HTR2B, ABAT, TYMS, COMT, PTGS2, SLC6A2, ACACB, SLC6A4, HSD11B2, PNP, CCND1, NPC1L1, CA9, DRD2, LYN, BCHE, ABCC1, HSP90AA1, STAT1, SRD5A1, PGF, CDK9, ADAM17, FABP3, RPS6KB1, LCK, CDK4, APEX1, BCL2, CDK1, MDM2, PPARG, TOP1, ABCG2.* |
| GO:0055114~oxidation-reduction process | 38 | 2.42738E-11 | *MAOB, MAOA, HSD17B3, AKR1B1, HMGCR, CYP2C19, PTGS2, CYP19A1, PTGS1, CYP17A1, HSD11B1, HSD11B2, HSD17B1, ALOX5, HSD17B2, CYP1B1, XDH, FDFT1, AOC3, CBR1, G6PD, NOS2, SRD5A1, AKR1C1, GSR, CYP51A1, AKR1C3, PGD, SQLE, DAO, AKR1B10, SCD, APEX1, TBXAS1, CYP1A2, NOX4, DHCR7, IDO1.* |
| GO:0045944~positive regulation of transcription from RNA polymerase II promoter | 38 | 1.10342E-05 | *HDAC4, TOP2A, HDAC5, APP, GSK3B, HDAC2, HDAC3, CCNT1, PLA2G1B, PRKDC, NR1I3, TXK, SERPINE1, RORA, PIK3R1, NR3C1, HIF1A, EGFR, RPS6KA3, RXRA, RPS6KA1, AKT1, DRD2, MAPK3, PARP1, STAT1, VDR, NR1H2, NR1H3, MAPK14, ESR1, SREBF2, VEGFA, CDK9, AR, PPARG, PPARA, MET.* |
| **Cell component** | | | |
| **Term** | **Count** | **P-Value** | **Genes** |
| GO:0005886~plasma membrane | 123 | 8.03158E-13 | *CHRM2, APP, CHRM1, SERPINE1, COMT, ATP12A, IGF1R, EDNRA, PLAU, ADORA3, ADORA1, PIM1, KDR, AKT1, EPHB4, CA14, PTGDR, PDGFRB, KCNH2, PRKCG, PRKCH, CSNK2A1, PRKCB, PRKCE, PRKCD, CSNK1D, TAAR1, GABRG2, AR, MTNR1A, ADAM17, MTNR1B, AGTR1, PRKCQ, ABCG2, PTGFR, SLC22A6, ABCB1, CHRNA7, PIK3R1, PLA2G5, SLC5A2, TERT, NPC1L1, GRK6, DRD2, CCR1, LYN, TAS2R31, INSR, BRAF, ESR1, PTK2, PTPRC, MDM2, ALPL, FGFR1, GABRB2, ACHE, GSK3B, FLT4, HTR2B, PIK3CD, ECE1, PIK3CB, SLC6A2, PTPRF, PIK3CG, SLC6A4, GRM2, CA2, CA4, CA9, ABCC1, PTGIR, HSP90AA1, NPY5R, SYK, MMP2, AVPR1A, TACR1, F2, ADRA2C, ADRA2A, DNM1, BACE1, FGR, FCER2, MMP14, PIK3CA, LCK, KCNMA1, MAPT, SGK1, MET, MGLL, PLEC, PTGER4, HDAC3, SRC, PTGER1, PTGER2, PTGER3, KCNA3, KCNA5, PLG, EGFR, GLRA1, CXCR1, CNR1, ERBB2, CA12, AOC3, PTPN1, GABRA1, GABBR1, CYP51A1, F11, PLA2G2A, OPRK1, P2RX3, TEK, PTPN2* |
| GO:0005829~cytosol | 118 | 4.75122E-18 | *PNMT, APP, COMT, RPS6KA3, PNP, CCND1, RPS6KA1, CHEK1, AKT1, MAP3K8, EPHB4, CA13, PRKCG, G6PD, PRKCH, CSNK2A1, PRKCB, PRKCE, PRKCD, CSNK1D, PGD, CDC25A, CDC25B, AR, CCNE2, CCNE1, PRKCQ, IDO1, CES1, PRKDC, PIK3R1, HIF1A, ACACB, LYN, PLK4, PLK1, BRAF, PTK2, DAO, AKR1B10, NAT1, CDK4, CDK2, BCL2, MDM2, CDK1, MAP3K14, BCL2L1, FGFR1, GABRB2, GSK3B, ITK, PIK3CD, AKR1B1, PIK3CB, PIK3CG, SLC6A4, CA1, CA3, CA2, CA7, CA6, JAK2, JAK3, YWHAG, JAK1, CBR1, PTGIR,* |

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**Table IV: Top five results of KEGG pathway dan Gene Ontology enrichment analysis of the potential targeted genes using DAVID v6.8.**

| | | | |
|---|---|---|---|
| | | | *HSP90AA1, DUSP3, SYK, TNNC1, TYK2, SREBF2, GAK, FGR, CCNA1, PIK3CA, LCK, TNNT2, PPARG, MAPT, SGK1, FBP1, MGLL, PLEC, HDAC4, HDAC3, SRC, NR1I3, ODC1, TYMS, AURKB, TYMP, AURKA, HSD17B1, ALOX5, MAPK1, TNNI3, XDH, MAPK3, PTPN1, NOS2, STAT1, CSNK1A1, AKR1C1, GSR, AKR1C3, PTPN11, MAPK14, MTOR, FABP1, FABP3, FABP4, RPS6KB1, FABP5, PTPN6, HSPA1A* |
| GO:0005634~nucleus | 110 | 0.006034134 | *CCNT1, HDAC10, PREP, RORC, RORA, NR3C1, RPS6KA3, PNP, CCND1, RPS6KA1, CHEK1, PIM1, KDR, AKT1, PDGFRB, PRKCG, G6PD, CSNK2A1, PRKCB, PRKCE, PRKCD, DYRK1A, DYRK1B, CSNK1D, PGD, CDC25A, AR, CCNE2, CCNE1, PLAA, ABCG2, DNMT1, KLK1, TXK, PIK3R1, HIF1A, ACACB, NUAK1, TERT, LYN, VDR, PLK1, CDC7, BRAF, ESR1, ESR2, PTK2, CLK1, CDK4, CDK2, BCL2, MDM2, CDK1, MDM4, FGFR1, TOP2A, ACHE, GSK3B, FLT4, PIK3CB, POLB, JAK2, JAK1, HSP90AA1, DUSP3, PARP1, SYK, MMP2, TYK2, SREBF2, CCNA2, NCOR2, CCNA1, PPARG, TOP1, SGK1, PPARA, PPARD, HDAC4, HDAC5, HDAC2, HDAC3, PCNA, SRC, NR1I3, PTGS2, TYMS, EGFR, AURKB, HDAC7, PTGS1, AURKA, RXRA, ERBB2, MAPK1, MAPK3, NOS2, STAT1, NR1H2, AKR1C3, NR1H3, PTPN11, MAPK14, MTOR, WEE1, FABP4, RPS6KB1, APEX1, PTPN6, PTPN2* |
| GO:0005737~cytoplasm | 107 | 0.005336642 | *APP, HDAC10, PREP, NR3C1, RPS6KA3, PNP, CCND1, RPS6KA1, PIM1, AKT1, MAP3K8, PIM3, PIM2, PDGFRB, G6PD, PRKCH, PRKCB, PRKCE, PRKCD, CDC25A, CDC25B, ERN1, AR, ADAM17, PLAA, PTGFR, TXK, PIK3R1, PLA2G7, HIF1A, NUAK1, LYN, PLK1, CDC7, BRAF, ESR1, PTK2, CDK9, CLK1, CDK2, BCL2, MDM2, CDK1, MAP3K14, BCL2L1, TOP2A, GSK3B, FLT4, HTR2B, AKR1B1, PIK3CG, POLB, CA1, CA3, CA2, JAK2, ACP1, JAK1, HSP90AA1, DUSP3, NPY5R, SYK, TYK2, ADRA2C, SREBF2, ADRA2A, CCNA2, MMP14, MAPT, SGK1, FBP1, PLEC, HDAC4, HDAC5, HDAC2, HDAC3, PCNA, SRC, NR1I3, ODC1, PTGS2, TYMS, EGFR, HDAC7, PTGS1, HSD17B1, ERBB2, MAPK1, AOC3, GABBR1, NOS2, STAT1, NR1H2, AKR1C3, PTPN11, MAPK14, MTOR, VEGFA, FABP1, WEE1, FABP4, RPS6KB1, FABP5, APEX1, PTPN6, TEK, HSPA1A* |
| GO:0016021~integral component of membrane | 101 | 0.026736903 | *APP, CISD1, COMT, ATP12A, IGF1R, ADORA3, ADORA1, KDR, CYP1B1, CA14, PTGDR, PDGFRB, KCNH2, TAAR1, GABRG2, AR, MTNR1A, ADAM17, MTNR1B, AGTR1, PTGES, ABCG2, PTGFR, SLC22A6, ABCB1, MAOB, MAOA, CHRNA7, SLC5A2, CYP19A1, FUT4, HSD11B1, HSD11B2, EBP, FUT7, NPC1L1, ST3GAL3, FDFT1, CCR1, TAS2R31, SRD5A1, ESR1, SQLE, PTPRC, TBXAS1, CYP1A2, BCL2, NOX4, ALPL, DHCR7, BCL2L1, FGFR1, ALK, GABRB2, ACHE, HTR2B, ECE1, SLC6A2, PTPRF, SLC6A4, GRM2, CA4, CA9, ACP1, ABCC1, PTGIR, GPR35, SIGMAR1, AVPR1A, TACR1, ADRA2C, BACE1, FCER2, BACE2, MMP14, MMP13, KCNMA1, MET, UGT2B7, PTGER4, HDAC2, PTGER1, PTGER2, PTGER3, HMGCR, EGFR, GLRA1, CXCR1, CNR1, ERBB2, HSD17B2, CA12, AOC3, BCHE, PTPN1, GABRA1, GABBR1, CYP51A1, OPRK1, SCD, PTPN2* |

**Table V: Top 20 hub genes with the highest degree score**

| No | Gene symbol | Full name | Score |
|---|---|---|---|
| 1 | *AKT1* | Serine/threonine-protein kinase AKT | 123 |
| 2 | *MAPK3* | MAP kinase ERK1 | 116 |
| 3 | *EGFR* | Epidermal growth factor receptor erbB1 | 107 |
| 4 | *SRC* | Tyrosine-protein kinase SRC | 102 |
| 5 | *MAPK1* | MAP kinase ERK3 | 101 |
| 6 | *VEGFA* | Vascular endothelial growth factor A | 98 |
| 7 | *HSP90AA1* | Heat shock protein HSP 90-alpha | 90 |
| 8 | *ESR1* | Estrogen receptor alpha | 84 |
| 9 | *PTGS2* | Cyclooxygenase-2 | 82 |
| 10 | *MTOR* | Serine/threonine-protein kinase mTOR | 76 |
| 11 | *PIK3CA* | PI3-kinase p110-alpha subunit | 74 |
| 12 | *APP* | Beta-amyloid A4 protein | 65 |
| 13 | *MMP9* | Matrix metalloproteinase 9 | 62 |
| 14 | *MAPK14* | MAP kinase p38 alpha | 59 |
| 15 | *AR* | Androgen Receptor | 58 |
| 16 | *ERBB2* | Receptor protein-tyrosine kinase erbB-2 | 57 |
| 17 | *MDM2* | p53-binding protein Mdm-2 | 57 |
| 18 | *KDR* | Vascular endothelial growth factor receptor 2 | 56 |
| 19 | *BCL2L1* | Apoptosis regulator Bcl-X | 55 |
| 20 | *STAT1* | Signal transducer and activator of transcription 1-alpha/beta | 55 |

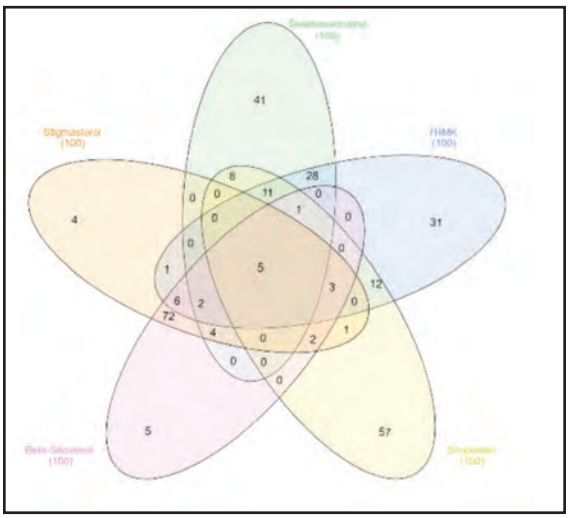


**Fig. 1:** Venn diagram of predicted genes target of 5 potential mahogany compounds, resulting in 294 potential targets.

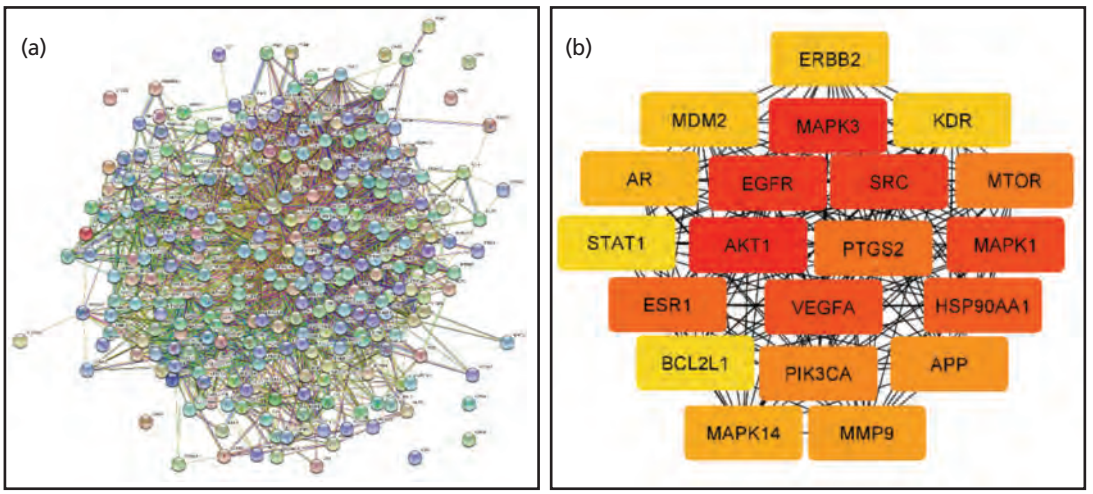


**Fig. 2:** (a) Protein–protein interaction networks of predictive mahogany metabolite target genes analyzed using STRING-DB and (b) the top 20 hub genes with the highest degree score analyzed using Cytoscape.

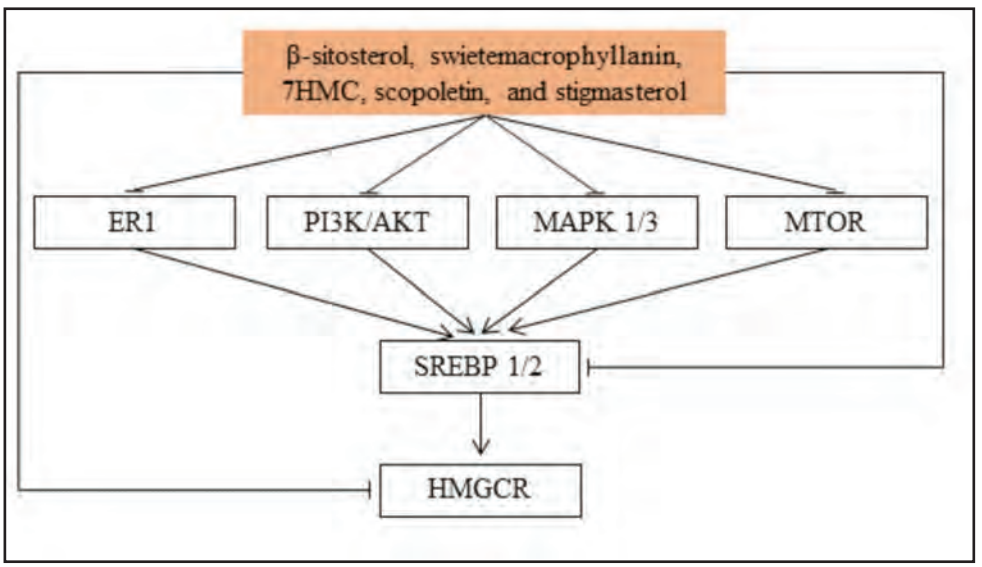


**Fig. 3:** Mechanism of antidyslipidemic activity of the 5 mahogany test compounds through the direct inhibition of HMGCR and indirect inhibition through SREBP, ER1, PI3K/AKT, MAPK 1/3, and MTOR.

target of mahogany compounds (Table II). SREBP was activated by MAPK, AKT1, PI3K, MTOR, and ER1.[28-30]

Intracellular mitogen-activated protein kinases (MAPKs) are an essential class of proline-dependent protein kinases involved in regulating various biological functions, such as inflammation, cell proliferation, and differentiation.[31] MAPK1 and MAPK3 (also known as ERK2 and ERK1, respectively) are the members of the MAPK family. The N-terminal domains of SREBP1a, SREBP1c, and SREBP2 are the substrates of MAPK1 and MAPK3.[32] The activation of the MAP kinase cascade increases the transcriptional activity of SREBP1a and SREBP2 to a similar degree (state). MAPK1/3 plays a role in regulating the lipid metabolism in the liver.[33] A past study also confirmed that the inhibition of MAPK3 had been known to reduce adipocytes and resistance to high-fat diet-induced obesity due to impaired adipocyte differentiation and higher postprandial metabolism.[34]

The phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) pathway is considered as one of the upstream pathways of SREBP.[29] The Akt pathway positively modulates the activation of SREBP2, which selectively activates *de novo* cholesterol synthesis.[35] The inhibition of the AKT signaling pathway is known to inhibit the formation of SREBP2 and the downstream target genes of SREBP2, such as LDLR and HMGCR.[35]

The mechanistic target of rapamycin (mTOR) is the catalytic subunit of two structurally distinct complexes: mTORC1 and mTORC2, both of which localize to different subcellular compartments and also affect different activation and function.36 During obesity and overnutrition conditions, mTORC1 is hyperactivated, resulting in the persistent activation of SREBP 1c in the liver, leading to the overproduction of lipids and hepatic steatosis hypertriglyceridemia.[37] The inhibition of mTORC1 is known to reduce the SREBP2 activity and cholesterol synthesis in the endoplasmic reticulum.[28] Moreover, the inhibition of mTORC1 is known to prevent lipid storage processes, increase low-density lipoprotein cholesterol levels, and activate lipolysis.38 Thus, the inhibition of mTORC1 can be a potential therapeutic target for metabolic syndromes such as dyslipidemia.

Estrogen receptor alpha (ERα) plays a significant role in adipocyte activity and sexual dimorphism in fat distribution.[7] For instance, ER alfa modulates the process of transcription and the activation of proteins that play a role in fat metabolisms, such as PPAR gamma, SREBP, HMGCR, and LDLR.[25,30,39] Past research has shown that ER-alpha activation plays a role in activating the SREBP2 expression, which is correlated with the exhibition of the HMGCR expression for cholesterol synthesis and LDLR for the cellular uptake of LDL cholesterol.[30,40] However, past studies have shown that phytoestrogen genistein activation of SREBP2 did not significantly increase HMGCR expression, rather it increases LDLR expression.[40]

Our research suggests that mahogany contains β-sitosterol, swietemachropyllanin, swietenia, 7-HMC, and scopoletin, which are responsible for the estrogenic and anti-dyslipidemic activity with the mechanism through direct or indirect inhibition of HMGCR. Indirect inhibition of the HMGCR activity through inhibition of the ERK1/2, AKT/PI3K, mTOR, and estrogen receptor alpha pathways mediated through SREBP1/2 (Fig. 3). This study demonstrates the potential of mahogany as an estrogenic agent and a dyslipidemia-preventing agent in postmenopausal women. This research was based on bioinformatics research that suspected the compound roles of its activity and mechanism pathways. Therefore, further research is warranted to confirm our results.

## CONCLUSION

In this study, a network pharmacology approach was proposed to explore the mechanism underlying the action of mahogany on dyslipidemia for menopausal conditions. The active ingredients of mahogany in the treatment of dyslipidemia consisted of five compounds β-sitosterol, swietemacrophyllanin, 7-hydroxy-2-(4-hydroxy-3-methoxy-phenyl)-chroman-4-one (7HMC), scopoletin, and stigmasterol. The possible molecular mechanisms mainly involved the direct inhibitory pathway of HMGCR and the indirect inhibitory pathway of HMGCR. The indirect inhibitory pathway was mediated through the PI3K/AKT, MAPK1/3, MTOR, ER1, and SREBP1/2 signaling pathways. Our findings suggested the use of mahogany as an alternative herbal therapy to prevent dyslipidemia in menopause conditions and exert estrogenic activity. This research provides new insights for further research on the anti-dyslipidemic effect of mahogany.

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# Assessment of association between PD-L1 expression and clinicopathological characteristics of Indonesian patients with high grade bladder urothelial carcinoma

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**ABSTRACT**

**Introduction: Urothelial carcinoma (UC) is the most common type of bladder cancer. One of the treatments that are currently being explored for UC involves the use of immune checkpoint inhibitors, especially those targeting PD–1/PD–L1 interaction. This interaction has been previously suggested to aid in the prediction of outcomes. This study was aimed to investigate PD–L1 expression in high grade UC cases in Indonesia, both at mRNA and protein levels, and assess its associated clinicopathological characteristics.**

**Materials and Methods: The study involved analysis of 51 formalin-fixed paraffin-embedded (FFPE) tissue samples, obtained from patients diagnosed with high grade UC. PD–L1 expression was measured using immunohistochemistry (IHC) and quantitative real-time polymerase chain reaction.**

**Results: PD-L1 IHC staining was found to be positive in five (9.80%) cases. Upregulated expression of PD-L1 mRNA was reported in the patients belonging to older age group ($p$ = 0.049) and mainly involved less invasive cases ($p$ = 0.019), when compared with normoregulated group. Age was positively correlated with PD-L1 expression, as observed in IHC (r = 0.281, $p$ = 0.046). In comparison to this, mitotic index was found to be inversely correlated with PD-L1 mRNA levels (r = −0.369, $p$ = 0.008).**

**Conclusion: IHC staining results showed that PD-L1 expression was lower as compared to previous studies, which might suggest poor response to anti PD-1/PD-L1 agents. The results of the study showed that higher mRNA levels were associated with lower proliferation and less invasive behavior. Thus, the study suggested the potential of PD-L1 mRNA levels to be used as a predictive factor for patient outcomes.**

**KEYWORDS:**
*Bladder Cancer, Immune Checkpoint Inhibitors, Immunohistochemistry, Immunotherapy, Real-Time PCR*

## INTRODUCTION

Urothelial carcinoma (UC) is known to be the most common type of bladder malignancy. It accounts for over 500,000 new cases and over 200,000 deaths per year.[1] Patient outcomes for UC patients at later stages remain dismal, necessitating the development of new treatment strategies. One of the treatment modalities that are currently being explored for treatment of UC involves the use of immune checkpoint inhibitors (ICI), especially the ones that target PD-1/PD-L1 interaction. Currently, there are five ICI agents targeting PD-1 or PD-L1 that have been approved for UC patients.[2]

Despite the advancements in this field, responses of patients toward ICI therapy have been reported to be significantly variable. It has been previously suggested that testing for PD-L1 expression using immunohistochemistry (IHC) staining might help in predicting therapy response and patient outcomes, in general.[3] However, very limited studies have previously explored patient characteristics and outcomes in relation to PD-L1 expression in UC cases. The results in most of these studies were mainly inconclusive. Interestingly, one of the studies reported that PD-L1 mRNA levels could predict prognosis better than PD-L1 expression assessed using IHC.[4]

In Indonesia, studies focused on the associations between PD-L1 status and clinicopathological features are still limited. In fact, the results for these studies are quite conflicting. A study involving colorectal cancer (CRC) patients reported lower levels of mRNA expression for PD-L1 in peripheral blood as compared to healthy controls. Importantly, these mRNA levels correlated with gender but showed no correlation with age, stage, histological type, patient status, and body mass index.[5] Inversely, Al Azhar et al. (2021) reported higher PD-L1 mRNA expression in peripheral blood of nasopharyngeal carcinoma patients as compared to healthy controls. These levels were not associated with any of the clinicopathological features that were analyzed.[6] A study involving analysis of formalin-fixed paraffin-embedded (FFPE) tissue of bladder UC showed an association of PD-L1 expression with depth of invasion.[7]

Although ICI treatment has not been routinely used in case of most of Indonesian patients, data for the expression of immune checkpoint molecules would assist clinicians in considering the potential effectiveness of these treatment strategies in Indonesia. This study was aimed to investigate PD-L1 expression, at mRNA and protein levels, in high grade UC cases in Indonesia, and assessed its association with clinicopathological characteristics.

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## MATERIAL AND METHODS

This cross-sectional study included 51 FFPE tissue samples, obtained from patients diagnosed with high grade UC at Dr. Sardjito Hospital, Yogyakarta, Indonesia (longitude of –7.768393056636577 and latitude of 110.37345273846378), during January 2014 to December 2019. Grading for tissue samples was determined on the basis of 2016 WHO Classification of Tumors of the Urinary System and Male Genital Organs.[8] Cases with incomplete clinicopathological data or degraded specimens were excluded from the study.

PD-L1 expression was measured using IHC and quantitative real-time polymerase chain reaction (RT-PCR). IHC staining was performed on 3 µm-thick tissue sections, using PD-L1 antibody clone 22C3 (Dako Agilent Technologies, Santa Clara, CA, USA). UltraTek® HRP Anti-Polyvalent Lab Pack (Scytek Laboratories, Logan, UT, USA) was used for visualization. Normal tonsil tissue was used as positive control. The expression of PD-L1 was determined using combined positive score (CPS), which was calculated using the following formula:

$$\text{CPS} = \frac{\text{number of PD-L1 staining cells (tumor cells, lymphocytes, and macrophages)}}{\text{total number of viable tumor cells}} \times 100$$

Samples with CPS ≥10 was classified as PD-L1 positive, while samples with CPS <10 were classified as PD-L1 negative.

Tumor RNA was extracted from FFPE tissue using GeneAll® Ribospin™ II (GeneAll Biotechnology, Seoul, South Korea). Quantification of PD-L1 mRNA was done using RT-PCR with AccuPower® GreenStar™ RT-qPCR PreMix on Exicycler™ 96 (Bioneer Corp., Daejeon, South Korea). RT-PCR was performed according to the protocol previously described by Vassilakopoulou et al.[9] In particular, this study provided sequences for the primer pairs and thermocycler conditions used in the present study. PD-L1 mRNA levels were calculated and normalized from the quantification cycle (Cq). GAPDH was used as an internal control. Samples were classified as normoregulated if the expression was lower or equal to the average of PD-L1 mRNA levels of the entire subject group, while the samples with levels above the average were classified as upregulated.

Detailed clinical data (age, sex, and lymph node metastasis) were obtained from the medical records. The collected pathological data included information regarding invasion of muscularis propria, mitotic index, and tumor-infiltrating lymphocytes (TILs). Muscle invasion and TILs were assessed from hematoxylin-eosin slides, while mitotic index was measured using Ki-67 IHC stained slides. Presence of muscle invasion was assessed in terms of whether tumor cells had invaded muscularis propria layer or not. If muscle invasion could not be assessed from the sample, the sample was excluded from analysis for muscle invasion. TILs were grouped as intense and non-intense using a 10% cut-off, according to previous studies.[10,11] The samples with 10% or more stromal TILs were classified as intense, while the samples with less than 10% stromal TILs were classified as non-intense. For mitotic index assessed using Ki-67 IHC stained slides, observed under the microscope, 20% cut-off value was used. The number of tumor cells with brown-stained nuclei was calculated for at least 1000 tumor cells.

Chi-square analysis was used to compare clinicopathological characteristics for PD-L1 positive and negative groups for the categorical variables. T-test and Mann–Whitney test were used to compare continuous variables for normally distributed variables and non-normal distribution, respectively. Correlation between continuous variables was analyzed using Pearson correlation. The experiments performed in this study were approved by the Medical and Health Research Ethics Committee of Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia (KE/FK/0733/EC/2019).

## RESULTS

The characteristic features for the whole group and group comparisons made on the basis of PD-L1 expression are presented in Table I. The study mainly included male patients (76.47%), with an average age of 65.78 years (range: 45–83 years). PD-L1 IHC staining was reported to be positive in five (9.80%) cases. Representative results for PD-L1 immunohistochemistry are shown in Fig. 1.

Fig. 2 shows the distribution of PD-L1 mRNA expression levels of the PD-L1 expression groups based on immunohistochemistry assessment. Patients with upregulated PD-L1 mRNA belonged to older age group ($p$ = 0.049). In fact, these cases were less invasive cases ($p$ = 0.019) as compared to normoregulated group (Table I). No other differences were found to be statistically significant in case of clinicopathological characteristics.

Pearson correlations between continuous parameters are shown in Table II. Age was found to be positively correlated with PD-L1 expression, as assessed using IHC examination (r = 0.281, $p$ = 0.046). In contrast to this, mitotic index was inversely correlated to PD-L1 mRNA levels (r = –0.369, $p$ = 0.008).

## DISCUSSION

This study investigated the associations between PD-L1 expression (both at mRNA and protein levels) and clinicopathological characteristics of high-grade UC cases in Indonesia. The results for IHC staining showed that PD-L1 expression was positive in 9.80% of the cases. Upregulated mRNA levels were found to be associated with less invasive behavior and lower mitotic index. Importantly, higher PD-L1 expression was associated with older age.

For IHC staining, five out of 51 samples tested positive for PD-L1, resulting in a prevalence of 9.80%. This rate is comparatively lower as compared to previous studies, wherein it ranged from 15.1%–46.39%.[12–15] It has been previously reported that differences in populations and histological variants might affect PD-L1 expression.[13] Another factor that might contribute is the variation in the antibody clone used, which is known to cause differences in sensitivity and specificity.[16] Low PD-L1 expression might suggest that ICI treatment in Indonesian patients would exhibit poorer response. However, the predictive role of PD-L1 still remains inconclusive, thus requiring further research.

**Table I:Associations between PD–L1 expression and clinicopathological characteristics**

| | All subjects (n = 51) | PD–L1 expression on IHC | | | PD–L1 mRNA expression | | |
|---|---|---|---|---|---|---|---|
| | | Positive | Negative | *p* ** | Upregulated | Normoregulated | *p* ** |
| Age, mean ± SD* | 65.78 ± 8.76 | 71.00 ± 9.54 | 65.20 ± 8.59 | 0.163 | 68.24 ± 8.53 | 63.4 ± 8.47 | 0.049 |
| Age category, n (%) | | | | | | | |
| <65 years | 22 (43.13) | 2 (3.92) | 20 (39.22) | 0.632 | 9 (17.65) | 13 (25.49) | 0.400 |
| ≥65 years | 29 (56.87) | 3 (5.88) | 26 (50.98) | | 16 (31.37) | 13 (25.49) | |
| Sex, n (%) | | | | | | | |
| Male | 39 (76.47) | 4 (7.84) | 35 (68.63) | 0.665 | 20 (39.22) | 19 (37.25) | 0.743 |
| Female | 12 (23.53) | 1 (1.96) | 11 (21.57) | | 15 (29.41) | 7 (13.73) | |
| Lymph node metastasis, n (%) | | | | | | | |
| Present | 7 (13.73) | 1 (1.96) | 6 (11.76) | 0.538 | 1 (1.96) | 6 (11.76) | 0.055 |
| Absent | 44 (86.27) | 4 (7.84) | 40 (78.43) | | 24 (47.06) | 20 (39.22) | |
| Muscle invasion, n (%) | | | | | | | |
| Yes | 37 (72.55) | 4 (8.89) | 33 (73.33) | 0.443 | 15 (33.33) | 22 (48.89) | 0.019 |
| No | 8 (15.69) | 0 (0.00) | 8 (17.78) | | 7 (15.56) | 1 (2.22) | |
| Indeterminate | 6 (11.76) | | | | | | |
| Mitotic index, mean ± SD* | 36.64 ± 15.80 | 47.40 ± 20.11 | 35.47 ± 15.07 | 0.110 | 33.04 ± 13.56 | 40.11 ± 17.24 | 0.111 |
| Mitotic index category, n (%) | | | | | | | |
| ≥20% | 42 (82.35) | 4 (7.84) | 38 (74.51) | 0.638 | 19 (37.25) | 23 (45.10) | 0.213 |
| <20% | 9 (17.65) | 1 (1.96) | 8 (15.69) | | 6 (11.76) | 3 (5.88) | |
| TILs, mean ± SD* | 17.21 ± 10.88 | 11.00 ± 8.21 | 17.89 ± 10.98 | 0.237 | 15.32 ± 11.62 | 19.03 ± 10.00 | 0.128 |
| TILs category, n (%) | | | | | | | |
| Intense (≥10%) | 35 (68.63) | 2 (3.92) | 33 (64.71) | 0.171 | 14 (27.45) | 21 (41.18) | 0.075 |
| Non-intense (<10%) | 16 (31.37) | 3 (5.88) | 13 (25.49) | | 11 (21.57) | 5 (9.80) | |
| PD-L1 mRNA, mean ± SD* | 9.78 ± 4.13 | 6.89 ± 2.54 | 10.10 ± 4.16 | 0.099 | | | |
| PD-L1 mRNA category | | | | | | | |
| Upregulated | 25 (49.02) | 1 (1.96) | 24 (47.06) | 0.187 | | | |
| Normoregulated | 26 (50.98) | 4 (7.84) | 22 (43.13) | | | | |

*SD = standard deviation.
**$p$ value < 0.05 was considered significant.

**Table II: Correlation between PD-L1 expression and clinicopathological parameters**

| | Mitotic Index | TILs | PD-L1 (IHC) | PD-L1 (mRNA) |
|---|---|---|---|---|
| Age | 0.007<br>(0.959) | -0.060<br>(0.676) | **0.281**<br>**(0.046)** | 0.133<br>(0.351) |
| Mitotic Index | | -0.049<br>(0.732) | 0.233<br>(0.101) | **-0.369**<br>**(0.008)** |
| TILs | | | -0.238<br>(0.093) | -0.027<br>(0.851) |
| PD-L1 (IHC) | | | | -0.196<br>(0.167) |
| PD-L1 (mRNA) | | | | |

Statistically significant values are indicated in bold

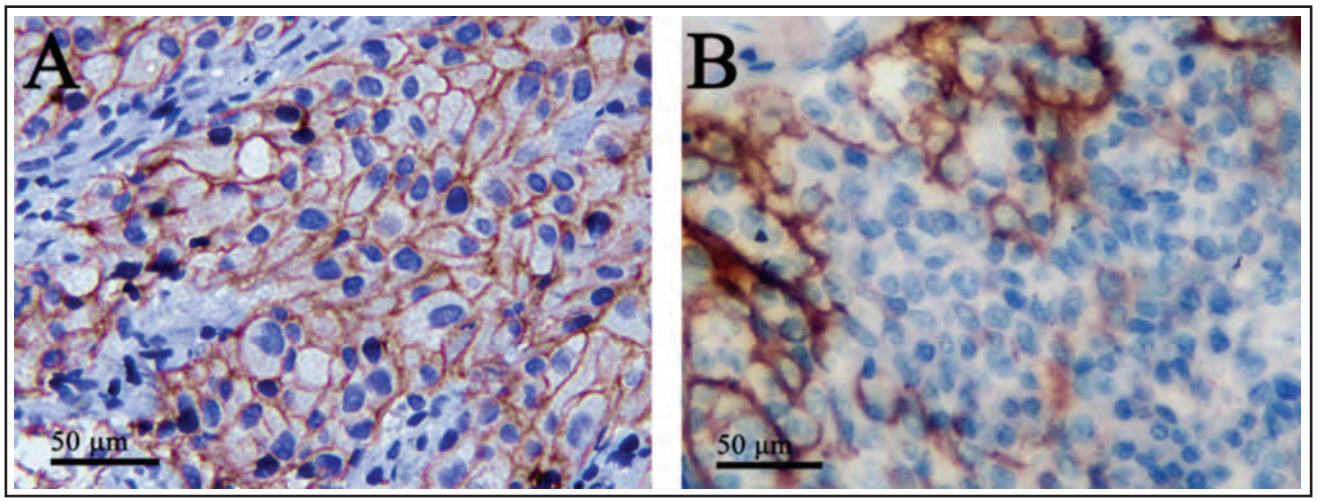


**Fig. 1:** (A) Immunohistochemistry staining image for representative urothelial carcinoma case stained positive for PD-L1, with combined positive score 100 (400× magnification). (B) Normal tonsil tissue as positive control showed positive membranous expression on reticulated epithelium of crypts.

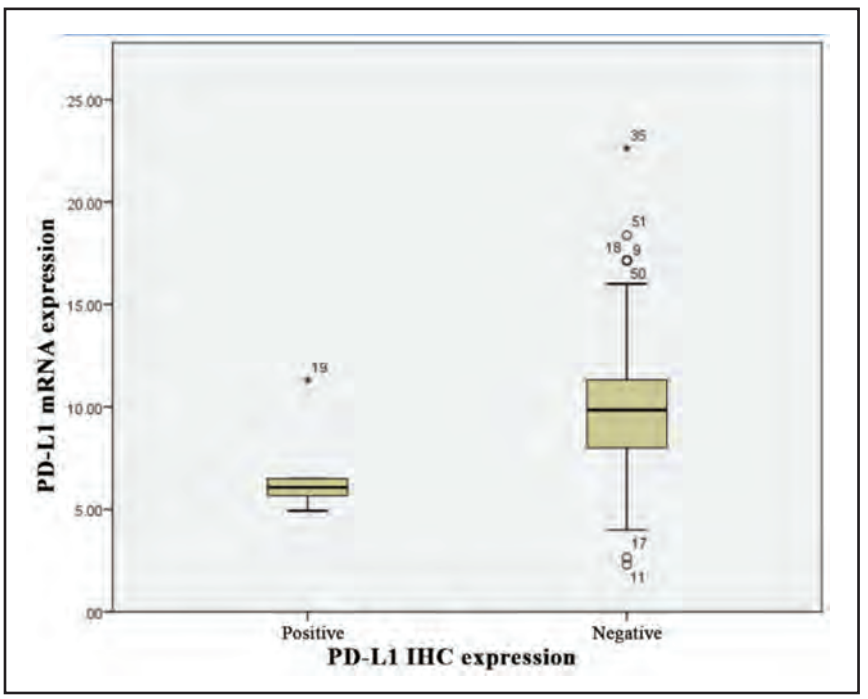


**Fig. 2:** Blox plot showing distribution of PD-L1 mRNA expression levels in positive and negative PD-L1 expression groups based on immunohistochemistry.

Importantly, it was observed that PD-L1 protein expression was not directly proportional to mRNA levels. PD-L1 expression levels observed for IHC and quantitative PCR even appeared to be negatively correlated. However, it was not statistically significant. Studies in UC have been previously shown to be variable in terms of the correlation between expression of PD-L1 mRNA and protein. Le Goux et al. reported a positive correlation between both these molecules.[17] However, Eckstein et al. reported that over 80% of urothelial bladder tumors that showed high expression of PD-L1 mRNA were negative for PD-L1 when IHC staining was performed.[4]

The type of PD-L1 expression (whether constitutive or inducible) might affect the correlation between the expression of PD-L1 mRNA and protein. In steady state conditions, mRNA levels tend to be proportional to protein levels.[18] However, in dynamic cell settings, such as proliferation, correlations between mRNA and protein expression become more variable. In fact, it can even reach negative correlation in certain cases. Variations in the findings of several studies might reflect the involvement of different expression modes in different populations. In this study, lack of positive correlation between PD-L1 mRNA and protein suggested that PD-L1 expression in Indonesian UC patients was inducible or reactive. The mode of expression might affect the prognostic role of PD-L1 in cancers, with reactive PD-L1 expression marking better prognosis and improved response to therapies targeting PD-L1.[19]

Post-transcriptional factors, including miRNA, involved in PD-L1 expression pathway might also affect the correlation between mRNA and protein levels. Several oncogenic miRNAs, such as miR-873 and miR-34a, are known to target the genes that encode PD-L1.[20] Consequently, inhibited translation could result in low protein expression, regardless of mRNA levels. Polymorphism of untranslated regions of PD-L1 mRNA can also affect the mRNA stability, which in turn can affect the measured mRNA levels.

Several post-translational processes, such as N-glycosylation, phosphorylation, and ubiquitination, can also affect PD-L1 protein levels. Glycosylation has been previously shown to protect PD-L1 from degradation, while phosphorylation and ubiquitination marked PD-L1 for degradation.[21] Increased degradation of PD-L1 protein might also account for a poor correlation between PD-L1 mRNA and protein levels.

This discrepancy between PD-L1 mRNA and protein expression might also explain the other associations that were found. Expression of PD-L1 in urothelial cancer cells is known to be associated with more aggressive tumors and higher recurrence.[22,23] Conversely, the results of this study showed that upregulated PD-L1 mRNA is associated with lower muscle invasiveness. Two previous studies also reported that bladder cancer cases with higher PD-L1 mRNA exhibit longer patient survival.[4,24] Differences in the prognostic role of protein and mRNA might arise from their different roles. A failure to express the PD-L1 protein, despite high levels of mRNA, might lead to stronger immune responses toward the tumor, thereby resulting in improved patient outcomes.

Interestingly, both upregulated PD-L1 mRNA and increased PD-L1 protein expression were found to be associated with older age. There is a possibility that PD-L1 transcription increases with age, which resulted in an overall increase in PD-L1 mRNA and protein expression levels. Very few studies have previously reported an association with age. Interestingly, one study conducted in United States of America reported that PD-L1 expression on IHC was linked to younger age.[10]

Although the samples analyzed in this study were limited and retrospective data collection might lead to information bias, the results of this study suggested that PD-L1 mRNA levels have the potential to be used as a prognostic factor for UC cases. Further research with larger sample size is required to investigate the correlation between PD-L1 expression (using both IHC and RT-PCR) and outcomes among subjects who do not receive immunotherapy.

## CONCLUSIONS

The results of this study suggested that PD-L1 expression at mRNA and protein levels exhibited different clinicopathological associations and possibly prognostic implications. The study particularly highlighted the potential of PD-L1 mRNA levels to be used as a predictive factor for patient outcomes. Studies with larger subject group are required to further ascertain the prognostic role of PD-L1 expression in Asian patients.


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## CONFLICT OF INTEREST

All authors declare no conflict of interest.

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# Neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and absolute lymphocyte count as mortality predictor of patients with Coronavirus Disease 2019

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## ABSTRACT

**Introduction: Coronavirus Disease 2019 (COVID-19) has a various spectrum of symptomsfrom asymptomatic to severe. Inprevious research, hematological examination could predict the severity of the infection. This study is aimed to evaluate Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR), and Absolute Lymphocyte Count (ALC) as a predictor of COVID-19 mortality.**

**Materials and Methods: The study was a cross sectional research with retrospective design by collecting data from medical records of the COVID-19 patients at a General Hospital Anwar Medika of East Java, for the period June 2020 to January 2021. A comparative test was performed using the Mann-Whitney test. The predictive ability was assessed using the ROC curve.**

**Results: A total of 70 subjects were involved in this study with 16 (22.9%) mortality. And the average NLR and PLR were 4.11 and 174.39. The Mann-Whitney test showed significant results (p<0.05) on leukocytes, NLR, PLR, and ALC of the patients. The ROC curve showed that under the curve of leukocytes, NLR, PLR, and ALC were 0.749, 0.731, 0.719, and 0.306, respectively.**

**Conclusion: With higher sensitivity and specificity by leukocytes 75% and 70.4%. Leukocytes, NLR, and PLR showed good ability to predict patient mortality from COVID-19.**

**KEYWORDS:**
*Coronavirus, mortality, predictive tools*

## INTRODUCTION

Coronavirus is a family of viruses found in humans and animals, the emergence of a new virus outbreak belonging to the coronavirus group was first discovered in the city of Wuhan, China. Within a few weeks, this virus spread to various parts of the continent. On February 20, 2020, the World Health Organization (WHO) announced that the outbreak caused by this new type of Coronavirus, namely Coronavirus Disease 2019 (COVID-19), was global. It is recorded that at this time, around 184 million people have been affected, and almost 4 million deaths due to Coronavirus, which has infected 222 countries around the world.[1]

In Indonesia alone, there were 2.3 million confirmed cases with nearly 60,000 deaths.[2] In SARS-CoV-2 infection, there are changes in several laboratory indicators which are also found in several other viral infections, because the hematopoietic system is a system that feels the impact of a viral infection.[3,4] Several previous studies have shown that there is lymphocytopenia, neutrophilia, eosinopenia, thrombocytopenia, or thrombocytosis, as well as the presence of reactive lymphocytes which indicate an acute infection.4Apart from the changes in the results of the hematological examination, the results can also be used to predict the severity of COVID-19. A meta-analysis stated that leukocytosis, lymphopenia, and thrombocytopenia are predictors of the severity of COVID-19 and increase the incidence of mortality with these results.[5]

Several other studies have also shown that NLR, PLR, and ALC can predict mortality from COVID-19 infection.[6,7] In this study, we evaluated the COVID-19 mortality hematologic predictors at Anwar Medika General Hospital as one of the COVID-19 referral hospitals in Indonesia

## MATERIAL AND METHODS

The study used an observational analytic cross-sectional research with retrospective design. The profile description is carried out retrospectively by collecting data from medical records of the COVID-19 patients' data consisting of age, gender, leukocytes and the differential count, platelets, length of stay, and mortality status. The hematological result was then calculated to produce NLR (neutrophil-to-lymphocyte ratio), PLR (platelet-to-lymphocyte ratio), and ALC (absolute lymphocyte count).

These were the following equation:

$$NLR = \frac{NLR=(\text{Absolute neutrophil count (Cells/}\mu L)}{(\text{Absolute lymphocyte count (Cells/}\mu L))}$$

$$PLR = \frac{\text{Thrombocyte count (Cells/}\mu L)}{\text{Absolute lymphocyte count (Cells/}\mu L)}$$

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The sample used in this study were patients infected with COVID-19 at the Anwar Medika General Hospital during the period of June 2020 to January 2021. With the inclusion criteria of patients who have been confirmed positive for COVID-19 through PCR examination results, while patients with a history of blood transfusions were excluded from this study.

Furthermore, comparative tests were conducted between the hematological profile to the length of stay (LoS) and patient mortality. Comparative tests were conducted using a two-sample independent t2 test on data with a normal distribution and using the Mann-Whitney test on data with an abnormal distribution. Variables that have significant values were then tested for predictive ability using the ROC curve then interpreted as sensitivity and specificity. The highest sensitivity and specificity are obtained by the curve reading that produced by IBM® SPSS®

## RESULTS

A total of 70 subjects were involved in this study. From the subjects, 10 subjects (14.3%) were males, and the rest were females (85.7%). The average age of the research subjects was 51.03 years with the youngest being 30 years old and the oldest 82 years old.

From the results of the hematological examination, the average leukocytes, platelets, neutrophils, and lymphocytes were 9,730/µL, 265,671/µL, 5,960/µL, and 1,830/µL. And from the calculation, the average NLR and PLR were 4.11 and 174.39. With an average length of stay (LoS) of 10.91 days.

From the mortality data, 16 patients (22.9%) died. The average age of the subjects who died was 53.19 years, higher than the group who survived 50.58 years. From the calculation of leukocytes, platelets, neutrophils, NLR, and PLR, it was found that the average death group was higher than the survival group. Meanwhile, ALC and LoS were lower in the mortal group than in the surviving group. The comparative test showed significant results ($p<0.05$) on leukocytes, NLR, PLR, ALC, and length of stay (LoS) of the patients.

The results of the receiver operating characteristic (ROC) curve the largest area under the curve (AUC) was produced by leukocytes, 0.749, followed by NLR and PLR. Meanwhile, ALC has the lowest AUC of 0.306. From the results of the highest sensitivity obtained PLR of 81.3% and the highest specificity is 70.4% leukocytes.

## DISCUSSION

The number of deaths due to COVID-19 is increasing every day in Indonesia, with the number of deaths reaching a thousand on 07/08/2021.[2] In addition, there are also more than 300,000 active cases of confirmed patients infected with COVID-19, which further reduces the ability of hospitals to handle patients.[2] However, clinical manifestations of the confirmed patients vary from asymptomatic to severe symptoms.[8,9] Because of this, it is necessary to stratify individuals with confirmed COVID-19 to optimize the capacity of the available hospitals.

From our research, it was found that from 70 subjects consisted of 16 subjects (22.9%) who died during the observation and the rest recovered. The mortality rate of 22.9% was higher if were looked at the death rate throughout Indonesia, which was 2.6%.[2] This finding could be related to community stigma regarding COVID-19 which causes patients to be late in seeking help to health centers which is associated with increased patient mortality.[10,11]

From the findings in our study, it was found that the average age of the covid patients we treated was 51.03 years, which is the age group at risk of severe infection in COVID-19.[12–14] This result is also supported by the finding of an older age in mortality cases compared to the survivors. However, in our study, there were no significant results between age and mortality status which was not in line with previous studies which stated that age was associated with patient mortality.[14]

The comparison test result from mortality status and hematological results were found that there was a relationship between mortality status with leukocytes, NLR, PLR, and ALC. There was also a significant relationship between mortality status and length of stay (LoS) of the patients. In the previous study, evaluation of the hematological component compared to the severity of COVID-19 infection showed that there was a relationship between either NLR, PLR, or ALC and infection severity with various degrees of association.[15–17] Our findings showed that leukocytes, NLR, and PLR were higher in subjects with mortality compared to surviving subjects. While the ALC showed the opposite results which decreased in subjects who experienced mortality. This finding is in line with research.[18] So that leukocytes, NLR, PLR, and ALC could be used as predictors of severity and mortality from COVID-19 patients. From the ROC curve, it was found that leukocytes, NLR, and PLR had AUC more than 0.50, but not with ALC. From the results of the best sensitivity and specificity, leukocytes were obtained with a sensitivity of 75% and a specificity of 70.4%, followed by PLR with a sensitivity of 81.3% and a specificity of 63%. Although ALC has a relationship with the patient's mortality status, the predictive ability of ALC on mortality status is quite low, seeing the AUC of only 0.306. In the previous predictive value testing in the research conducted by Yang et al. the sensitivity from NLR and PLR were 88% and 77%, respectively.[6] These results indicate that this modality can be used as a basis for predicting mortality in COVID-19 patients.

The association of leukocytes and NLR in infection cases in previous studies has been associated with the occurrence of cytokine storms that often occur in severe COVID-19 patients.[19] Although in this study no further investigation was carried out regarding the cause of death suffered by the patients, the increase in the number of leukocytes, which were dominated by polymorphonuclear cells, indicated that this finding was a predictor of increased immune activity in the patients' body which could be a sign of a cytokine storm. In line with the investigation Xiong et al. stated that the increase in neutrophil was a result of the release of CXCL-2 and CXCL-8 which increased neutrophil recruitment and indicated high cytokine activity in the patient's body.[20]

From our findings, it was found that there was a higher PLR

**Table I: Characteristic of the subject**

| Variable | Mortality status | |
|---|---|---|
| | Survive, n (%) | Mortal, n (%) |
| Gender | | |
| Male | 7 (70%) | 3 (30%) |
| Female | 47 (78.3%) | 13 (21.7%) |

**Table II: Comparison variable between mortality status**

| Variable | Mean±SD | | | *p* |
|---|---|---|---|---|
| | Total subject | Survived subject | Dead subject | |
| Age (years old) | 51.03±11.77 | 50.58±12.814 | 53.19±7.332 | NS |
| Leukocyte (/µL) | 9,730±4,720 | 8,672±3,687 | 13,200±6,219 | 0.003 |
| Thrombocyte (/µL) | 265,671±101,263 | 259,113±106,998 | 286,812.5±82,743.1 | NS |
| Neutrophil (/µL) | 5,960±3,310 | 5,183.8±2,287.4 | 8,505.3±4,820.2 | NS |
| NLR | 4.11±3.40 | 3.23±2.10 | 11±5.02 | 0.005 |
| PLR | 174.40±97.80 | 160.76±94.797 | 223.55±96.64 | 0.008 |
| ALC (/µL) | 1,830±981.5 | 1,895.4±967.3 | 1,572.4±1,035.8 | 0.019 |
| Length of stay (days) | 10.91±4.52 | 11.91±4.42 | 75±3.45 | 0.001 |

NS, Not significant

**Table III: The highest sensitivity and specificity of the variable**

| Predictive variable | AUC | Sensitivity | Specificity |
|---|---|---|---|
| Leukocyte | 0.749 | 75% | 70.4% |
| NLR | 0.731 | 75% | 57.4% |
| PLR | 0.719 | 81.3% | 63% |
| ALC | 0.306 | 56.3% | 20.4% |

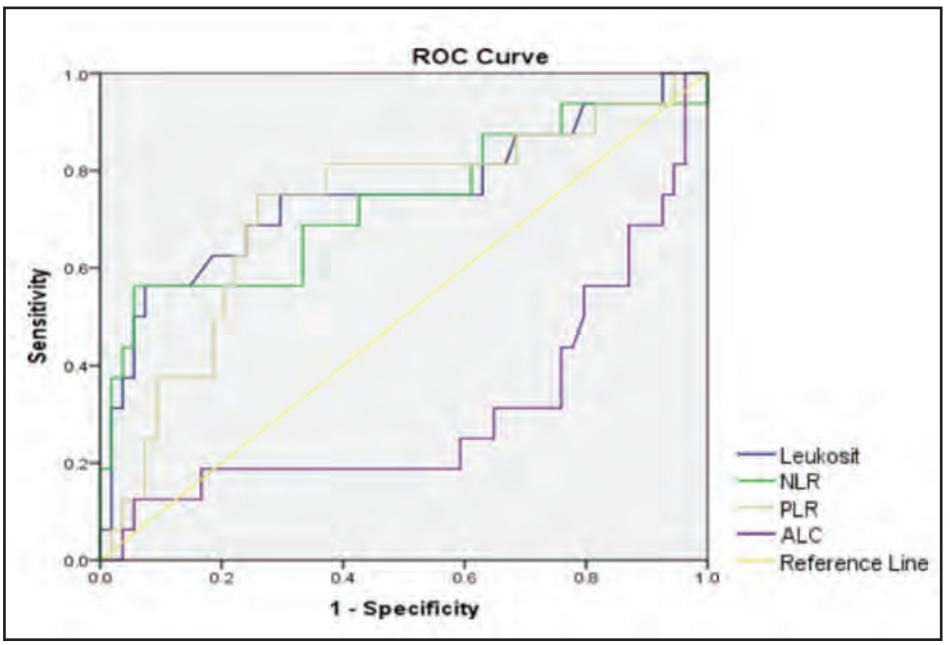


**Fig. 1:** ROC Curve of the predicting variable

in the mortal group compared to the surviving group. This finding is in line with previous research.[21] However, the increase in PLR was not in line with increase in platelets, there was no significant difference between the survivors and the mortality group from the platelet count. The increase in PLR is due to a decrease in the denominator component which is ALC. Several hypotheses that the decrease in ALC in COVID-19 patients is due to the P53 signaling process and FAS signaling which causes lymphocytes to undergo apoptosis.[20,22]

The findings in this study can be used to detect the possible severity and mortality of COVID-19 patients. So that it can be used as consideration for determining the room and therapy to be given to patients. However, this study has limitations mainly because the focus of this study was to evaluate the predictive ability of NLR, PLR, and ALC on COVID-19 mortality in one of the COVID-19 referral sites in Indonesia. Therefore, the subjects involved in this study are limited and cannot represent subjects in Indonesia in general. However, this study can be the basis that NLR, PLR, and leukocytes can be used as predictors of COVID-19 mortality that are easy and inexpensive.

## CONCLUSION

Hematological components (Leukocytes, NLR, PLR, and ALC) have a relationship with mortality status in COVID-19 patients. However, only leukocytes, NLR, and PLR showed good ability to predict patient mortality so it could be used as a triage consideration for COVID-19 patients in the conclusions with the goals of the study but avoid unqualified statements and conclusions not completely supported by the data.

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