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Original Articles

Case Report

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Original Articles

119 Effectiveness of Mar'ke Bilar Drink Formulation on Pro- Anti-Free Radicals in The Blood of Women of Childbearing Age Operators in Gas Stations

Ginta Siahaan[1], Fauzi Romeli[1], R.R. Sri Arini Winarti Rinawati[2], Suriani br Ginting[2],
Kenya Putri Kasi Sembiring Pandia[1], Ifta Maulida Tanjung[1]
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Mar'ke Bilar affects blood Pb, MDA, and SOD levels and formulation 1 is the most effective in increasing SOD levels. It is necessary to consume colored foods such as Marke' Bilar which are very good for avoiding diseases caused by free radicals by air and water pollution, especially those caused by heavy metals.

131 Spatial and Temporal Analysis of Gallbladder Stone Cases at dr. Soekardjo Hospital, West Java: An Evidence-Based Study

Mudatsir[1], Muhammad Raka El Ghifari[2],
Fahmi Rahmat Amanulloh[2], Tryantini Sundi Putri[3],
Andhy Romdani[2]
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[2]Department of Civil Engineering, Faculty of Engineering, Siliwangi University, Tasikmalaya, Indonesia
[3]Department of Civil Engineering, Faculty of Engineering, Halu Oleo University, Kendari, Indonesia

The spatial distribution of gallbladder stone was concentrated in the certain clusters. Moreover, there were significant correlations between monthly variations, age, and gender with gallbladder stones.

140 Comparison of Line Dance and *Senam Lansia* to Improve Cardiorespiratory Endurance in Elderly

Stephanie Indrawati Sugiarto[1], Rudy Handoyo[1,2],
Rahmi Isma Asmara Putri[1,3]
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[2]Department of Physical Medicine and Rehabilitation, Kariadi Hospital, Semarang, Indonesia
[3]Medical Rehabilitation Installation, Diponegoro National Hospital, Semarang, Indonesia

The increase of cardiorespiratory endurance in line dance group and *senam lansia* group did not differ statistically.

146 The Risk of Bag-Carrying Related to Musculoskeletal Pain in Medical Students

R. Ayu Aisyah Hanifah[1], Indri Seta Septadina[2],
Ramadhan Ananditia Putra[3]
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[3]Department of Orthopedy and Traumatology, Faculty of Medicine, Sriwijaya University, Palembang

There is no significant relationship between bag-carrying habits, which include how to carry a bag, bag weight, and the duration of using a bag, with neck, shoulder, and back pain among medical students of Sriwijaya University.

152 The Correlation of Vitamin D Levels and Ghrelin, Adiponectin, and Resistin Levels Among Post-Ischemic Stroke Patients

Santoso Jaeri[1], Retnaningsih Retnaningsih[2,3],
Amin Husni[3], Nani Maharani[4], Gemala Anjani[5]
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There is no significant correlation between serum vitamin D levels and serum ghrelin, adiponectin, or resistin levels among post-ischemic stroke patients.

160 Correlation of Single Breath Count Test with Peak Expiratory Flow Rate in Adult Patients Post COVID-19 Infection

Marcherly Wardi[1], Siti Chandra Widjanantie[1],
Heidy Agustin[2], Cleopas Martin Rumende[3],
Andari Perwira Putri[4], Sri Wahyudati[5]
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[5]Department of Physical Medicine and Rehabilitation, Faculty of Medicine, Diponegoro University, Semarang, Indonesia

This research demonstrates a significant, moderate positive correlation between SBCT and PEFR in adult post-COVID-19 patients, indicating that higher SBCT values correspond to higher PEFR measurements.

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Editorial

Artificial Intelligence (AI) technology was rapidly development, it is brings wave changes in various sectors, including the medical world. AI technology has utilized in various application , start from imaging medical, machine learning-based diagnosis, management of electronics patient data, up to prediction epidemiology disease. Challenges and opportunities that arise from AI penetration requires community medical to respond the phenomenon in a way critical and wise .

The use of AI can increase diagnostic accuracy through analysis radiology image and histopathology more fast and consistent. Deep learning algorithms, for example, have proven capable detect abnormalities on chest radiography or results digital pathology with sensitivity and specificity that are close to or even beyond doctor specialist. AI also simplifies the triage and prediction process of critically condition in emergency, and give recommendation therapy precision based on big data and electronics medical records. Efficiency in hospital administration helped, for example in scheduling, claim coding, up to monitoring quality service.

However , the adoption of AI in the medical field facing diverse challenge significant. Validation clinically suboptimal in many AI algorithms create the result prone to against dataset bias and lack of generalization in the different population . Issues of patient data privacy as well as security cyber become attention primary, considering the very large volume of medical data sensitive. From the aspect ethics, AI is not yet can fully replace consideration clinical a doctor in relation full doctor - patient empathy and trust . Risk dehumanization service health and loss personal touch is necessary beware.

Doctors and staff health competence in understand as well as leveraging AI to become prerequisite absolute for collaboration between human and machine running optimally. Curriculum of medical education must introduced basics of data science , machine learning, as well as principles ethics and law use of AI in medicine.

Responding dynamics mentioned , some step strategic can be taken :

- **AI Education and Literacy :** Medical faculty, service health institutions, and profession organizations must together increasing AI literacy among medical practitioners and students. Workshops, training, and collaboration multidisciplinary become need urge.
- **Regulations and Ethics Standards :** Government and authorities health need set regulations that govern certification AI algorithms , patient data protection , and liability aspect if happen misdiagnosis or therapy .
- **Collaboration Human-Machine :** AI is not competitors, but rather tool support clinical decision making. In fact, doctors positioned as holder control and taker decision end on every intervention medical.
- **Research and Local Innovation :** Increasing collaboration of AI research in the field health based on Indonesian population data so that AI models and innovations are relevant with public local need.

AI has potential big usher in a new era transformation of better health services and efficient. However, AI adoption needs to be addressed in a way critical –– optimizing its advantages at a time mitigate the risks. Close Collaboration between AI developers, health staff, regulators, and society is the key to making AI truly become catalyst for progress nation health, without lost essence humanity a doctor.

Editor

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OPEN ACCESS

Original Article

Association of Pre-treatment Serum Fibrinogen-Albumin Ratio Index (FARI) and Concurrent Chemoradiotherapy (CCRT) Therapeutic Response in Patients with Locally Advanced Cervical Cancer (LACC)

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Abstract

Background : Cervical cancer is currently the second most prevalent women malignancy cases in Indonesia. High prevalence of cases diagnosed as locally advanced cervical cancer / LACC (FIGO Stage IIB-IVA), where concurrent chemoradiotherapy (CCRT) is the main treatment modality. Several therapeutic efficacy predictors in other malignancy cases including the pre-treatment serum fibrinogen-albumin ratio index (FARI) have been associated with therapeutic response to CCRT. However, there were no recent studies in cervical cancer cases. The aims of this study was to determine the association of pre-treatment FARI and CCRT therapeutic response in patients with LACC.
Methods : This is a prospective cohort study in patients with LACC from January – May 2024 whose clinical stage was determined. The pre-treatment FARI was calculated in patients who had met both inclusion and exclusion criteria, and undergone the CCRT regimen. Patients who completed the regimen were evaluated for therapeutic response. Data processing was carried out using SPSS 25 for Windows software.
Results : In this study, it was found that the complete response (CR) group with the best outcome had the smallest pre-treatment FARI (9.79 ± 1.71), on the other hand the progressive disease (PD) group had largest pre-treatment FARI (33.72 ± 12.78). In addition, all CCRT therapeutic response groups had significantly different FARI values (*P value* < 0.05) and the FARI cut point value of 12.44 had a sensitivity of 100% and a specificity of 78.1% for predicting complete response (CR) to CCRT.
Conclusion : Low pre-treatment FARI is significantly associated with the likelihood of patients having a complete response (CR), which is the best outcome to CCRT.

Keywords : Fibrinogen-albumin ratio index, concurrent chemoradiotherapy, therapeutic response, cervical cancer

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INTRODUCTION

Cervical cancer until recently is still considered as one of the health problems in women, ranked as the 4th and the 2nd most malignant cases worldwide and in Indonesia, consecutively.[1,2] Based on the data obtained by the Global Burden of Cancer Study (GLOBOCAN) in 2020, it was globally estimated that there were around 604,000 new cases with 342,000 deaths per year, and in Indonesia it was estimated that there were around 36,633 new cases with 21,003 deaths per year.[2–4]

The main contributor to the high incidence and mortality of cervical cancer in Indonesia is predominantly the relative low coverage of HPV vaccination and early screening of cervical pre-cancerous lesions. Based on the data obtained from The World Health Organization-International Agency for Research on Cancer (WHO-IARC) in 2023, it was estimated that only 5–6% of women have been vaccinated against HPV and only 8–9% of women have undergone early screening for cervical pre-cancerous lesions in the last 5 years.[5,6] Another contributor to the high incidence and mortality of cervical cancer in Indonesia is the higher prevalence of cases diagnosed in later stages as locally advanced cervical cancer / LACC (Stage IIB-IVA), A meta-analysis by Monk BJ *et al*. (2022) found that around 37% of cervical cancer cases were LACC with poorer prognosis as the stage increases.[7]

Until recently, concurrent chemoradiotherapy (CCRT) is still the mainstay treatment for LACC.[1,5] A study by Cohen PA *et al*. (2019) found that the 5-year survival/overall survival (OS) of LACC after CCRT was around 70%, however approximately 35% of LACC cases still experienced disease progression or development after CCRT.[8,9] Several prognostic factors that influence the therapeutic response to CCRT in patients with LACC include age, tumor histology type, tumor size, and cancer stage (lymph node involvement and location).[9] Several inflammatory biomarkers from hematological examinations have been studied to be related to CCRT therapeutic response, including hemoglobin (Hb) levels and HPV-DNA viral load.[10–12]

Inflammation is a part of the pathophysiological processes in malignancy, several studies have found that there is more than one inflammatory mediator that is related to the process of tumor formation, development (tumorigenesis), and pre-metastasis process; these mediators include: neutrophils, platelets, lymphocytes, fibrinogen, albumin, and a combination of mediators in the ratio. Recently, several studies have been conducted on the routine mediators above as diagnostic and prognostic modality of prostate, digestive (gastric and colorectal), lung (non-small cell lung cancer), and central nervous (high-grade glioma) malignancies.[13–18] Recent studies by Sabur YA *et al*. (2023), An Q *et al*. (2020) and Huang L *et al*. (2020) have shown a significant increase in the fibrinogen-albumin ratio index (FARI) in cases of precancerous cervical lesions to cervical cancer compared to the control population of healthy patients; so that it can be applied as a promising diagnostic and prognostic modality in cervical cancer cases.[19–21]

In addition to being a diagnostic and prognostic modality for malignancy, several other studies have also found a significant association between pre-treatment FARI and overall survival (OS) prognosis from surgical management to CCRT therapeutic response in breast and digestive (esophageal and colorectal) cancer cases. However, until now there has been no research that assesses the association between pre-treatment FARI and CCRT therapeutic response in cervical cancer patients.[22–25] We conducted a study to determine the association of pre-treatment FARI and CCRT therapeutic response in patients with LACC.

METHODS

Study design and population

This study is a prospective cohort study performed in Prof. Dr. R.D. Kandou Central General Hospital, Manado, North Sulawesi, to determine the association of pre-treatment FARI and CCRT therapeutic response in patients with LACC. Eligible study participant included newly diagnosed and clinically staged patients with LACC (Stage IIB-IVA) who undergone CCRT regimen provided by the hospital from December 2023 – April 2024. The patients were recently diagnosed based on history taking, physical, and histopathological examinations, abdominal-pelvic magnetic resonance imaging (MRI), cystoscopy, rectoscopy, laboratory examination (full blood count, renal function, liver functions, and electrolytes) and chest X-Ray (distant metastasis screening) results. The patients were clinically staged based on The 2018 International Federation of Gynecology and Obstetrics (FIGO) staging of cancer of the cervix uteri.[1] Study participant with anemia (Hb < 11.0 g/dL), leucopenia (< 3,500 /uL); thrombocytopenia (< 100,000 /uL); elevated liver functions (2x increase in SGOT/AST and/or SGPT/ALT); electrolyte imbalance (Natrium < 135 or > 145 mmol/L, Kalium < 3.5 or > 5.2 mmol/L, or Chloride < 97 or > 111 mmol/L); previous history of hypertension or diabetes on treatment; previous malignancy history; previous hysterectomy history; any usage of blood product transfusion, albumin supplementation or fibrate medication before or while undergoing CCRT; and patients who didn't complete the whole regimen or diseased patients while undergoing CCRT were excluded from the study. Pre-treatment serum albumin and fibrinogen were analysed and FARI was calculated before the study participant underwent the CCRT regimen. Study participants who have completed the whole CCRT

regimen were then evaluated for therapeutic response using abdominal-pelvic MRI based on Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1).[26]

Sample Collection

Study participants underwent venipuncture to collect blood for serum fibrinogen analysis (≥ 3 mL in a plasma citrate tube) and serum albumin analysis (≥ 5 mL in a heparin tube), pre-treatment FARI was then calculated. Blood sample collection was performed after the patient had signed the informed consent form but prior to undergoing the CCRT regimen. Patient sociodemographic information such as: patient's name, medical record number, date of birth, age, phone number, body mass index (BMI), tumor histopathologic type, baseline tumor size based on initial abdominal-pelvic MRI result, and LACC stage (FIGO Stage IIB-IVA) were collected prior to undergoing CCRT regimen. All patient data, including sociodemographic data, pre-treatment serum fibrinogen and albumin analysis, calculated pre-treatment FAR, and prospectively collected therapeutic response data were collected and stored in Microsoft Excel (Version 16.59).

Serum Fibrinogen and Albumin Analysis

Serum fibrinogen and albumin analysis was carried out by Prodia Laboratory Manado. For fibrinogen analysis, a minimum of ≥ 3 mL of blood sample collected in a plasma citrate tube; while a minimum of ≥ 5 mL of blood sample collected in a heparin tube for albumin analysis. Both samples were delivered within 30 minutes to an hour time frame to the laboratory, then centrifuged in 1500 g / 3100 rpm for 15 minutes. The plasma/serum separated was analysed, and blood sample collection was repeated when hemolysis, lipemia, or icterus occurred in the post-centrifuged serum. Fibrinogen was analysed from the serum using STart-Max coagulation analyser with Both STA®-Liquid Fib reagent and STA®-Owren-Koller buffer reagent (Stago, Diagnostica Stago, Inc., US), which utilizes the electromechanical clotting method of Clauss; normal serum albumin levels were considered to be 34–48 g/L. Albumin was analysed from the serum using Cobas® C501 analyser with ROCHE Albumin Gen.2 (ALB2) reagent (Roche, F. Hoffmann-La Roche AG, CH), which utilizes the bromocresol green (BCG) colorimetry; normal serum fibrinogen levels were considered to be 2–4 g/L.

Fibrinogen-albumin ratio index (FARI)

Fibrinogen and albumin ratio index (FARI) was calculated by dividing the serum fibrinogen levels (g/L) over the serum albumin levels (g/L) and multiplied by 100%. Both serum fibrinogen and albumin levels were analysed as described above.

Concurrent Chemoradiotherapy (CCRT) Regimen Protocol

Concurrent Chemoradiotherapy (CCRT) regimen for LACC patients were given according to the standard protocol by The Radiotherapy Department of Prof. Dr. R.D. Kandou Central General Hospital, Manado, North Sulawesi. The protocol consisted of External Beam Radiotherapy (EBRT) regimen for 5 days every week with 2-field boxes using Cobalt-60 teletherapy or Linear Accelerator (LINAC) covering the entire pelvic region until a total dose equivalent of 70 Gy was achieved, and chemotherapy regimen with Cisplatin (dose 40 mg/m^2 body surface area/BSA) was carried out once a week for 6 cycles. Study participants who did not complete the whole CCRT regimen were excluded.

Therapeutic Response Evaluation

Therapeutic response of LACC patients after the whole CCRT regimen completed was then evaluated by measuring the tumor (target lesion) size comparing it to the pre-treatment baseline tumor size. Tumor size was assessed by measuring the longest tumor dimension diameter (cm) using post-treatment abdominal-pelvic MRI. Therapeutic response evaluation was classified according to the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1) criteria, into four categories[26]:

- Complete Response (CR): Disappearance of the tumor (target lesion).
- Partial Response (PR): At least a 30% decrease in tumor size compared to the baseline tumor size.
- Stable Disease (SD): Neither sufficient tumor shrinkage to be qualified as PR nor sufficient increase to be qualified as progressive disease (PD).
- Progressive Disease (PD): At least a 20% or a 0.5 cm increase in tumor size compared to the baseline tumor size.

Statistical Analysis

Association was evaluated measuring the significant difference between pre-treatment serum FARI and CCRT therapeutic response with the analysis of variance (ANOVA) using Cohen's F test or Mann-Whitney U, if the first was not fulfilled. Consecutive sampling was used as the sampling method, with a minimum of 12 study participants in each 4 groups of therapeutic responses, calculated with 95% confidence level and 80% power of test with expected drop-out of 20%. Continuous variables were expressed as mean ± standard deviations (SD) for normally distributed variables, and median and ranges

for non-normally distributed variables. Categorical variables were expressed as total and percentages. Receiver operating curve (ROC) analysis were performed. Multivariate regression analysis were performed to control possible confounding factors. All statistical analyses were conducted using Statistical Product and Service Solutions (SPSS) 25.0 software (SPSS, Inc., Chicago, IL, NY., USA) with *P-value* of < 0.05 considered as statistically significant.

Ethics

This study has obtained an ethical clearance and research permit from the Health Research Ethics Commission of Prof. Dr. R.D. Kandou Central General Hospital (Ethical Approval No. 019/EC/KEPK-KANDOU/I/2024, Research Permit No. DP.04.03/DXV/654/2024). All study participants were asked for their consent by signing a written informed consent.

Funding

This study was supported by The Department of Obstetrics and Gynecology, Prof. Dr. R.D. Kandou Central General Hospital Faculty of Medicine, Sam Ratulangi University. The funding sources had no role in the study design; in the collection, analysis, or data interpretation; in the writing of the report; and in the decision to submit the study for publication.

RESULTS

Baseline Characteristics

There were 78 study participants recruited in a 6 month period of December 2023 April 2024 in our Obstetrics and Gynecology department through our prospective cohort model. A total of 28 patients were excluded while undergoing the CCRT regimen: 8 patients died due to terminal complications, 12 patients received transfusion of blood products, 5 patients received albumin transfusion, and 3 patients refused to finish the whole CCRT regimen (Figure 1). The final cohort of 50 study participants who finished the whole CCRT regimen were evaluated for therapeutic responses. Out of the final cohort, the average age of study participants was 45.56 ± 11.88 years. The average FARI of study participants in the final cohort was 14.88 ± 8.08, However, because these data were not normally distributed (Kolmogorov-Smirnov normality test value <0.05), these data were presented in the form of a median and ranges of 12.44 (44,38) (Table 1).

Of the 50 study participants in the final cohort, it showed that BMI values were generally normal (42%) and baseline tumor size was mostly >4 cm (68%). The histological types obtained were almost equally distributed, only slightly dominant in the squamous cell carcinoma (SCC) type (40%); and the LACC stage was generally higher than stage IIIB (52%). The overall CCRT therapeutic response of patients at Prof. Dr. R. D. Kandou Hospital was still fairly good, with 38 patients (76%) classified as complete response (CR) or partial response (PR) and only 12 patients (24%) were classified as stable disease (SD) or progressive disease (PD) (Table 1).

The association of pre-treatment FARI and CCRT therapeutic response

Based on the results of the serum albumin, fibrinogen and subsequently calculated pre-treatment FARI comparison in each response group, it was found that the complete response (CR) group had the smallest average serum fibrinogen levels of 3.90 ± 0.92 g/L and the largest average serum albumin levels of 39.67 ± 6.22 g/L. This combination produced the smallest average FARI of 9.79 ± 1.71. On the other hand, the progressive disease (PD) group had the largest average serum fibrinogen levels of 7.60 ± 1.78 g/L and the smallest average serum albumin levels of 23.6 ± 3.71 g/L. This combination produced the largest average FARI of 33.72 ± 12.78. Therefore based on these data, it can be indicated that patients with smaller average FARI have a greater possibility of experiencing a complete response (CR) (Table 2).

The results of the multivariate analysis between CCRT therapeutic response groups were performed using the Mann-Whitney U test due to the data not being normally distributed. From this bivariate analysis, it was found that all groups had FARI values that were significantly different and statistically significant (Table 3). This is evidenced by the results of the *P-value* which all showed a value <0.05. The only statistically insignificant difference was found in the albumin value between the complete response (CR) and partial response (PR) groups. However, these two groups still had significant differences in FARI values. Looking at the average difference between groups, it was also found that the worse the CCRT therapeutic response, the greater difference in FARI obtained. The FARI value between CR and PR differed by 3.71; while with SD it differed by 8.67; and with PD it differed by 23.93. This bivariate analysis was continued into the ROC curve analysis (Figure 2).

From the results of this ROC curve analysis, it was found that the FARI value had a significant association with CCRT therapeutic response (Figure 2). The area under the curve (AUC) value of 0.926 produced by this study was also quite large. This value was statistically significant indicated by a *P-value* <0.005. From this curve, it was found that for screening purposes, the best FARI cut-off value to predict the outcome of complete response was 12.44. This cut-off point was found to have the highest sensitivity of 100% with a specificity of 78.1%. that was still considered excellent. In the future, this value can be a reference point used by clinicians in predicting CCRT

TABLE 1
Baseline Characteristics and Distributions of Locally Advanced Cervical Cancer Patients

Characteristics	Final Cohort Study Participants (n = 50)*
Age (years)	46.56 ± 11.88
Hemoglobin/Hb (g/dL)	11.30 (3,60)
FARI	12.44 (44,38)
BMI (kg/m^2)	
Underweight (<18.5 kg/m^2)	7 (14%)
Normal (15.8–22.9 kg/m^2)	21 (42%)
Overweight (23–24.9 kg/m^2)	6 (12%)
Obese (≥25 kg/m^2)	16 (32%)
Histologic type	
SCC	20 (40%)
NSCC	18 (36%)
Adenocarcinoma	12 (24%)
Baseline tumor size (cm)	
≤ 4 cm	16 (32%)
> 4 cm	34 (68%)
Cervical cancer stage	
IIB	10 (20%)
IIIA	6 (12%)
IIIB	8 (16%)
IIIC1R	13 (26%)
IIIC2R	5 (10%)
IVA	8 (16%)
CCRT response	
CR	18 (36%)
PR	20 (40%)
SD	7 (14%)
PD	5 (10%)

***Note:** Continuous variable such as age is displayed in *Mean ± SD*, while Hb and FARI are displayed in *Median (Range)* since data is not evenly distributed (normality test *Kolmogorov-Smirnov* < 0.05). Categoric variables such as BMI, histology types, baseline tumor size, cervical cancer stage, and CCRT response are displayed in *Total (Percentage)*.
Abbreviations: Hb = Hemoglobin, FARI = Fibrinogen-Albumin Ratio Index, SCC = Squamous Cell Carcinoma, NSCC = Non-Keratinizing Squamous Cell Carcinoma, CCRT = Concurrent Chemoradiotherapy, CR = Complete Response, PR = Partial Response, SD = Stable Disease, PD = Progressive Disease

therapeutic response in patients with locally advanced cervical cancer (LACC). Besides the FARI value, there are also other indicators that can be used and need to be considered by clinicians so that a more comprehensive scoring system can be formed in predicting patient chemoradiation outcomes.

DISCUSSION

The average age of participants in this study was 45.56 + 11.88 years and most participants (21 people, 42%) had a normal body mass index (BMI). A study in Pontianak also showed similar findings where most participants were

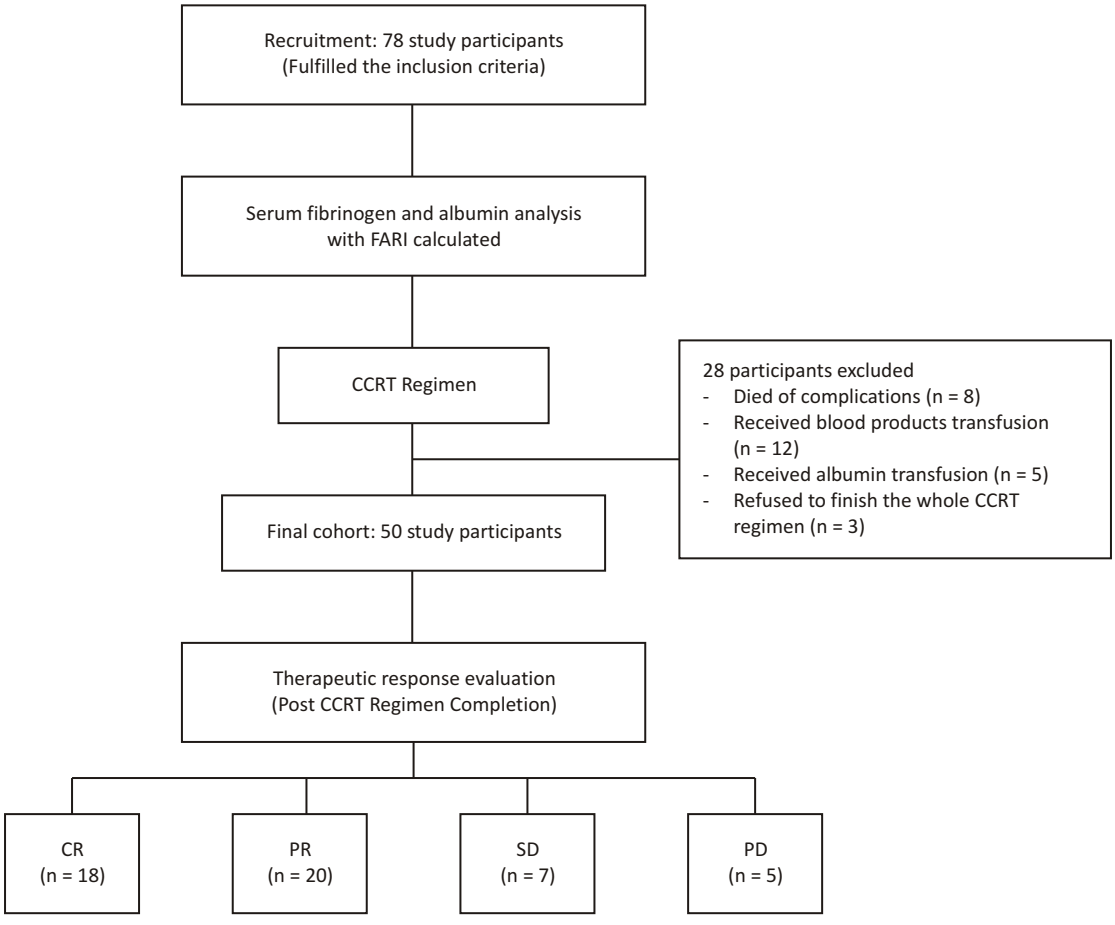

Figure 1. Study participant flowchart. Abbreviations: FARI = Fibrinogen-Albumin Ratio Index, CCRT = Concurrent Chemoradiotherapy, CR = Complete Response, PR = Partial Response, SD = Stable Disease, PD = Progressive Disease

TABLE 2
Pre-treatment fibrinogen-albumin ratio index (FARI) comparison in all CCRT therapeutic response groups for locally advanced cervical cancer/LACC (FIGO Stage IIB-IVA)

Variables*	*CR* (n=18)	*PR* (n=20)	*SD* (n=7)	*PD* (n=5)
Fibrinogen (g/L)	3.90 ± 0.92	4.90 ± 0.93	5.67 ± 0.88	7.60 ± 1.78
Albumin (g/L)	39.67 ± 6.22	36.65 ± 4.95	27.63 ± 11.28	23.6 ± 3.71
FARI	9.79 ± 1.71	13.50 ± 2.39	18.46 ± 1.97	33.72 ± 12.78

***Note:** fibrinogen, albumin and FARI in each CCRT therapeutic response group (CR, PR, SD, and PD) are displayed in Mean ± SD
Abbreviations: CCRT = Concurrent Chemoradiotherapy, FARI = Fibrinogen-Albumin Ratio Index, CR = Complete Response, PR = Partial Response, SD = Stable Disease, PD = Progressive Disease

aged 41–50 years (40.2%).[28] The most common histological type found in this study was squamous cell carcinoma (20 people, 40%). Studies in Surabaya and Pontianak also revealed that most of the histological types of cervical cancer found were squamous cell carcinoma, but in these studies the proportions were much higher at 82.39% and 70%, respectively. Squamous cell carcinoma is a cancer that originates from squamous epithelial cells and is divided into keratin and non-keratin SCC. The non-keratin type is more associated with human papillomavirus (HPV) infection than the keratin type.[28,29] Most tumors were larger than 4 cm (50 people, 68%) and most participants had stage III cervical cancer (32 people, 64%). Studies in China and

TABLE 3
Multivariate analysis of pre-treatment fibrinogen, albumin, and FARI comparison in other CCRT therapeutic response groups (PR, SD, and PD) vs complete response (CR) group

Variables*	***PR vs CR***	***P value***
Fibrinogen (g/dL)	-1.00 ± 0.30	0.02
Albumin (g/dL)	3.02 ± 1.81	0.20
FARI	-3.71 ± 0.68	0.0005
Variables*	***SD vs CR***	***P value***
Fibrinogen (g/dL)	-1.76 ± 0.41	0.001
Albumin (g/dL)	12.04 ± 3.50	0.001
FARI	-8.67 ± 0.79	0.0005
Variables*	***PD vs CR***	***P value***
Fibrinogen (g/dL)	-3.70 ± 0.58	0.001
Albumin (g/dL)	16.07 ± 2.95	0.002
FARI	-23.93 ± 2.93	0.001

***Note:** Comparison of fibrinogen, albumin and FARI in other chemoradiotherapy response groups (PR, SD, and PD) vs CR are displayed in Mean Difference ± SD
Abbreviations: CCRT = Concurrent Chemoradiotherapy, FARI = Fibrinogen-Albumin Ratio Index, CR = Complete Response, PR = Partial Response, SD = Stable Disease, PD = Progressive Disease

Pontianak also showed that most cervical cancers diagnosed were in stage III, 52.5% and 55.7%, respectively.[28,30]

The complete response (CR) group had the lowest fibrinogen levels (3.90 ± 0.92 g/L) and the highest albumin levels (39.67 ± 6.22 g/L) while the progressive disease (PD) group had the highest fibrinogen levels (7.60 ± 1.78 g/L) and the lowest albumin levels (23.6 ± 3.71 g/L). These findings are in accordance with the research by Li *et al.*, namely that low fibrinogen values have a positive prognostic value in cervical cancer patients undergoing chemoradiotherapy. When a tumor-related inflammatory response occurs, various events occur that trigger tumor growth and metastasis, starting from increased release of cytokines and inflammatory mediators, inhibition of apoptosis, and immunosuppressive effects. Inflammatory mediators can trigger pre-metastatic conditions to not be recognized by immunological surveillance and play a role in every stage of tumorigenesis and tumor development.[19,31]

Fibrinogen is an acute phase protein that increases during inflammation or infection and plays an important role in blood clotting, cell adhesion, and thrombosis. Tumor cells and inflammatory cells from tumors can activate the coagulation cascade and there is a relationship between hemostasis factors and advanced tumor stage, large tumor size, and tumor biology. Fibrinogen triggers adhesion, proliferation, and cell migration responses during angiogenesis and tumor cell growth through its role in extracellular matrix formation and increasing binding to growth factors. Other studies have also shown that fibrinogen is an independent parameter for cervical cancer patients and hyperfibrinogenemia can also be used as a parameter to predict early cervical cancer recurrence.[31,32] Fibrinogen is associated with high tumor burden and is often found in patients with advanced cervical cancer.[33]

Albumin is a negative acute phase protein associated with inflammation and tumorigenesis. Hypoproteinemia is associated with poor quality of life in cancer patients and poor prognosis in cancer patients. The findings related to albumin values in this study are also in accordance with a study by Li, *et al.* where high albumin levels are associated with higher survival rates so that it can be concluded that albumin is a prognostic indicator of cervical cancer patients.[31] Albumin values decrease due to systemic inflammatory responses and malnutrition in advanced tumors and can be a predictive factor for therapeutic response and prognostic survival in several gynecologic cancers. Other studies have also shown that hypoalbuminemia is associated with lower progression free survival (PFS) and overall survival (OS).[32–34] Approximately 20% of cancer-related deaths are caused by malnutrition. Malnutrition and inflammation can suppress serum albumin synthesis which reflects the patient's nutritional status, severity, progression, and

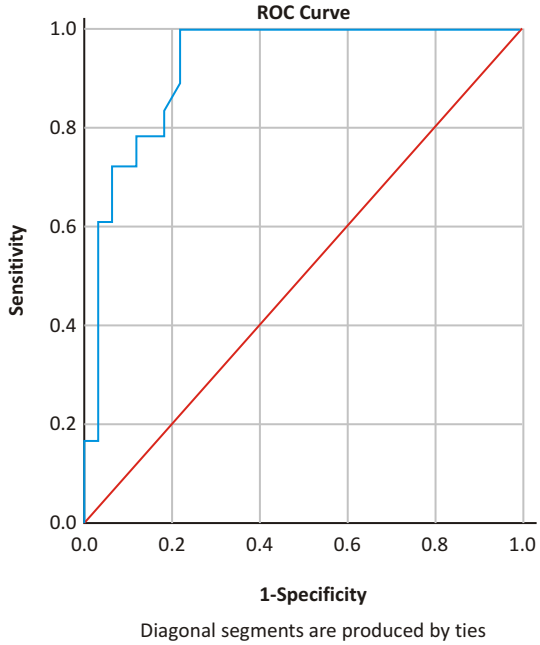

Figure 2. Study participant flowchart. Abbreviations: FARI = Fibrinogen-Albumin Ratio Index, CCRT = Concurrent Chemoradiotherapy, CR = Complete Response, PR = Partial Response, SD = Stable Disease, PD = Progressive Disease

prognosis.[35]

Based on the research results, the lower the FARI values, the greater the possibility of the patient experiencing a complete response (CR) outcome. This finding was established based on the fibrinogen and albumin values of each group. In addition, the greater the difference in FARI between groups, the worse the patient's chemoradiation response. So far, there has been no study that specifically examine the relationship between FARI and CCRT therapeutic response in cervical cancer, but higher FARI is associated with lower recurrence-free survival (RFS) and overall survival (OS).[20] The relationship between FARI and chemoradiotherapy response has been studied in other cancers. A study showed that high FARI values are associated with chemotherapy resistance, low PFS, and low OS in ovarian cancer. The fibrinogen albumin ratio index (FARI) is associated with curative effects and high FARI is associated with poor chemoradiation effects in esophageal squamous cell cancer.[23,36] A low albumin fibrinogen ratio is also associated with better chemotherapy responses in metastatic colorectal cancer and clear-cell ovarian cancer.[37,38] A high FARI is associated with poor adjuvant chemoradiation responses in rectal cancer.[39]

The fibrinogen albumin ratio index (FARI) has a better prognostic value compared to high fibrinogen values and low serum albumin. In addition, FARI also describes systemic inflammation, nutrition, and coagulation status.[32] Systemic inflammation not only plays a role in tumor initiation and metastasis but also in the initiation of chemotherapy resistance.[40] Chronic inflammatory responses are related to tumor proliferation, development, metastasis, and angiogenesis. Patients with malignant tumors experience hypoalbuminemia due to the release of cytokines such as interleukin-6 (IL-6) which inhibits albumin synthesis and secretion from liver cells and TNF-α which is also related to albumin levels. The adverse effects of fibrinogen and hypoalbuminemia may be the reason why the fibrinogen and albumin ratios are able to predict the efficacy of therapy and survival in patients receiving chemoradiation.[23]

Based on the receiver operating characteristic (ROC) curve analysis, the best FARI cut-off value for predicting complete response was 12.44. This figure is not much different from other studies on clear cell ovarian cancer where in that study, the FARI cut-off value of 12 was the optimal number for predicting platinum chemotherapy resistance with a sensitivity of 73.3% and a specificity of 68.2% while this study had a higher sensitivity and specificity of 100% and 78.1%, respectively.[37] This is different from the findings by Zhang *et al*. that the FARI cut-off value for assessing chemotherapy outcomes in metastatic colorectal cancer was 10.63; lower than this study.[38]

CONCLUSION

There was a significant association between pre-treatment FARI and CCRT therapeutic response in LACC stage IIB-IVA patients; the lower the FARI, the higher the

likelihood of the patient having a complete response (CR) outcome to CCRT (*P-value* < 0.05), the pre-treatment FARI cut-off value of 12.44 has a sensitivity of 100% and a specificity of 78.1% to predict a complete response (CR) outcome to CCRT.

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Author Contribution

GNH was involved in the conception of study and manuscript preparation; BJL and JMMS coordinated the study; GNH and BJL analysed the data and interpreted the results; all authors performed data acquisition, table design and laboratory analysis. All authors participated in the critical revision of the manuscript.

Conflict of Interest

All the authors declare that they have no conflicts of interest that might be perceived as influencing the impartiality of the reported research.

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Original Article

Effect of R-CHOP Chemotherapy on Creatinine Clearance in Diffuse Large B-Cell Lymphoma

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Abstract

Background : Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin's lymphoma (LNH). First-line therapy for DLBCL is a combination regimen of Rituximab, Cyclophosphamide, Hydroxydoxorubicin, Vincristine (Oncovin), and Prednisone (R-CHOP). The CHOP regimen causes acute kidney injury (AKI) directly or mediated by reactive oxygen species (ROS). AKI is associated with significant morbidity and mortality in cancer patients, including high mortality, increased hospitalization, and a lower cancer remission. The aims of this study was to analyzing the effect of R-CHOP chemotherapy on creatinine clearance values in DLBCL lymphoma patients

Methods : Analytical observational study with a retrospective design involved 38 patients diagnosed with DLBCL who underwent R-CHOP chemotherapy at RSUP Dr. Kariadi Semarang. The independent variable of the study was the frequency of chemotherapy. The dependent variables of the study were serum creatinine levels and creatinine clearance which were measured in 4 phases, namely before, post 3x-, post 4x- and post 5x- R-CHOP chemotherapy. Analysis used the Friedman, T-dependent, and Wilcoxon tests.

Results : The creatinine clearance value of DLBCL lymphoma patients before administering R-CHOP had a normal value with an average of 68.98 mL/min. The creatinine clearance value of DLBCL lymphoma patients after administering R-CHOP after 3x chemotherapy was found to be an average of 78.26 mL/min, after 4x chemotherapy the average was 75.50 mL/min and after 5x chemotherapy the average was 73.24 mL/min. There was a significant difference in creatinine clearance values for DLBCL lymphoma patients before and after administration of R-CHOP (p=0.018).

Conclusion : Administration of R-CHOP chemotherapy has a significant effect on the creatinine clearance value of DLBCL lymphoma patients.

Keywords : Creatinine clearance; Diffuse large B-cell lymphoma; R-CHOP

INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin's lymphoma (LNH), accounting for approximately 30–40% of LNH cases.[1] First-line therapy for DLBCL is a combination regimen of Rituximab, Cyclophosphamide, Hydroxydoxorubicin, Vincristine (Oncovin), and Prednisone (R-CHOP).[2]

Previous research stated that rituximab could cause acute kidney injury (AKI). Rituximab has been associated with electrolyte imbalance and AKI in patients with high circulating tumor cells (>25,000/mm^3) or advanced tumor conditions. This generally occurs within 12–24 hours after the first dose and is thought to be caused by acute tumor lysis syndrome (ATLS).[3] Cyclophosphamide as one of the chemotherapy regimens for DLBCL is also known to have nephrotoxic properties. Cyclophosphamide in long-term use has the side effect of inflammation and damage to the structure of kidney tissue.[4] Hydroxydoxorubicin toxicity affects various organs including the kidneys. Numerous studies have shown that hydroxydoxorubicin-induced toxicity is caused by oxidative stress, which results in oxidation and cross-linking of thiol groups as well as peroxidation of cell membrane lipids.[5] Vincristine (Oncovin) is known to contribute to drug-induced thrombotic microangiopathy (DITMA) through immune system-mediated mechanisms and toxicity. Vincristine is one of the common chemotherapeutic agents responsible for DITMA.[6] However, prednisone (corticosteroid) is known to be one of the R-CHOP therapy regimens but is also used in the treatment of acute kidney injury (AKI), more specifically acute interstitial nephritis (AIN), namely drug-induced AKI (75%), infection (5–10%) or autoimmune (15–20%).[7]

AKI is a common but significant complication in cancer patients. AKI is often caused by a combination of conditions including tumor lysis syndrome (TLS), urinary tract obstruction, use of nephrotoxic drugs, and sepsis. Patients with poor kidney function are less likely to receive optimal care. AKI became an important risk factor for all-cause mortality in all cancer patients in a dose-dependent manner.[8] Two recent studies described an overall one-year incidence of AKI in cancer patients between 11–20%, with higher risks in patients with hematological cancers. Some studies have noted much higher rates of AKI (60%),but are biased with a larger number of critically ill patients with hematological malignancies.[9] Research related to the effects of R-CHOP chemotherapy on the evaluation of kidney function with increasing frequency of chemotherapy in DLBCL patients has never been conducted in Indonesia. This study aims to analyze the effect of R-CHOP chemotherapy on creatinine clearance values in DLBCL lymphoma patients.

METHODS

Analytical observational study with a retrospective design involved 38 patients diagnosed with DLBCL who underwent R-CHOP chemotherapy at RSUP Dr. Kariadi Semarang between January 2022 to January 2023. The research sample used medical record data obtained using the whole sampling method, namely taking all patients who met the inclusion criteria and did not have exclusion criteria during the period January 2022 to January 2023 to be used as research subjects. Inclusion criteria were 1) patients diagnosed with Diffuse Large B-cell Lymphoma (DLBCL), 2) patients receiving R-CHOP therapy, and 3) complete medical record data. Exclusion criteria were 1) patients with a history of amputation, 2) patients with hypothyroidism/hyperthyroidism, and 3) patients who were pregnant. The independent variable in this study was the frequency of chemotherapy. The dependent variables of the study were serum creatinine levels and creatinine clearance which were measured in 4 phases, namely before, post 3x-, post 4x- and post 5x- R-CHOP chemotherapy. Creatinine clearance evaluation is used as an indicator of kidney function. Calculations are performed using the Cockcroft-Gault formula using baseline data of age, weight, serum creatinine levels and gender. Patients are said to have AKI if the creatinine clearance value is <60 mL/minute/1.73m^2 based on The Kidney Disease Improving Global Outcomes (KDIGO) criteria. Data analysis was carried out using the statistical application SPSS Edition 29. Analysis was carried out using the Friedman because the analysis was conducted on >2 groups, the data was on a numeric scale, the data distribution was not normal, and the evaluation was pre-post. Results are significant if p<0.05.

The research has obtained ethical permission from the Health Research Ethics Committee RSUP Dr. Kariadi Semarang with no. 1461/EC/KEPK-RSDK/2023 and Research Permit Letter from RSUP Dr. Kariadi Semarang with no. DP.04.01/I.II/7592/2023

RESULTS

The assessment was carried out on 38 patients diagnosed with DLBCL who underwent R-CHOP chemotherapy at RSUP Dr. Kariadi Semarang, the following results were obtained.

DLBCL patients at RSUP Dr. Kariadi Semarang is dominated by women (55.3%) with an average age of 56 years. The most reported stages of DLBCL were stage II (60.5%) and stage IV (21.1%). The serum creatinine values before chemotherapy, post-3x-, post-4x- and post-5x- were 1 mg/dL, 0.8 mg/dL, 0.92 mg/dL and 0.92 mg/dL. Evaluation of comorbidities found that 2 patients (5.3%) had diabetes mellitus, and 7 patients (18.4%) had hypertension.

TABLE 1
Demographics of DLBCL patients

Variable	n (%)	Mean ± SD	Median (min–max)
Gender		–	–
Male	17 (44.7)		
Female	21 (55.3)		
Age	–	–	58 (21–77)
DLBCL Stage		–	–
Stage I	7 (18.4)		
Stage II	23 (60.5)		
Stage III	0 (0)		
Stage IV	8 (21.1)		
Creatinine serum	–		
Before chemotherapy		–	1 (0.6-1.7)
After 3x chemotherapy		–	0.8 (0.4-1.7)
After 4x chemotherapy		0.92 ± 0.289	–
After 5x chemotherapy		0.92 ± 0.247	–
Diabetes mellitus		–	–
Yes	2 (5.3)		
No	36 (94.7)		
Hypertension		–	–
Yes	7 (18.4)		
No	31 (81.6)		

TABLE 2
Creatinine clearance of DLBCL patients

Variable	Mean ± SD	Median (min – max)	*P*
Creatinine clearance value			0.018
Before chemotherapy	68.98 ± 23.440	–	
After 3x chemotherapy	–	73.12 (32.22 – 202.81)	
After 4x chemotherapy	–	66.87 (34.24 – 198.33)	
After 5x chemotherapy	–	67.33 (36.52 – 198.33)	

Friedman's test; significant p<0.05

Evaluation of creatinine clearance values before chemotherapy, after 3x-, after 4x- and after 5x- showed a value of 68.98 mL/min/1.73m2, 73.12 mL/min/1.73m^2, 66.87 mL/min/1.73m^2 and 67.33 mL/min/1.73m^2. Analysis found that there was a significant difference (p=0.018) in creatinine clearance values between R-CHOP chemotherapy phases.

Evaluation of creatinine clearance status showed that before chemotherapy most patients had creatinine clearance values > 60 mL/min (60.5%). After 3x-, 4x- and

TABLE 3
Creatinine clearance status of DLBCL patients

Variable	n (%)
Creatinine clearance before chemotherapy	
<60 mL/min/1.73m^2	15 (39.5)
≥60 mL/min/1.73m^2	23 (60.5)
Creatinine clearance after 3x chemotherapy	
<60 mL/min/1.73m^2	8 (21.1)
≥60 mL/min/1.73m^2	30 (78.9)
Creatinine clearance after 4x chemotherapy	
<60 mL/min/1.73m^2	12 (31.6)
≥60 mL/min/1.73m^2	26 (68.4)
Creatinine clearance after 5x chemotherapy	
<60 mL/min/1.73m^2	13 (34.2)
≥60 mL/min/1.73m^2	25 (65.8)

5x- chemotherapy, there was an increase in the number of patients who experienced a decrease in creatinine clearance values, where patients who had creatinine clearance values <60 mL/min were 21.1%, 31.6% and 34.2%.

DISCUSSION

In this study, the research subjects had a median value of 58 years where the youngest was 21 years and the oldest was 77 years. Research assessing the influence of age on the final clinical course of DLBCL patients, found that from an assessment of 7166 patients, the average patient age was 68 years (60–79 years). A total of 2,343 subjects received R-CHOP therapy. Subjects aged 60–69 years have a 3.38x (CI95%= 2.65–4.11) higher risk of death from DLBCL than subjects aged 20–29 years.[10] Another study assessing age as a predictor of late clinical outcome in DLBCL patients in Sweden found that from an assessment of 1169 adult patients the median age value was 64.6 years.[11] The results of this study showed that the average age of DLBCL patients when the diagnosis was made was 51.83 years, where the youngest age was 22 years and the oldest age was 77 years.[12]

DLBCL is the most common B-cell non-Hodgkin's lymphoma (LNH).[13] The incidence rate increases with increasing age and the incidence is most often found in patients aged >65 years. DLBCL usually occurs in patients with comorbid diseases or very advanced age (≥80 years). Many of these patients receive R-CHOP type chemotherapy (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone) or R-CHOP-like therapy based on the patient's general condition.[14]

In this study it was found that most patients were women, this is thought to be due to the high mortality rate in male patients, so that most patients who received complete chemotherapy treatment until the end were women. Estrogen is known to play a role in the inhibition of nuclear factor kappa B (NF-κB). NF-κB regulates the transcription of interferon regulatory factor 4 (IRF4). IRF4 is involved in B and T cell differentiation and is overexpressed in B cell malignancies resulting from NF-κB hyperactivation.[15] Thus, the combination of IRF4 polymorphisms and estrogen deficiency can cause men to be more susceptible to immune cell malignancies, one of which is DLBCL. Estrogen is also known to increase the expression of B-cell lymphoma-extra-large (Bcl-XL), a well-known anti-apoptotic protein, compared with testosterone.[16]

The cancer stages most reported in this study were stage II and stage IV. Research finds that most DLBCL patients have stages IV and II. Subjects with stage IV DLBCL had a 1.37x (95% CI=1.19–1.54) higher risk of death from DLBCL than subjects with stage I DLBCL.[10] Another study reported that the most common stage of DLBCL was stage I-II (61.1%).[12]

There was a significant difference in creatinine clearance values at all stages of chemotherapy (p=0.018). Increased frequency of R-CHOP chemotherapy is associated with increased creatinine clearance in DLBCL patients. Before R-CHOP chemotherapy, most study subjects (60.5%) had good creatinine clearance values (>60 mL/minute). Administration of R-CHOP chemotherapy after cycles 1, 3, 4 and 5 showed an increase

in creatinine clearance in 5.8% of subjects, 18.4% of subjects, 7.9% of subjects and 5.3% of subjects, respectively.

A study assessing the effects of minimal renal toxicity in DLBCL patients receiving R-DHAP chemotherapy found that a decrease in creatinine clearance was observed after each cycle of R-DHAP. A statistically significant decrease in GFR was obtained after the third and fourth cycles of R-DHAP administration.[17] Research that has been conducted reports the incidence of acute kidney injury in DLBCL patients who received the R-CHOP regimen.[18] Evaluation of 653 newly diagnosed DLBCL patients who received R-CHOP regimen therapy found that 133 (20.3%) patients had creatinine clearance levels of less than 60 mL/min. Patients with low creatinine clearance levels were generally older, had advanced stages of DLBCL, extranodal involvement, high lactate dehydrogenase (LDH) levels, and a high proportion of nongerminal cell (GC) subtypes, which are indicators of poor prognosis. Therefore, patients with lower creatinine clearance levels showed worse PFS (5-year PFS 74.2% vs. 80.8%, $p = 0.13$) and OS (5-year OS 77.1% vs. 86.9%, $p = 0.013$).[19] Gamez DLG, *et al* who assessed the incidence of secondary CKD due to chemotherapy in NHL patients found that from the evaluation of 16 NHL patients there were 4 different types of chemotherapy were administered, R-CHOP was administered to 10 patients (62.5%), ERCHOP to 3 patients (18.75%), ESHAP to 1 patient (6.25%) and Metrotexate + Rituximab to 2 patients (12.5%). Of the 16 patients analyzed, 7 had AKI (43.75%), 6 of them received R-CHOP, of which 4 developed CKD (52.14%).[20]

Rituximab is a chimeric anti-CD20 monoclonal antibody used for various cases of LNH. Rituximab is administered as monotherapy but can also be used in conjunction with other regimens including CHOP. The CHOP regimen is used as standard first-line treatment in the management of NHL. The standard CHOP regimen consists of day 1 VCR 1.4 mg/m^2, day 1 DXR 50 mg/m^2, day 1 CPA 750 mg/m^2, and days 1–5 PSL 100 mg/body. VCR is a vinca alkaloid that is primarily metabolized in the liver. VCR and its metabolites are excreted 69% in the feces and 12% in the urine 72 hours after administration. The anthracycline DXR and its major metabolite doxorubicinol are largely eliminated by the kidneys. CPA is an alkylating agent that undergoes biotransformation into active metabolites in the liver. The half-life of CPA in plasma varies greatly between individuals, and ranges from 1.8 to 9.2 hours. Approximately 30–60% of CPA is eliminated by the kidneys in the initial form of the drug or as its metabolite products.[21]

Patients with low creatinine clearance levels are generally older, have advanced DLBCL, extranodal disease, high lactate dehydrogenase (LDH) levels, and a high proportion of non-germ cell (GC) subtypes, which are indicators of poor prognosis.[19]

The limitation of this study was only discusses DLBCL patients within the scope of ENT-KL science but does not include DLBCL patients in other scientific scopes, such as Internal Medicine.

CONCLUSION

Administration of R-CHOP chemotherapy has a significant effect on the creatinine clearance value of DLBCL lymphoma patients. The use of R-CHOP chemotherapy in DLBCL patients is safe for the patient's kidney function. However, evaluation of kidney function should be carried out continuously to monitor kidney function as optimally as possible because decreased kidney function requires adjustment of the dose of R-CHOP chemotherapy, thus limiting the optimal benefits obtained by the patient.

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Original Article

Integrating Neck Massage and Progressive Muscle Relaxation: A Comprehensive Approach for Reducing Migraine Disability and Enhancing Quality of Life

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Abstract

Background : Migraine is a highly disruptive and common neurological disorder characterized by intense headache and transient somatosensory and motor disturbances that affects approximately 1 billion people worldwide with many common causative factors and often results in disability and reduced quality of life. This study aims to determine the effect of combined neck massage and progressive muscle relaxation therapy on disability and quality of life of migraine sufferers.
Methods : This quasi-experimental study used a pre-test-post-test control group design over eight weeks (May to July 2024), selecting 50 participants aged 18–60 years with migraines for over three months based on ICHD-3 criteria. The study utilized the Migraine Disability Assessment Questionnaire and the Migraine-Specific Quality of Life Questionnaire as instruments.
Results : The combination therapy of neck massage and progressive muscle relaxation both has a significant effect on reducing the severity, frequency, and duration of migraine attacks, as well as improving migraine-related disability (p =0.000) and quality of life (p-*value* = 0.001).
Conclusion : Combination therapy of neck massage and progressive muscle relaxation is effective in reducing migraine disability and improving the quality of life of migraine sufferers.

Keywords : Headache, Migraine, Neck Massage, Quality of Life

INTRODUCTION

Migraine is a highly disruptive and prevalent neurological condition that affects roughly 1 billion individuals globally,[1] with multiple common causative factors and often resulting in disability.[2] Migraine is characterized by repeated bouts of moderate to severe pulsating headache, which can occur on one or both sides of the head. These headaches are aggravated by regular physical activity and can last between 4 to 72 hours.[3] Pathophysiologically, migraine is a neurovascular disorder influenced by genes ,[4] and various genetic and environmental factors.[4-6] Migraine has clinical manifestations, namely severe headache and temporary somatosensory and motor disorders.[7] Additional migraine symptoms include a headache that can persist for 4 to 72 hours, vertigo, and a heightened sensitivity to external stimuli, including light and sound (photophobia and phonophobia).[8] Various aspects of life, including physical and mental health, social relationships, work, and financial conditions, can be affected and lead to a decline in quality of life when a person experiences disruptions due to migraine symptoms.[9]

According to the 2019 Global Burden of Disease (GBD) survey, headache problems were the second leading cause of years lived with disability (YLD), following back pain, with migraines contributing 88.2% of the total cases.[10] Migraine conditions can occur episodically or chronically, and are sometimes accompanied by or without aura.[5] Compared to the general population, people with a family history of migraines have a 1.9-fold increased chance of experiencing migraines.[11] Migraine affects at least 11% of men and 19% of women worldwide. Although this condition is not fatal, for individuals aged less than 50 years, frequent attacks with great intensity rank third in cause of handicap.[12] Migraine with aura, a type of migraine, can also be influenced by genes.[13] Monozygotic twins have a 34% chance, while dizygotic twins have a 12% chance.[14]

Based on how long they correlate with the headache, migraine episodes can be separated into four phases: the premonitory phase (before the headache), the aura phase (just before or accompanying the headache), the headache period, and the postdrome phase.[15] The phases of a migraine episode might overlap and vary, but this explanation is useful. During an attack, some migraine symptoms (sensory sensitivity and neck pain) may be present, while others (aura) may come and go. The various stages of a migraine episode allow the individual to describe and distinguish between the physiological changes that occur at the start of the attack, the changes that produce the headache, and the changes that occur during recovery.[16] During a migraine attack, the premonitory phase typically includes symptoms like yawning, frequent urination, mood swings, irritability, light sensitivity, neck pain, and trouble concentrating.[17]

Research into the possible mechanisms underlying migraine shows agreement that activation of the trigeminovascular system plays an important role,[18] often caused by circulating pro-inflammatory chemicals and oxidative conditions.[19] Although efforts continue to find safe and effective prevention and treatment strategies, currently available therapies, including non-pharmacological approaches,[20] only provide partial relief, so migraine management remains a challenge.[21] In a holistic approach to migraine management, physical therapy interventions are an important aspect. Physical therapy can improve the general health and quality of life for those who are coping with this complicated neurological condition in addition to helping to alleviate symptoms.[22] For a tailored and effective approach to managing and reducing migraine symptoms, physical therapy can be a helpful adjunct.[23,24] The disability referred to in this study refers to the level of limitation of daily activities caused by migraine attacks, both in the context of work, household tasks, and social activities. Migraine Disability Assessment (MIDAS) Questionnaire instrument to measure the number of missed days, the number of days with significant disruption (≥50% decrease in productivity), Total score reflects the level of disability: the higher the MIDAS score, the greater the disability due to migraine. Quality of life in the context of this study refers to an individual's perception of how much migraine affects their daily functioning and emotional well-being. Measurements were made using the Migraine-Specific Quality of Life Questionnaire (MSQOL), which assesses the impact of migraine in three main domains: Role Restriction (RR), Role Prevention (RP), and Emotional Function (ER).

Massage therapy has emerged as a promising alternative for migraine treatment. Massage has become increasingly popular due to its ability to reduce migraine symptoms and improve overall well-being.[25] Some evidence supports that massage may help migraines, but more in-depth scientific research is needed.[26] Research on the effectiveness of massage therapy in treating migraines could yield important information, evidence-based practice, and a deeper comprehension of supplementary migraine treatment modalities.[27] Progressive muscle relaxation (PMR), a muscle tension reduction technique[28] that aims to achieve deep relaxation and relieve anxiety and tension,[28,29] has grade-A evidence as an effective relaxation method for managing migraines.[30] Recent research shows that a multidisciplinary integrative approach to managing migraines, which includes medication therapy, and relaxation, is the most successful technique for lowering headache frequency and severity, enhancing patients' quality of life, and maximizing the efficacy of pharmaceutical treatment.[31,32] Despite the data supporting progressive muscle relaxation and neck massage to treat migraines, additional studies and

targeted analyses are still necessary to assess the impact on patients' quality of life and disability.[33] This study aims to examine the integrating effect of progressive muscle relaxation and neck massage therapy on migraine-related disability and the quality of life of migraine sufferers in the Surabaya area, using a pre-test-post-test treatment with an intervention group.

METHODS

Study Design

Design of this study is quasy-experimental, pre-post test with control group.The duration of the study is 8 weeks, from May to July 2024. Participants were selected through consecutive sampling, where individuals or groups were chosen based on specific criteria aligned with the research objectives, following the ICHD-3 guidelines and modified for inclusion and exclusion criteria. Migraine Disability Assessment (MIDAS) Questionnaire instrument to measure the number of missed days, the number of days with significant disruption (≥50% decrease in productivity), Total score reflects the level of disability: the higher the MIDAS score, the greater the disability due to migraine. Quality of life in the context of this study refers to an individual's perception of how much migraine affects their daily functioning and emotional well-being. Measurements were made using the Migraine-Specific Quality of Life Questionnaire (MSQOL), which assesses the impact of migraine in three main domains: Role Restriction (RR), Role Prevention (RP), and Emotional Function (ER).

Participants

A sample of 50 person who is in the Surabaya City area. Inclusion criteria: participants who have experienced migraines for over 3 months, aged between 18 and 60 years. The exclusion criteria include participants who are currently experiencing or recovering from neck or spinal disorders, have a diagnosis of psychological disorders, or have chronic illnesses. All participants had given their consent for the study research.

Intervention

The intervention group was given a protocol of neck massage therapy combined with progressive muscle relaxation tailored to alleviate migraines. The massage was performed by several certified nurses acting as complementary therapy professionals. To ensure the ability of the massage therapists was the same, all nurses involved had undergone the same intensive training and followed a standardized therapy protocol. In addition, regular monitoring and evaluation were carried out to ensure the consistency and quality of the therapy provided to the study participants. The pre-test phase includes the initial condition where participants have not yet received any intervention. The control group receives only standard care, and all participants undergo an initial assessment before starting the intervention. The implementation of the intervention includes the subject sitting and leaning against the chair's backrest in a relaxed position with their arms hanging at their sides. The backrest extends up to the upper chest. The therapist then stands behind the chair with their hands on the patient's shoulders. A maximum of 5 minutes is needed to complete the neck massage session.[34] Then it continues with progressive muscle relaxation therapy. The guidelines for progressive muscle relaxation, as well as the relaxation exercise steps for headache sufferers, are taken from the London Headache Centre.[28] The participants perform this exercise three times a week and record their progress. For the first four weeks, training sessions consist of weekly group meetings. The video program is used to demonstrate neck massage techniques and progressive muscle relaxation. During the registration session, participants received 20 minutes of therapy (5 minutes of neck massage and 15 minutes of progressive muscle relaxation). They were then told to follow the researchers' instructions for eight weeks to complete the exercises, with the goal of completing at least 60 sessions and recording their headache data every day. Instructional fliers, weekly phone calls, and a WhatsApp group that tracks the amount of time left to study between classes and the final assessment are all used to maximize compliance with the intervention group. The post-test phase occurs after participants have completed the intervention, which includes neck massage and progressive muscle relaxation therapy over eight weeks. The final assessment is conducted to evaluate changes in migraine-related disability and quality of life, using recorded headache data and compliance tracking.

Outcome Measures

Dissability Assesment

A quick self-assessment tool called the Migraine Disability Assessment (MIDAS) Questionnaire is used to gauge patients' headache-related disability over three months. Five questions covering three activity domains make up the MIDAS score: While questions 2 and 4 measure the number of extra days with significant activity limitations (defined as a productivity decrease of at least 50%) in the domains of paid work and household tasks, questions 1, 3, and 5 measure the number of days missed because of headaches at school or paid work, household tasks, as well as family, social, or recreational activities. The total of the responses to questions 1 through 5 is the MIDAS score. Healthcare professionals can obtain additional clinical information about the

frequency of headaches and the average level of pain during the previous three months by answering two supplementary questions (A and B).

Quality of Life (QoL) Assessment

The impact of migraines on participants' daily lives is evaluated using the Migraine-Specific Quality of Life Questionnaire (MSQOL). The survey was administered at the start of the intervention, at the conclusion of the intervention, and at the follow-up assessment. The 14 items on this particular migraine tool are intended to gauge the influence and/or restrictions that migraines have on day-to-day functioning. The questionnaire includes three domains: Role Restriction (RR) with 7 items that evaluate how migraines limit social and daily work activities; Role Prevention (RP) with 4 items that assess how migraines prevent the execution of activities; and Emotional Role (ER) with 3 items that evaluate the emotional impact of migraines. The total raw score is calculated and rescaled from 0 to 100, with higher scores indicating a better Quality of Life.

Data Collection

Baseline quality of life (QoL), medical history, migraine disability scores, and basic demographic data were gathered. Participants in the study learn how to keep a headache journal. Data on headache features, including how often and how long the attacks occur, what triggers them, the accompanying symptoms, the number of days affected by migraines, and the severity of the attacks, are gathered. After the three-month intervention period, a follow-up evaluation is carried out. This research was conducted after obtaining ethical approval from the STIKES Adi Husada Research Ethics Committee through Ethical Clearance letter No. 481.2/Ket/PPM/STIKES-AH/VIII/2024.

Statistical Analysis

Basic qualities are summarized using descriptive statistics. The Wilcoxon test, the proper statistical test, was used to examine the variations in pain intensity and QoL scores both within and between groups. A statistically significant *p-value* is one that is less than 0.05.

RESULTS

Features of the Demographics

Participants were split equally between The study included an experimental group (n=25) and a control group (n=25).

Migraine Disability

The participants in both the massage and control groups initially provided their MIDAS scores. Figure 1 and Table 2 illustrate the significant impact of the combined therapy of neck massage and progressive muscle relaxation regarding migraine-related disability (MIDAS Score), which is divided into five categories: (05) Little or No Disability, (610) Mild Disability, (1120) Moderate Disability, and (more than 21) Severe Disability. Figure 2 shows a decreased frequency of headaches before and after the intervention study. Figure 3 illustrates a reduction in pain intensity experienced before and after the research (Intervention).

Based on the normality test, the data was not normally distributed, so the next statistical test used the Wilcoxon Signed Rank Test. Initial assessment of the treatment group for migraine disability status showed a mean score of 2.36 ± 0.67, demonstrating the early influence of migraines on participants' overall disability status. An exceptional decline in migraine-related disability status was observed following the intervention; the average score increased to 3.84 ± 0.82, indicating a decreasing disability status. The findings of the Wilcoxon test analysis in the intervention group, as shown in Table 2, show that the combined therapy of progressive muscle relaxation and neck massage is beneficial in reducing migraine-related impairment (MIDAS score). In contrast, in the control group, participants started with an average disability score due to initial migraines of 2.52 ± 0.62, and experienced a non-significant decrease in migraine-related disability to 2.51 ± 0.58.

Quality of life

Based on the normality test, the data was not normally distributed, so the next statistical test used the Wilcoxon Signed Rank Test. The intervention group's initial quality

TABLE 1
Comparison of clinical and sociodemographic information

Sociodemographic information*	Intervention Group (n=25)	%	Control Group (n=25)	%
Gender				
Male	5	20	5	20
Female	20	80	20	80

TABLE 1. Continued.

Sociodemographic information*	Intervention Group (n=25)	%	Control Group (n=25)	%
Age (years)				
17–25	5	20	5	20
26–35	15	60	14	56
36–45	5	20	6	24
Marital Status				
Single	11	44	13	52
Married	14	56	12	48
Education				
Illiterate	0	0	0	0
Primary	0	0	0	0
Secondary	3	12	4	16
Intermediate	10	40	9	36
University	12	48	12	48
Occupation				
Student	1	4	1	4
Manual Work	5	20	6	24
Semi Professional/Professional	16	64	11	44
Housewife	3	12	7	28
Migraine headache Onset (years)*	0	0	0	0
Current Smoking				
Yes	3	12	4	16
No	22	88	21	84
Type of Migraine				
Low Frequent Episodic Migraine (LFEM)	14	26	16	64
High Frequency Episodic Migraine (HFEM)	11	44	9	36
Chronic Migraine (CM)	0	0	0	0
Presence of Aura				
With Aura	9	36	10	40
Without Aura	16	64	15	60

of life (QoL) examination revealed an average score of 34.6 ± 6.2, demonstrating the early effect of migraines on the patients' overall QoL. Following the intervention, the average QoL score increased to 72.1 ± 7.6, indicating an exceptional improvement. The Wilcoxon Test results showed a statistically significant rise from baseline to post-intervention (p<0.001). On the other hand, participants in the control group began with an average initial quality of life score of 36.2 ± 5.6. QoL improved slightly after the intervention, reaching 37.1 ± 5.2, suggesting some improvement, but this difference was not very noticeable. Nevertheless, Table 3 shows that this change in the control group is not statistically significant.

DISCUSSION

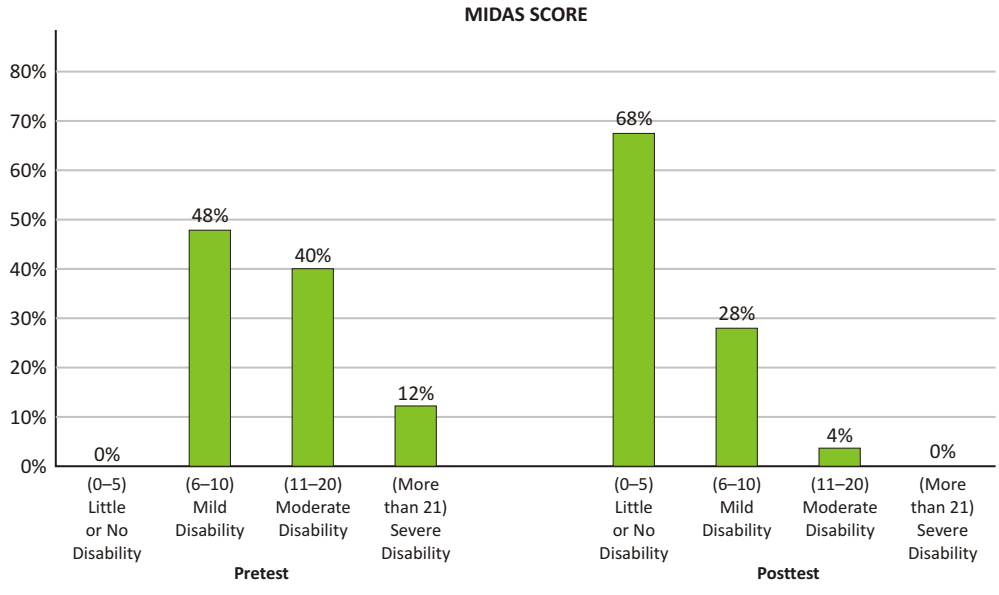

Figure 1. MIDAS Score score before and after the study performed (Intervention).

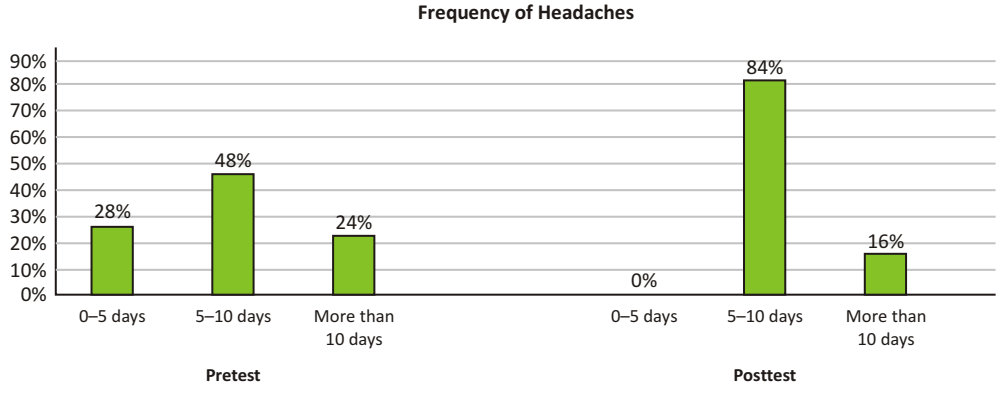

Figure 2. Frequency of headaches before and after the study performed (Intervention).

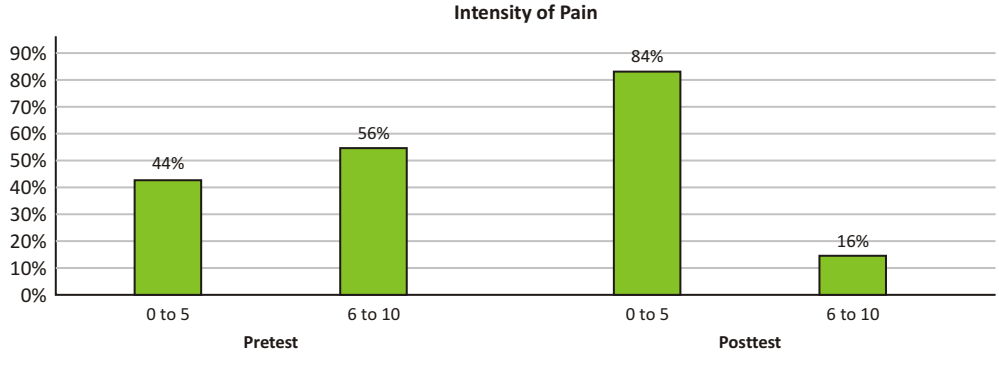

Figure 3. Intensity of pain experienced before and after study performed (Intervention)

The intervention program significantly decreased the severity, frequency, and duration of migraine episodes and enhanced the participants' overall quality of life. The majority of participants of this study are women. This result is consistent with a study conducted in India by Mehta and colleagues, in which a larger proportion of women than males participated in order to examine the impact of additional physical therapy on traditional pharmaceutical treatment for migraine sufferers. There

TABLE 2
Results of Migraine Disability (MIDAS Score)

Sociodemographic information*	Intervention Group (n=25)		Control group (n=25)	
	Pre-test	Post-test	Pre-test	Post-test
Mean	2.64	1.36	2.52	2.95
Standar Deviation	0.70	0.56	0.71	0.57
Normality (sig)	0.000	0.000	0.000	0.000
Homogenity (sig)	0.137		0.021	

TABLE 3
Wilcoxon Signed Rank Test Migraine Disability (MIDAS Score)

Sociodemographic information*	Intervention Group (n=25)		Control group (n=25)	
	Pre-test	Post-test	Pre-test	Post-test
Mean	2.36	3.84	2.52	2.51
Standar Deviation	0.67	0.82	0.60	0.58
P Value	0.000			

Description: *Significant (p <0.05); Wilcoxon Signed Rank Test

are two main forms of migraine, according to the International Headache Society: migraine without aura, which accounts for about 70% of attacks, and migraine with aura, which accounts for around 30% of incidents. This is consistent with the majority of patients in the current study experiencing migraine without aura.[35]

Common symptoms of migraines include neck pain. This symptom may lead to a number of migraine-related difficulties and can start in the premonitory period and last into the postdrome phase.[36] Neck massage therapy has been well studied as a prophylactic measure against migraines. Similarly, progressive muscle relaxation therapy has shown substantial effects on reducing migraines, with a notable decline in migraine occurrences.[37] Migraines are often associated with muscle tension around the neck and shoulders. Neck massage helps relieve this tension by manipulating the muscle tissue and reducing muscle spasms, which can alleviate pain and migraine symptoms. Neck massage can stimulate the parasympathetic nerves, which are responsible for the body's relaxation response. Activating this nervous system can help reduce stress and anxiety, which are often triggers for migraines. In addition, neck massage helps improve blood flow to the muscles around the neck and head. This increase in circulation can help reduce muscle tension and improve tissue oxygenation, which in turn can decrease the intensity and frequency of migraines.[38]

In progressive muscle relaxation therapy, it involves the gradual contraction and relaxation of various muscle groups.[28] The majority of participants in this study experienced significant functional disability due to their frequent episodic migraines. This disability was reflected in the disruption of daily activities, decreased quality of life, limitations in social interactions, and emotional impacts such as stress and fatigue. The most common complaints were neck pain and muscle tension, which contributed significantly to the level of disability. The developed therapy program, namely neck massage therapy and progressive muscle relaxation, directly addressed these main sources of complaints. Neck massage focused on relieving muscle tension and increasing circulation in areas that are often the focus of pain, while progressive muscle relaxation helped reduce stress and improve the body's response to chronic pain. Thus, both therapies were not only symptomatic, but also aimed to reduce the overall level of disability, so that participants could experience improvements in function, emotional well-being, and overall quality of life.

This technique can help reduce stress and anxiety by promoting a deep sense of relaxation,[39] which can prevent or lessen migraine attacks. PMR helps individuals increase their awareness of muscle tension in their bodies by focusing on muscle contraction and relaxation.[29] This awareness enables them to identify and address tension that can trigger migraines more quickly. Because PMR causes the sympathetic nervous system to become less active and the parasympathetic nervous

TABLE 4
Results of Quality of Life Assessment

Sociodemographic information*	Intervention Group (n=25)		Control group (n=25)	
	Pre-test	Post-test	Pre-test	Post-test
Mean	1.88	1.00	1.52	1.48
Standar Deviation	0.33	0.00	0.50	0.50
Normality (sig)	0.000	0.000	0.000	0.000
Homogenity (sig)	0.000		1.000	

TABLE 5
Wilcoxon Signed Rank Test Quality of Life Assessment

	Intervention Group (n=25)		Control group (n=25)	
	Pre-test	Post-test	Pre-test	Post-test
Mean	34.6	72.1	3.61	37.1
Standar Deviation	6.2	7.6	5.6	5.2
P Value	0.001			

Description: *Significant (p <0.05); Wilcoxon Signed Rank Test

system to become more active, which is linked to the stress response, it can help regulate the autonomic nervous system. Migraines may become less frequent and less severe as a result. Migraines can have a detrimental influence on social and professional lives, but they can also significantly increase emotional well-being when symptoms are reduced.[33]

The study's findings are consistent with randomized clinical trials that offer solid clinical proof that activities for the neck muscles, manual therapy, and pain neuroscience instruction can be used in conjunction as a non-pharmacological migraine treatment.[40] In addition, other research explains that a combination therapy program of neck massage and progressive muscle relaxation has a significant effect on reducing the severity, frequency, and duration of migraine attacks, enhancing patients' quality of life and migraine-related impairment.[24] The intervention program significantly reduces the intensity, frequency, and duration of migraine attacks, while also improving the quality of life for patients.[24] Along with lessening the negative effects of stress on the body and mind, progressive muscle relaxation improves circulation and cranio-cervical musculoskeletal function, as well as decreasing the frequency, intensity, and duration of pain.[23] According to the terms of time interaction and significant intervention for migraine attacks and days with migraines, migraine patients who receive regular relaxation treatment have a lower frequency of migraines compared to individuals who do not receive relaxation treatment. The findings of this study are consistent with previous research, indicating that an integrated education and relaxation program significantly reduces the frequency of migraine attacks in the intervention group compared to the control group. Moreover, participants receiving the intervention reported a significantly lower number of migraine days per month and shorter attack durations than those in the control group.[41] The integration of neck massage and progressive muscle relaxation offers a promising non-pharmacological strategy to reduce migraine-related disability and improve patients' quality of life. These findings underscore the relevance of holistic, patient-centered approaches in chronic pain management and support the implementation of such interventions as complementary therapies in clinical physiotherapy practice. Healthcare providers are encouraged to incorporate these techniques into individualized care plans, while future research with larger samples and extended follow-up is needed to confirm their long-term effectiveness and facilitate standardized clinical application.

CONCLUSION

Adult migraine sufferers' quality of life can be enhanced and migraine impairment can be decreased by combining progressive muscle relaxation with neck massage therapy.

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Original Article

Analysis of Category I Cesarean Section Response Time on Maternal-Neonatal Outcomes at Adam Malik Hospital Medan

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Abstract

Background : Maternal and neonatal mortality remain significant global health concerns. Category I cesarean sections are performed in life-threatening obstetric emergencies, with a recommended decision-to-incision interval (DII) of ≤30 minutes. The aims of this study was to evaluate the proportion of category I emergency cesarean sections performed ≤ 30-minute response time at Adam Malik Hospital, Medan, and to identify factors contributing to delays.

Methods : This retrospective cohort study included 44 consecutively selected cases of category I emergency cesarean sections performed at Adam Malik Hospital, Medan, between January 1 and December 31, 2023. Eligible cases involved immediate threats to maternal or fetal life and had complete documentation of surgical timings. Time data including decision-to-incision interval and its components were extracted from electronic medical records and cross-validated with operating room and delivery ward logs. The primary outcome was decision-to-incision interval (DII), dichotomized at 30 minutes. Variables associated with DII (p <0.25) were included in multivariate analysis using Firth's penalized logistic regression to account for small sample size and data separation.

Results : 43.2% of cases achieved a DII of ≤30 minutes. Multivariable analysis identified patient transfer time (AOR = 16.91, 95% CI: 2.19–358.36) and anesthesia duration (AOR = 27.21, 95% CI: 2.29–889.18) as significant predictors of delay. No significant associations were found between DII and adverse maternal or neonatal outcomes.

Conclusion : Delays in patient transfer and anesthesia were the main contributors to prolonged DII in emergency cesarean sections. While these delays did not significantly impact short-term clinical outcomes, targeted improvements in emergency obstetric workflows may help hospitals meet national response time standards and enhance quality of care.

Keywords : Emergency Caesarean Section, Decision to incision interval, Response time, Maternal Mortality rate, Neonatal mortality rate, Medan

INTRODUCTION

Maternal mortality rates remain high, with approximately 287,000 women dying during or after pregnancy and childbirth in 2020. Nearly 95% of all maternal deaths occurred in low- and lower-middle-income countries in 2020, and most of them were preventable.[1] In Indonesia, the 2020-2024 National Medium-Term Development Plan (RPJMN) targets reducing the maternal mortality ratio (MMR) to 183 per 100,000 births, down from 305 per 100,000 births.[2] Currently, Indonesia has an MMR of 189 per 100,000 live births, which is significantly higher than other countries in Southeast Asia.[3] In North Sumatra, maternal deaths in 2020 reached 195 per 100,000 live births, based on the population census long form.[4] According to data from the 2018 Sampling Registration System (SRS), 76% of maternal deaths occurred during labor and postpartum phases, with 24% during pregnancy, 36% during labor, and 40% postpartum. Over 62% of maternal and infant deaths occurred in hospitals.[5]

Based on data from the Central Statistics Agency (BPS), Indonesia's infant mortality rate (IMR) was 16.9 per 1,000 live births according to the 2020 population census, a 1.74% decrease compared to the previous year. Papua Province had the highest IMR at 38.17, while DKI Jakarta had the lowest at 10.38.[6] The high maternal mortality rate indicates that there is a need to improve the quality of antenatal care and delivery services. The National Institute for Health Care Excellence (NICE) divides emergency cesarean sections into four categories.[7] Category I cesarean section refers to a procedure where there is a direct threat to the life of the mother or fetus. The time from when the operation decision is made to the start of the surgical incision must be ≤ 30 minutes.[8]

Response time refers to the time required to respond to an incident. Specifically, the Decision to Incision Interval (DII) is the time elapsed (in minutes) from the obstetrician's decision to perform a cesarean section until the surgical incision is made.[9] While the response time system for category 1 cesarean sections has been implemented at Adam Malik Hospital, delays in achieving the target Decision-to-Incision Interval (DII) may still occur due to various contributing factors. According to the Indonesian Ministry of Health Regulation Number 30 of 2022, the national target is for 80% of category 1 cesarean sections to be performed within 30 minutes as a national indicator for hospital service quality. However, evidence from several Indonesian studies indicates that even vertical hospitals have struggled to meet this benchmark, and international literature similarly shows that few countries have achieved the 80% target. This suggests systemic and institutional barriers that warrant further investigation. Therefore, this study aims to evaluate whether Adam Malik Hospital meets the national DII performance target, to identify and analyze the factors contributing to delayed response times, and to assess how significantly these delays impact maternal and neonatal outcomes. By understanding these associations, the study seeks to inform targeted improvements in emergency obstetric care delivery.

METHODS

This retrospective cohort study was conducted at Adam Malik Hospital Medan, Indonesia, covering the period from January 1 to December 31, 2023. The study aimed to evaluate response times for category I cesarean sections and their associations with maternal and neonatal outcomes. All women undergoing emergency category I cesarean sections which defined as operations performed for immediate threats to maternal or fetal life were screened for inclusion. Indications included persistent fetal distress, umbilical cord prolapse, failed instrumental delivery (vacuum or forceps), signs of uterine rupture, antepartum hemorrhage with hypovolemic shock, placental abruption, and failed vaginal birth after cesarean (VBAC). Exclusion criteria were extrauterine pregnancies, major fetal anomalies, and maternal death occurring before the decision for cesarean section was made.

Time data were extracted primarily from electronic medical records and validated against operating room and delivery ward logs. Key time intervals were calculated from recorded timestamps and verified across data sources. Records with missing key timestamps (e.g., pediatric or anesthesia team arrival times) were excluded or flagged as incomplete to ensure accurate calculation of time intervals. No imputation or substitution was performed for missing values; only verifiable data were used. Cases were consecutively selected based on inclusion criteria, and only those with complete and internally consistent time data were retained for analysis (n=44). The primary outcome – decision-to-incision time – was defined as the interval from the clinical decision for cesarean delivery to the initial skin incision. This variable was first treated continuously, then dichotomized at >30 minutes to reflect clinically relevant delay. Component intervals were also calculated, including administrative preparation time (decision by obstetricians to anesthesia and/or pediatric consults answered), patient transfer time (decision to transfer to arrival in preoperative room), surgical preparation (arrival in preoperative room to anesthesia start), and anesthesia duration (start of anesthesia to incision). Regarding the maternal outcomes included postpartum hemorrhage (>1000 mL), blood transfusion, ICU admission, and in-hospital maternal death. Neonatal outcomes included low Apgar scores (<7 at 5 minutes), need for neonatal resuscitation, NICU admission, and neonatal death. These were extracted from surgery report

and electronic medical records.

Descriptive statistics were used to summarize baseline characteristics. All analyses were performed using R version 4.3.0. Categorical variables were reported as frequencies and percentages, and continuous variables as medians with minimum and maximum due to non-normal distributions (verified by the Shapiro-Wilk test). To test the robustness of our delay definitions, alternative percentile thresholds (75^{th}, 90^{th}) were explored. In addition, log-transformed linear regression models were used to analyze decision-to-incision time as a continuous variable. These complementary analyses ensured that findings were not dependent on arbitrary dichotomization thresholds. The final delay-related predictors, we then dichotomized the variables based on the median of time distributions due to its robustness; for example, transfer delays were defined as durations exceeding 15.5 minutes. The univariate analysis including anesthesia type (general vs. regional) and time of operation (day vs. night) were done using chi-square and fisher exact tests. Variables with *p-value* <0.25 were included into the multivariable analysis and adjusted ORs (AORs) with 95% confidence intervals (CIs) were reported. Firth's penalized logistic regression was used to mitigate bias from small sample sizes and separation.

RESULTS

A total of 44 cases that met the inclusion criteria were included in this study, 9 cases (20%) originated from the

TABLE 1
Characteristics of subjects with category I cesarean section at Adam Malik Hospital Medan

Maternal Neonatal Characteristics	(n = 44)
Mother age (years old)	30.95 ± 5.92
Education	
Primary school	2 (4.5%)
Junior high school	9 (20.5%)
Senior high school	23 (52.3%)
Bachelor	9 (20.5%)
Uneducated	1 (2.3%)
Parity	
Primigravidae	11 (25.0%)
Secundigravidae	6 (13.6%)
Multigravidae	24 (54.6%)
Grande Multigravidae	3 (6.8%)
History of C-Section	
None	21 (47.7%)
1 time	8 (18.2%)
2 times	9 (20.5%)
3 times	6 (13.6%)
Gestational age (week)	34.54 ± 4.41
Aterm	21 (47.7%)
Preterm	23 (52.3%)
Birth weight (gram)	2.540 ± 772
Normal	25 (56.8%)
Low birthweight (<2500 gr)	14 (31.8%)
Very low birthweight (<1500 gr)	2 (4.5%)
Extremely low birthweight (<1000 gr)	3 (6.8%)

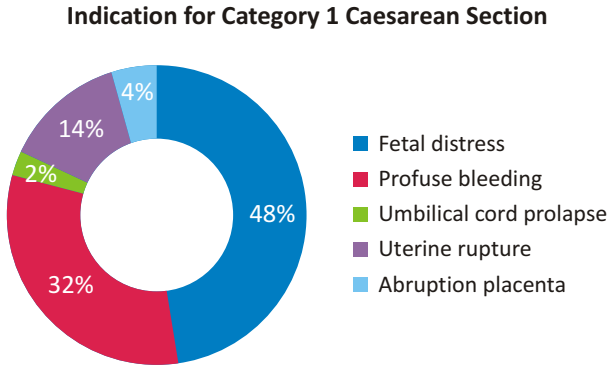

Figure 1. A pie chart shows indication of category 1 caesarean section in Adam Malik General Hospital Medan

TABLE 2
Response time and limitation to category I caesarean section

Response time and limitation	(n=44) (%)
Response time	
Decision to incision interval (median (min–max))	41 (22 – 250)
≤ 30 minute	19 (43.2%)
31 – 74 minute	16 (36.4%)
≥ 75 minute	9 (20.5%)
Factors affecting delay (median (min–max))	
Time for administration preparation	6 (2 – 79)
Time for transfer	15.5 (3 – 175)
Time for surgery preparation	13.5 (5 – 75)
Anesthesia time	15 (5 – 70)
Incision to delivery	15 (4 – 80)

obstetrics ward, while the remaining 35 cases (80%) were referred from the emergency department of Adam Malik Hospital in Medan. The monthly distribution of category I cesarean sections showed that August had the highest number of cases, with 7 cases (16%), while February and December each recorded only 1 case (2%). The demographic characteristics of the study population are presented in Table 1. The mean maternal age was 30.95 years with a standard deviation of 5.92 years. Most mothers had a high school level of education (52.3%), were multigravida (54.6%), and had no prior history of cesarean section (47.7%). The average gestational age was 34.54 ± 4.41 weeks, and the mean birth weight of the newborns was 2.540 grams ± 772 grams.

The indications for performing category I cesarean sections are illustrated in Figure 1. The three most common indications were fetal distress, which accounted for 21 cases (47.7%), profuse vaginal bleeding in 14 cases (31.8%), and uterine rupture in 6 cases (13.6%). The median decision-to-incision interval was 41 minutes, ranging from 22 to 250 minutes. A total of 19 mothers (43.2%) underwent surgery within 30 minutes, 16 mothers (36.4%) between 31 and 74 minutes, and 9 mothers (20.5%) after 75 minutes. The time required for instrument preparation had a median of 13.5 minutes, ranging from 5 to 75 minutes. Patient transfer required a median of 15.5 minutes, with the shortest time being 3 minutes and the longest 175 minutes. The duration of anesthesia had a median of 15 minutes, ranging from 5 to 70 minutes. Lastly, the time from incision to delivery of the baby was also a median of 15 minutes, with a minimum of 4 minutes and a maximum of 80 minutes (Table 2).

Table 3 presents the factors associated with delayed response time in category I cesarean sections. In the univariate analysis, patient transfer time, surgery

TABLE 3
Factors that affecting response time

Delaying factors	Response time		COR (95%CI)	*p-value*	AOR (95% CI)	*p-value*
	≤30 minute (n=19)	>30 minute (n=25)				
Time of decision			1.026 (0.31 – 3.39)	0.967[a]	NA*	NA*
Working hour	9 (47.4%)	12 (48.0%)				
Shift hour	10 (52.6%)	13 (52.0%)				
Type of Anesthesia			3.82 (0.94 – 15.55)	0.088[b]	2.55 (0.28 – 44.83)	0.423
General	8 (42.1%)	4 (16.0%)				
Regional	11 (57.9%)	21 (84.0%)				
Administration prep			5.29 (1.42 – 23.91)	0.0144[b]	5.94 (0.87 – 74.06)	0.07
≤ 6 minutes	15 (79%)	10 (40%)				
> 6 minute	4 (21%)	15 (60%)				
Patient transfer time			15.06 (3.57 – 87.37)	0.0002[b]	16.91 (2.19 – 358.36)	0.005
≤ 15.5 minute	16 (84%)	6 (24%)				
> 15.5 minute	3 (16%)	19 (76%)				
Surgery prep			8.87 (2.31 – 42.06)	0.0019[b]	1.55 (0.17 – 12.56)	0.677
≤ 13.5 minute	15 (79%)	7 (28%)				
> 13.5 minute	4 (21%)	18 (72%)				
Anesthesia time			26.42 (4.29 – 709.71)	0.0001[b]	27.21 (2.29 – 889.18)	0.007
≤ 15 minute	18 (95%)	9 (36%)				
> 15 minute	1 (5%)	16 (64%)				

[a] = chi-square, [b]= fisher-exact, NA=Not included in multivariate model ($p > 0.25$ in univariate analysis); AOR = Adjusted Odds Ratio from multivariable Firth logistic regression; COR = Crude Odds Ratio from bivariate Firth logistic regression.

preparation time, anesthesia duration, and administration preparation time were significantly associated with whether the decision-to-incision interval was within or beyond 30 minutes. Patients with a transfer time of 15.5 minutes or less were significantly more likely to receive surgery within 30 minutes (COR: 15.06; 95% CI: 3.57–87.37; $p = 0.0002$), and this remained significant in the multivariate model (AOR: 16.91; 95% CI: 2.19–358.36; $p = 0.005$). Similarly, shorter anesthesia duration (≤15 minutes) was strongly associated with timely intervention (COR: 26.42; 95% CI: 4.29–709.71; $p = 0.0001$), and this association persisted after adjustment (AOR: 27.21; 95% CI: 2.29–889.18; $p = 0.007$). While surgery preparation time (≤13.5 minutes) showed a strong association in univariate analysis (COR: 8.87; 95% CI: 2.31–42.06; $p = 0.0019$), it was not statistically significant in the multivariate model (AOR: 1.55; 95% CI: 0.17–12.56; $p = 0.677$). Administration preparation time (≤6 minutes) was also significant in univariate analysis (COR: 5.29; 95% CI: 1.42–23.91; $p = 0.0144$) but lost significance after adjustment (AOR: 5.94; 95% CI: 0.87–74.06; $p = 0.07$). Neither time of decision (working hours vs. shift hours) nor type of anesthesia (general vs. regional) showed significant associations with response time and were excluded from the multivariate model due to *p-values* greater than 0.25 in univariate analysis.

In the Firth logistic regression analysis, none of the maternal or neonatal outcomes showed a statistically significant crude association with delayed decision-to-incision time, although maternal death approached significance (COR = 0.09, 95% CI: 0–1.04, $p = 0.055$). After adjusting for other clinical variables using multivariable Firth logistic regression, no significant adjusted associations were observed. Notably, neonatal resuscitation had an elevated, though imprecise, adjusted odds ratio (AOR = 7.13, 95% CI: 0.63–119.60, $p = 0.112$), suggesting a potential signal that warrants further investigation. Other outcomes such as transfusion, low

TABLE 4
Comparison of maternal and neonatal outcomes based on decision to incision interval

Outcomes	Decision to incision interval		COR (95% CI)	*p-value*	AOR (95% CI)	*p-value*
	≤30 minute (n=19)	>30 minute (n=25)				
Maternal						
Estimated blood loss > 1000 mL			0.55 (0.16 – 1.87)	0.366[a]	1.31 (0.15 – 11.55)	0.800
Yes	8 (42.1%)	7 (28.0%)				
No	11 (57.9%)	18 (72.0%)				
Blood Transfusion			0.72 (0.22 – 2.31)	0.578[a]	1.25 (0.21 – 0.45)	0.818
Yes	10 (52.6%)	11 (44.0%)				
No	9 (47.4%)	14 (56.0%)				
Hysterectomy**			0.66 (0.19 – 2.21)	0.495[a]	–	–
Yes	8 (42.1%)	8 (32.0%)				
No	11 (57.9%)	17 (68.0%)				
Maternal death			0.09 (0 – 1.04)	0.0547[b]	0.14 (0 – 2.56)	0.197
Yes	3 (15.8%)	0 (0%)				
No	16 (84.2%)	25 (100.0%)				
Neonatal						
APGAR <7			0.52 (0.14 – 1.81)	0.3[b]	0.76 (0.11 – 4.76)	0.765
Yes	7 (36.8%)	4 (16.0%)				
No	12 (63.2%)	21 (84.0%)				
Resuscitation			1.41 (0.42 – 4.94)	0.582[a]	7.13 (0.63 – 119.6)	0.112
Yes	6 (31.6%)	10 (40.0%)				
No	13 (68.4%)	15 (60.0%)				
NICU admission			1.07 (0.33 – 3.54)	0.907[a]	0.33 (0.03 – 3.15)	0.332
Yes	8 (42.1%)	11 (44.0%)				
No	11 (57.9%)	14 (56.0%)				
Neonatal death			0.30 (0.08 – 1.03)	0.0549[a]	0.29 (0.03 – 1.97)	0.207
Yes	10 (52.6%)	6 (24.0%)				
No	9 (47.4%)	19 (76.0%)				

[a] = chi-square, [b]= fisher-exact; AOR = Adjusted Odds Ratio from multivariable Firth logistic regression; COR = Crude Odds Ratio from bivariate Firth logistic regression. **The variable Hysterectomy was excluded from the multivariable model due to collinearity or model convergence limitations.

Apgar score, NICU admission, and neonatal death also demonstrated no statistically significant associations with surgical delay. These findings may reflect limited statistical power due to the small sample size (n = 44), and thus larger studies are needed to confirm or refute these trends (Table 4).

DISCUSSION

In this study, we found that mothers who underwent category 1 cesarean sections had a mean age of 30.95 ± 5.92 years. This finding can be compared with several international studies on similar populations. For instance, a study by Andisha and Cronje in South Africa involving

153 patients who underwent category 1 cesarean sections reported that 52.9% of the mothers were aged between 2029 years, and 69.3% had a gestational age of 37–42 weeks.[10] Similarly, a retrospective cross-sectional study by Dorjey *et al*. at a tertiary hospital in Bhutan analyzed 78 category 1 cesarean sections performed in 2020.[11] most mothers were between 26–35 years old, with 47.4% being multigravida and 74.4% delivering at term. In another study conducted by Wangwe *et al*. in a Tanzanian tertiary hospital, 427 emergency cesarean sections were performed over a three-month period, of which 40 were classified as category 1.[12] Among these, 37% of the patients were aged 25–29 years, 65.6% had a parity of 2–4, 45.7% had a primary school education, and 65.3% were at term (gestational age 37–42 weeks). These comparative findings highlight variations in maternal demographics across different regions, underscoring the importance of local context in obstetric care and response system evaluations.

The most common indication for category I cesarean section was fetal distress, with decision-to-incision interval (DII) ranging from 22 to 250 minutes, and a median of 41 minutes. Similar indications have been reported in previous studies. For example, Shahwar *et al*. conducted a study at a tertiary hospital in Pakistan and identified only two primary indications for category I cesarean sections: fetal distress (59 cases) and placental abruption (34 cases).[13] Likewise, a retrospective cohort study by Dunn *et al*. in Singapore involving 390 pregnant women found that fetal distress was the leading indication (61.8%), followed by breech presentation (13.3%), umbilical cord prolapses (8.7%), and placental abruption (8.5%).[14] Response times for category I cesarean sections have also been documented in various studies. Dunn *et al*. reported a mean DII of just 9.4 ± 3.2 minutes, with a mean transfer time of 3.9 ± 2.6 minutes, anesthesia time of 3.3 ± 2.2 minutes, and surgery start time of 2.3 ± 1.3 minutes.[14] However, longer response times exceeding 30 minutes are more commonly reported in studies from developing countries. In the present study, one extreme case recorded a DII of 250 minutes. This patient, previously hospitalized for third-trimester abnormal vaginal bleeding, developed fetal distress while being treated in the obstetric ward. The significant delay was attributed to several logistical challenges: the obstetric ward was located in a separate building far from the operating theater, and both the anesthesia and perinatology teams were occupied with other emergencies during the night shift. Additionally, administrative preparations and the physical transfer of the patient contributed to the prolonged delay.

In the study by Dorjey *et al*.[11], only 29.5% of patients underwent category I caesarean section within the recommended 30-minute decision-to-delivery interval (DDI). Their reported median times were: transfer 13 (12–15) minutes, anesthesia 20 (15–28) minutes, anesthesia-to-delivery 3 (2–5) minutes, and total delivery time 25 (23–30) minutes. Similarly, Wangwe *et al*.[12] found a mean DDI of 126.73 minutes, with only 0.5% of cases meeting the ≤ 30-minute target. Kitaw *et al*.[1] reported that just 20.3% of procedures met the recommended interval. Our study showed a slightly better outcome, with 43.2% of cases achieving a response time ≤ 30 minutes, a higher proportion than in previous studies. Yeni *et al*.[1], at Zainoel Abidin Hospital, reported a mean response time of 36.29 ± 8.59 minutes for 19 eligible category I cases, while Gunawan *et al*.[1] at Sardjito Hospital observed a much higher mean of 115 ± 52 minutes, with only 40% of cases falling within 30–90 minutes.

In our bivariate analysis, several factors were associated with response time delays, including administrative preparation, transfer time, surgical setup, and anesthesia. However, multivariate analysis identified only transfer time and anesthesia as statistically significant contributors. These findings are supported by Kitaw *et al*.[1], who noted that transfer time (AOR = 5.26, 95% CI: 2.65–10.46), referral status, and type of anesthesia significantly impacted DDI. Similarly, Tebeu *et al*.[1] and Temesgen *et al*.[1] reported transfer delays and surgical preparation as significant contributors to DDI >30 minutes. Chow *et al*.[2] also emphasized patient transfer and theater preparation as key delays ($p < 0.001$), as did Mariam *et al*.[21]. The wide confidence intervals in multivariate analysis likely stem from a small sample size––only 44 category I caesarean sections were performed over one year––highlighting a limitation in our study. Additionally, accurate DII records were only available from January 2023 onward.

Regarding maternal outcomes, we found no significant differences in postoperative bleeding, transfusion, or hysterectomy between the ≤ 30 and > 30-minute groups. However, maternal mortality was higher in the ≤ 30-minute group (3 cases), this may occur because the severity of patients in the ≤ 30 minutes group is generally more severe than in the group with response times >30 minutes. 3 cases were diagnosed with respectively loss of consciousness d/t massive bleeding ec placenta accreta spectrum, profuse bleeding ec placenta accreta spectrum, and profuse bleeding ec placenta accreta spectrum with anemia (6.0 gr/dL). So the maternal condition before the caesarean section was already in a poor prognosis condition, it can be said that whether the surgery was carried out or not, the possibility of maternal death remains equally high.

For neonatal outcomes, there were no significant differences in 5-minute APGAR scores, resuscitation, NICU admission, or mortality. This contrasts with Yeni *et al*.[1], who reported significant differences in fever, ICU need, and maternal mortality favoring the ≤ 30-minute group. Their study also found worse neonatal outcomes––including APGAR scores, NICU need, and

intubation––in the > 30-minute group. On the other hand, Dorjey *et al*.[11] reported no significant differences in maternal or neonatal outcomes between groups, although neonates in the ≤30-minute group had more frequent low 1-minute APGAR scores and meconium presence. Grace *et al*.[22] found worse neonatal outcomes overall in category I versus non-category I caesareans but did not analyze outcomes by DDI. Radhika *et al*.[23] similarly found no significant neonatal outcome differences between DDI ≤ 30 and > 30-minute groups. While our findings align with previous studies showing that response time frequently exceeds 30 minutes, we observed a comparatively higher proportion of timely interventions. Maternal and neonatal outcomes were largely similar across groups, except for maternal mortality, which may reflect greater clinical severity in faster-managed cases. Differences across studies may be due to variations in patient characteristics, severity, and sample sizes. Future research with larger, more representative samples and improved recordkeeping is recommended to validate and generalize these findings.

This study contributes valuable insights into the real-world execution of category I caesarean sections in a tertiary hospital in Indonesia, particularly in terms of documenting detailed delay components and exploring both maternal and neonatal outcomes. The inclusion of multivariate analysis allowed us to adjust for confounding variables and better identify independent factors contributing to delayed response times. However, several limitations must be acknowledged. The small sample size may have limited the statistical power to detect differences, especially in maternal and neonatal outcomes. Additionally, the retrospective nature of the study, reliance on hospital records, and incomplete DII documentation prior to 2023 may have introduced information bias. The study was also conducted in a single center, which may limit its generalizability to other settings with different logistical challenges or healthcare infrastructure. Furthermore, while maternal mortality was higher in the ≤ 30-minute group, we were not able to explore individual clinical trajectories or contextual factors underlying those deaths. Similarly, extreme delay cases (e.g., DII >75 minutes or the 250-minute case) deserve more in-depth qualitative assessment in future research. Prospective, multi-center studies with larger samples and richer clinical data are recommended to validate and extend these findings. For further study, after we have done evaluating decision to incision interval (DII), we will proceed to next step to evaluate decision to delivery interval (DDI) for better performance in our hospital settings.

CONCLUSION

This study found that (43.2%) of category I caesarean sections were performed within the recommended 30-minute Decision-to-Incision Interval (DII), reflecting persistent delays despite slightly better performance compared to previous studies. Transfer time and anesthesia were identified as the most significant contributors to these delays. While no statistically significant differences were observed in maternal and neonatal outcomes between those managed within and beyond 30 minutes, the higher maternal mortality rate in the ≤ 30-minute group suggests that clinical urgency may have influenced response time prioritization. These findings underscore the need for systematic improvements in emergency response processes, particularly in patient transfer and anesthesia initiation. Strengthening these aspects may improve timely delivery and potentially enhance outcomes in high-risk obstetric cases. Further research with larger samples and more comprehensive data collection is recommended to confirm and expand upon these findings.

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Original Article

The Association between Atopy, and Family History of Asthma Patient and Severity Asthma based on Spirometry

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Abstract

Background : Asthma is a chronic inflammatory respiratory condition characterized by fluctuating symptoms, which often leads to diagnostic challenges. Accurate assessment and interpretation of clinical and functional parameters are essential to improve disease management and patient outcomes. This study aims to analyze the demographic and clinical profiles of asthma patients, particularly investigating the relationships among the degree of airway obstruction, spirometric indices, smoking exposure, environmental risk factors, atopic history, and family history, to better understand the multifactorial nature of asthma.
Methods : A retrospective descriptive-analytic study was conducted using medical records of 107 asthma patients treated at the Outpatient Installation of the Lung Polyclinic, Wlingi General Hospital, Blitar Regency, from 2021 to 2022. Descriptive statistics were used to summarize demographic and clinical data, and Spearman correlation tests assessed associations between variables.
Results : A high proportion of patients had a history of smoking, with 22.4% being active and 52.3% passive smokers. However, no significant correlation was found between smoking status and asthma control. Common environmental risk factors included exposure to dust and cold air, though these did not consistently correlate with asthma exacerbations. Significant correlations were identified between the severity of airway obstruction and spirometric parameters such as FEV1, FVC, FEV1/FVC ratio, PEF, FEF 25, FEF 50, and FEF 75, confirming the impact of airflow limitation on lung function. No significant associations were found between asthma stability and atopic or family history.
Conclusion : The findings underscore the complexity of asthma pathophysiology, where airflow obstruction is clearly linked to reduced spirometric function, but other factors such as smoking, environmental exposures, atopy, and family history do not show consistent predictive value for asthma control. These results highlight the need for a comprehensive and individualized approach in asthma diagnosis and management.

Keywords : Asthma, Atopy History, Degree of Obstruction, Environmental Risk Factors, Spirometric Parameters

INTRODUCTION

Asthma is a chronic inflammatory disease of the airways, characterized by symptoms such as wheezing, breathlessness, chest tightness, and coughing.[1,2] This inflammation leads to recurrent episodes of coughing, wheezing, breathlessness, and a feeling of heaviness in the chest.[3]

The symptoms of asthma are fluctuating and may include wheezing, coughing, shortness of breath, and a sense of chest heaviness.[1] Mismanagement often occurs due to the incorrect assumption that all abnormal breath sounds are wheezing, leading to a conclusion of asthma.[4] Accurate recording of wheezing and other consistent symptoms is crucial to prevent misdiagnosis and improper management.[5,6]

The diagnosis of asthma is established based on a thorough medical history, physical examination, and diagnostic tests. The medical history includes inquiries about respiratory complaints such as wheezing, shortness of breath, chest pain, and cough, as well as a family history of allergy-related diseases. Physical examination involves listening for expiratory wheezing during auscultation, observing for tachypnea, tachycardia, or the tripod sitting position. Trigger factors such as cold air, exercise, and pollution also need to be noted.[7]

The primary supporting examination is spirometry, measuring FEV1. In asthma patients, reversible FEV1 indicates airway obstruction (FEV1 < 0.8 and FEV1/FVC ratio < 0.70). The severity of asthma correlates with lower FEV1 and FEV1/FVC values. FEV1 increases by >12% and >200 ml after salbutamol administration.[1,8]

Several factors contribute to asthma or airway hyperreactivity, including environmental allergens such as dust mites, pet dander, cockroaches, and molds, triggering allergic reactions in sensitive individuals, leading to asthma symptoms.[7] Additionally, respiratory viral infections, like colds or flu, can narrow airways and exacerbate asthma symptoms.[8] Exercise-induced hyperventilation can trigger bronchoconstriction, especially in individuals with asthma.[1] Conditions such as gastroesophageal reflux disease (GERD), chronic sinusitis, and rhinitis can worsen asthma symptoms.[7] Certain medications like Aspirin, NSAIDs, and beta-blockers may induce asthma in some individuals.[8] Other factors such as obesity, air pollution, tobacco smoke, occupational exposure, irritants, stress, and perinatal factors (such as prematurity) can also increase asthma risk.[6,7,9–18]

Several related studies have been conducted, including research by,[19] utilizing an analytical observational design with a cross-sectional approach. Data were collected from 100 pediatric asthma patients at Dr. Soetomo Hospital in Surabaya. Atopy history was identified using the ISAAC questionnaire. Asthma severity levels were categorized based on GINA 2019. Chi-Square tests were employed for data analysis. The study found a significant relationship between atopy history and the severity of asthma in pediatric patients ($p < 0.05$). Pediatric patients with atopy history had a 2.5 times higher risk of experiencing severe asthma compared to those without atopy history.

Another study conducted by Cetemen involved ninety children aged 6–18 years. Sixty obese children and thirty healthy control children formed the study groups. Obesity was defined as a body mass index (BMI) ≥ the 95th percentile for age. Physical examinations and lung function tests (FEV1, FVC, PEF, FEF25-75) were conducted.[20] Questionnaires investigating obesity, allergic diseases, and asthma were administered. Blood samples were taken for total IgE and specific allergen measurements. The results indicated a significantly higher prevalence of asthma attacks in the past 12 months ($p = 0.049$) and family members with obesity ($p = 0.001$) among obese children compared to the control group. Specific IgE levels for food and inhalant allergens increased in the obese group, although this increase was not statistically significant ($p = 0.136$ and $p = 0.392$, respectively). There were no statistically significant differences in lung function tests ($p > 0.05$) and total IgE levels ($p = 0.619$) between the study and control groups.

London also conducted a similar study, focusing on children with both parents having asthma compared to those without parental asthma history.[21] The prevalence ratio (PR) for early-onset persistent asthma was 12.1 [95% confidence interval (CI) 7.91–18.7], compared to 7.51 (95% CI 2.62–21.5) for early-onset transient asthma and 5.38 (95% CI 3.40–8.50) for late-onset asthma. Maternal smoking during pregnancy was predominantly associated with the risk of early-onset persistent asthma if there was a history of allergies and asthma in the parents, and the combined effect was more than mere addition (interaction contrast ratio = 3.10, 95% CI 1.45–4.75). The findings of this study reinforce previous data that parental history of asthma and allergies is most strongly linked to early-onset persistent asthma, indicating that in children with genetic predisposition, early-life environmental exposure, such as maternal smoking during pregnancy, promotes the development of persistent early-onset asthma into late childhood. Similar research was also conducted by.[22–26]

The objectives of this research are to present an overview of the demographic and clinical characteristics of asthma patients and to analyze the correlation between the degree of obstruction and spirometry results, as well as the history of atopy and family history related to asthma/atopy. Through this approach, the study aims to provide deeper insights into the factors influencing asthma within the examined population.

METHODS

This research used a descriptive-analytic design with a retrospective approach. The main data source were collected from medical records of patients who underwent treatment at the Outpatient Installation of the Lung Polyclinic, Wlingi General Hospital, Blitar Regency, from 2021 to 2022. A total of 107 patients were included as the study sample.

Asthma diagnosis in this study was based on spirometry testing, which was performed both before and after administration of a bronchodilator (pre- and post-bronchodilator spirometry), in accordance with the recommended diagnostic gold standard. Spirometry was conducted at the time of diagnosis and subsequently repeated every three months as part of routine clinical follow-up.

The data analysis was carried out in two stages. The first stage involved descriptive analysis, including frequency and percentage distributions for demographic variables such as age, gender, occupation, and education. Clinical variables such as the degree of airway obstruction, spirometry results, history of atopy, and family history of asthma or atopy were also described using frequency and percentage distributions. For numerical variables like FEV1, FVC, and FEV1/FVC, mean values and standard deviations were calculated.

The second stage involved analytical statistics using the Spearman correlation test to determine the relationship between the degree of obstruction and spirometry parameters, history of atopy, and family history of asthma or atopy. The degree of airway obstruction was categorized based on FEV1/FVC values into normal, mild, moderate, and severe categories. Spirometry parameters included FEV1, FVC, and FEV1/FVC.

RESULTS

Based on data in Table 1, from the analysis of smoking history characteristics in 107 research samples, it is revealed that 22.4% of patients are active smokers, 52.3% are exposed to passive smoking, 8.4% are former smokers, while 16.8% have unknown smoking status (NA). Additionally, in analyzing Stable Asthma Diagnoses, it is observed that 14.0% of patients are categorized as Fully Controlled Stable Asthma, 63.6% are Partially Controlled, and 22.4% are Uncontrolled.

The comorbidities were found that 0.9% suffer from CHF, 0.9% DM, 2.8% GERD, 3.7% HF, 26.2% HT and the percentages vary for conditions such as Lung Tumor, Obesity, Rhinitis, and TB. Based on Atopy History, 57.0% of patients have no history of atopy, while the remaining 43.0% have atopy history, including types such as Dust Allergy, Cold, Food Allergy, Weather, Dermatitis, Eczema, Rhinitis, and HT.

Finally, in the assessment of Asthma Risk among 107 patients, it is found that 39.3% have Dust exposure risk, 43.9% are exposed to Cold Air, 10.3% have Allergy History, 7.5% are influenced by Stress, and 0.9% respond to Dry Air. Exacerbation Asthma Diagnosis illustrates that 31.8% of patients experience Mild-Moderate exacerbation, 3.7% have Severe exacerbation, 0.9% have Life-Threatening exacerbation, and in 63.6% of patients, the exacerbation level is unknown (NA). Meanwhile, the distribution for restriction, obstruction, and mixed is 14.0%, 11.2%, 41.1%, and 33.6% respectively. In terms of obstruction degree, 64.6% of patients have a mild obstruction degree, while 35.4% fall into the moderate degree among the 48 research sample participants.

Based on the data in Table 2, for information on Spirometric Results, from the 107 research samples, it is noted that the average for FEV1 (%) is 51.41%, for FVC is

TABLE 1
Characteristics of Observed Parameters

Variables	Frequency	Percentage (%)
Smoking history		
NA	18	16.8%
Active	24	22.4%
Passive	56	52.3%
Former	9	8.4%
Stable Asthma Diagnosis		
Well-Controlled	15	14.0%
Partially Controlled	68	63.6%
Uncontrolled	24	22.4%

TABLE 1. Continued.

Variables	Frequency	Percentage (%)
Comorbid		
CHF (Congestive Heart Failure)	1	0.9%
DM (Diabetes Mellitus)	1	0.9%
GERD (Gastroesophageal Reflux Disease)	3	2.8%
HF (Heart Failure)	4	3.7%
HT (Hypertension)	28	26.2%
Lung Tumor	1	0.9%
Obesity	3	2.8%
Rhinitis	6	5.6%
TB (Tuberculosis)	2	1.9%
Patient's ATOPY History		
No	61	57.0%
Yes	46	43.0%
Type of Atopy: Dust Allergy	4	3.7%
Type of Atopy: Cold Allergy	5	4.7%
Type of Atopy: Food Allergy	1	0.9%
Type of Atopy: Weather-Related Allergy	1	0.9%
Type of Atopy: Dermatitis	6	5.6%
Type of Atopy: Eczema	6	5.6%
Type of Atopy: Rhinitis	28	26.2%
Type of Atopy: Hypertension (HT)	1	0.9%
Family History of ASTHMA/Atopy		
No	71	71
Yes	36	36
Asthma (family atopy)	27	27
Rhinitis (family atopy)	5	5
Dermatitis (family atopy)	1	1
ASTHMA RISK ASSESSMENT		
Type of risk factor: Dust	42	39.3%
Type of risk factor: Cold air	47	43.9%
Type of risk factor: History of allergies	11	10.3%
Type of risk factor: Stress	8	7.5%
Type of risk factor: Dry air	1	0.9%
Diagnosis of Exacerbated Asthma		
NA	68	63.6%
Mild-Moderate	34	31.8%

TABLE 1. Continued.

Variables	Frequency	Percentage (%)
Severe	4	3.7%
Life-threatening	1	0.9%
RESTRICTION/OBSTRUCTION/MIXED		
Normal	15	14.0%
Obstruction	12	11.2%
Restriction	44	41.1%
Mixed	36	33.6%
Degree of Obstruction		
Mild	31	64.6%
Moderate	17	35.4%

Source: Researcher's data analysis (2023)

TABLE 2
Characteristics of Spirometry Results

Variables	Frequency
FEV1 (%)	51.41 ± 20.13
FVC (%)	67.13 ± 20.73
FEV1/FVC (%)	61.65 ± 10.47
PEF (%)	28.74 ± 13.58
FEF 25 (%)	26.52 ± 16.23
FEF 50 (%)	20.02 ± 12.30
FEF 75 (%)	17.81 ± 11.71

Source: Researcher's data analysis (2023)

67.13%, for FEV1/FVC is 51.41%, for PEF is 28.74%, for FEF 25 is 26.52%, for FEF 50 is 20.02%, and for FEF 75 is 17.81%.

Table 3 shows the correlation test results between stable asthma diagnosis and spirometric outcomes (FEV1, FVC, FEV1/FVC, PEF, FEF 25, FEF 50) along with the patient's atopy history and family history of asthma/atopy yielded *p-values* greater than 0.05 (p>0.05, accept Ho). Therefore, it can be concluded that there is no significant relationship between stable asthma diagnosis and spirometric outcomes (FEV1, FVC, FEV1/FVC, PEF, FEF 25, FEF 50), the patient's atopy history, and family history of asthma/atopy. In other words, regardless of the stable asthma diagnosis (Well-Controlled, Partially Controlled, Uncontrolled), there is no association with the level of spirometric outcomes (FEV1, FVC, FEV1/FVC, PEF, FEF 25, FEF 50), nor with the presence or absence of the patient's atopy history or the family history of asthma/atopy.

For the correlation test results between stable asthma diagnosis and spirometric outcome FEF 75, a correlation coefficient of -0.213 with a *p-value* of 0.028 (p<0.05, reject Ho) was obtained. It can be concluded that there is a significant relationship between stable asthma diagnosis and spirometric outcome FEF 75. The negative correlation coefficient is moderately strong, indicating that the more uncontrolled the asthma, the lower the spirometric outcome FEF 75, and conversely, the more stable the asthma, the higher the spirometric outcome FEF 75.

Table 4 shows the correlation test results indicate that the Restriction/Obstruction/Mixed pattern has a significant correlation with spirometric parameters such as FEV1, FVC, FEV1/FVC, PEF, FEF 25, FEF 50, and FEF

TABLE 3
Correlation Test Results Between Stable Asthma Diagnosis and Spirometric Results, Atopic History, and Family History of Asthma/Atopy in the Family

Variables	Correlation Coefficients	*P-value*
The relationship between Stable Asthma Diagnosis and FEV1 (%)	-0.165	0.089
The relationship between Stable Asthma Diagnosis and FVC (%)	-0.098	0.317
The relationship between Stable Asthma Diagnosis and FEV1/FVC (%)	-0.179	0.065
The relationship between Stable Asthma Diagnosis and PEF (%)	-0.186	0.055
The relationship between Stable Asthma Diagnosis and FEF 25 (%)	-0.144	0.140
The relationship between Stable Asthma Diagnosis and FEF 50 (%)	-0.145	0.137
The relationship between Stable Asthma Diagnosis and FEF 75 (%)	-0.213	0.028
The relationship between Stable Asthma Diagnosis and Atopy History	-0.159	0.103
The relationship between Stable Asthma Diagnosis and Family History	-0.063	0.517

Source: Researcher's data analysis (2023)

TABLE 4
Results of Correlation Test Between Restriction/Obstruction/Mixed with Spirometric Outcomes, Atopic History, and Family History of Asthma/Atopy

Variables	Correlation Coefficients	*P-value*
The relationship between Restriction/Obstruction/Mixed and FEV1 (%)	-0.614	0.000
The relationship between Restriction/Obstruction/Mixed and FVC (%)	-0.546	0.000
The relationship between Restriction/Obstruction/Mixed and FEV1/FVC (%)	-0.556	0.000
The relationship between Restriction/Obstruction/Mixed and PEF (%)	-0.413	0.000
The relationship between Restriction/Obstruction/Mixed and FEF 25 (%)	-0.574	0.000
The relationship between Restriction/Obstruction/Mixed and FEF 50 (%)	-0.669	0.000
The relationship between Restriction/Obstruction/Mixed and FEF 75 (%)	-0.530	0.000
The relationship between Restriction/Obstruction/Mixed and Atopy History	0.017	0.863
The relationship between Restriction/Obstruction/Mixed and Family History	-0.029	0.770

Source: Researcher's data analysis (2023)

75. The strong negative correlation coefficients for each parameter indicate that patients with obstruction to a mixed pattern tend to have lower spirometric values, while patients with restriction have relatively higher values. This conclusion is supported by the significant *p-values* ($p<0.05$) in all tests, validating the rejection of the null hypothesis. Analysis of spirometric parameters reveals that the Restriction/Obstruction/Mixed pattern is significantly associated with a decline in lung function. The strong negative correlation coefficients depict that patients with this pattern consistently experience a decrease in the tested spirometric parameters. Although there is a significant correlation with spirometric parameters, the relationship between the Restriction/Obstruction/Mixed pattern and the patient's and family's history of Atopy is not proven to be significant. The *p-values* greater than 0.05 ($p>0.05$) in both correlation tests indicate that there is no substantial correlation between this pattern and the presence or absence of Atopy history in patients or their family history.

Table 5 shows results of the correlation tests indicate a significant relationship between the Degree of Obstruction and the spirometric parameter FEV1. A strong negative correlation coefficient suggests that patients with moderate obstruction tend to have lower FEV1 values, while those with mild obstruction have relatively higher values.

TABLE 5
Correlation Test Results Between Degree of Obstruction and Spirometric Outcomes, Patient Atopy History, and Family History of Asthma/Atopy

Variables	Coefficient of correlation	*P-value*
Relationship between Degree of Obstruction and FEV1 (%)	-0.489	0.000
Relationship between Degree of Obstruction and FVC (%)	-0.300	0.038
Relationship between Degree of Obstruction and FEV1/FVC (%)	-0.829	0.000
Relationship between Degree of Obstruction and PEF (%)	-0.428	0.002
Relationship between Degree of Obstruction and FEF 25 (%)	-0.514	0.000
Relationship between Degree of Obstruction and FEF 50 (%)	-0.508	0.000
Relationship between Degree of Obstruction and FEF 75 (%)	-0.308	0.033
Relationship between Degree of Obstruction and Atopy History	0.074	0.615
Relationship between Degree of Obstruction and Family History	0.039	0.793

Source: Researcher's data analysis (2023)

TABLE 6
Results of Correlation Tests Between Degree of Obstruction and Atopy Types, Family Atopy Types, and Risk Factor Types (Asthma Risk Assessment)

Variables	Coefficient Correlation	*P-value*
The relationship between the Degree of Obstruction and the Type of Dust Allergy	0.197	0.180
The relationship between the Degree of Obstruction and the Type of Cold Allergy	0.064	0.668
The relationship between the Degree of Obstruction and the Type of Food Allergy	–	–
The relationship between the Degree of Obstruction and the Type of Weather Allergy	-0.108	0.465
The relationship between the Degree of Obstruction and the Type of Dermatitis Allergy	0.197	0.180
The relationship between the Degree of Obstruction and the Type of Eczema Allergy	-0.154	0.295
The relationship between the Degree of Obstruction and the Type of Rhinitis Allergy	0.031	0.835
The relationship between the Degree of Obstruction and the Type of Hypertension Allergy	-0.108	0.465
The relationship between the Degree of Obstruction and Asthma (family atopy)	-0.110	0.457
The relationship between the Degree of Obstruction and Rhinitis (family atopy)	0.175	0.233
The relationship between the Degree of Obstruction and Dermatitis (family atopy)	-0.108	0.465
The relationship between the Degree of Obstruction and ACT SCORE	-0.011	0.940
The relationship between the Degree of Obstruction and GINA SYMPTOMS CONTROL	-0.084	0.571
The relationship between the Degree of Obstruction and the Type of Dust Risk Factor	-0.074	0.615
The relationship between the Degree of Obstruction and the Type of Cold Air Risk Factor	0.113	0.444
The relationship between the Degree of Obstruction and the Type of Allergy History Risk Factor	0.019	0.895
The relationship between the Degree of Obstruction and the Type of Stress Risk Factor	0.169	0.252
The relationship between the Degree of Obstruction and the Type of Dry Air Risk Factor	–	–
The relationship between the Degree of Obstruction and the Diagnosis of Asthma Exacerbation	0.075	0.610
The relationship between the Degree of Obstruction and Restriction / Obstruction / Mixed	0.226	0.122

Source: Researcher's data analysis (2023)

TABLE 7
Results of Correlation Test Between the Degree of Obstruction and Types of Patient Comorbidities

Variables	Coefficient correlation	*P-value*
Relationship between Degree of Obstruction and CHF (Congestive Heart Failure)	-0.108	0.465
Relationship between Degree of Obstruction and DM (Diabetes Mellitus)	–	–
Relationship between Degree of Obstruction and GERD	-0.011	0.940
Relationship between Degree of Obstruction and HF (Heart Failure)	–	–
Relationship between Degree of Obstruction and HT (Hypertension)	-0.092	0.535
Relationship between Degree of Obstruction and Lung Tumor	–	–
Relationship between Degree of Obstruction and Obesity	0.064	0.668
Relationship between Degree of Obstruction and Rhinitis	0.092	0.534
Relationship between Degree of Obstruction and TB (Tuberculosis)	–	–

Source: Researcher's data analysis (2023)

TABLE 8
Results of Correlation Test Between Stable Asthma Diagnosis and Types of Patient Comorbidities

Variables	Coefficient of correlation	*P-value*
Relationship between Stable Asthma Diagnosis and CHF	0.153	0.116
Relationship between Stable Asthma Diagnosis and DM	0.153	0.116
Relationship between Stable Asthma Diagnosis and GERD	0.070	0.475
Relationship between Stable Asthma Diagnosis and HF	0.138	0.156
Relationship between Stable Asthma Diagnosis and HT	0.054	0.581
Relationship between Stable Asthma Diagnosis and Lung Tumor	0.153	0.116
Relationship between Stable Asthma Diagnosis and Obesity	-0.019	0.843
Relationship between Stable Asthma Diagnosis and Rhinitis	-0.042	0.671
Relationship between Stable Asthma Diagnosis and TB	0.097	0.321

Source: Researcher's data analysis (2023)

The correlation test between the Degree of Obstruction and FVC spirometric results shows a significant relationship, with a moderately strong negative correlation coefficient. Patients with moderate obstruction have lower FVC spirometric values compared to those with mild obstruction.

Furthermore, the correlation test between the Degree of Obstruction and the spirometric parameter FEV1/FVC reveals a highly significant relationship, with a very strong negative correlation coefficient. Patients with moderate obstruction have lower FEV1/FVC spirometric values compared to those with mild obstruction.

Similarly, there is a significant relationship between the Degree of Obstruction and other spirometric parameters such as PEF, FEF 25, FEF 50, and FEF 75. Strong negative correlation coefficients for each parameter indicate that patients with moderate obstruction tend to have lower spirometric values, while those with mild obstruction have relatively higher values.

Table 6 shows the correlation test results indicate that there is no significant relationship between the Degree of Obstruction and Allergy Types such as Dust, Cold, Food, Weather, Dermatitis, Eczema, Rhinitis, and Hypertension (HT). The obtained results show a p-value greater than 0.05 (p>0.05, accept Ho), indicating that mild or moderate Obstruction is not significantly associated with the presence or types of allergic atopy.

In the correlation test with Family Atopy Types, such as Asthma, Rhinitis, and Dermatitis, it also shows that there is no significant relationship with the Degree of Obstruction. The *p-value* greater than 0.05 (p>0.05, accept Ho) confirms that the Degree of Obstruction in patients

TABLE 9
Results of Correlation Test Between Restriction/Obstruction/Mixture and Types of Patient Comorbidities

Variables	Coefficient of correlation	*P-value*
Relationship between Restriction/Obstruction/Mixture and CHF	0.118	0.224
Relationship between Restriction/Obstruction/Mixture and DM	-0.015	0.878
Relationship between Restriction/Obstruction/Mixture and GERD	0.075	0.443
Relationship between Restriction/Obstruction/Mixture and HF	-0.101	0.302
Relationship between Restriction/Obstruction/Mixture and HT	0.146	0.133
Relationship between Restriction/Obstruction/Mixture and Lung Tumor	-0.015	0.878
Relationship between Restriction/Obstruction/Mixture and Obesity	0.129	0.184
Relationship between Restriction/Obstruction/Mixture and Rhinitis	-0.062	0.525
Relationship between Restriction/Obstruction/Mixture and TB	-0.021	0.827

Source: Researcher's data analysis (2023)

does not have a significant correlation with the types of atopy that may exist in the patient's family.

Furthermore, the correlation test with Asthma Control Test (ACT) Score and Gina Symptoms Control indicates that there is no significant relationship between the Degree of Obstruction and the high or low ACT score or the presence or absence of symptom control according to Gina Symptoms Control. The *p-value* greater than 0.05 (p>0.05, accept Ho) indicates that the level of asthma control in patients is not dependent on the Degree of Obstruction.

Correlation tests for Asthma Risk Assessment, including risk factors such as Dust, Cold Air, Allergy History, Stress, and Dry Air, show that there is no significant relationship with the Degree of Obstruction. The *p-value* greater than 0.05 (p>0.05, accept Ho) confirms that the Degree of Obstruction in patients does not have a significant correlation with the measured asthma risk factors in the study.

Finally, correlation tests with the Diagnosis of Asthma Exacerbation and the Restriction/Obstruction/Mixed category also show that there is no significant relationship with the Degree of Obstruction. The *p-value* greater than 0.05 (p>0.05, accept Ho) confirms that mild or moderate Obstruction is not significantly associated with the Diagnosis of Asthma Exacerbation or the Restriction/Obstruction/Mixed category in patients.

Table 7 shows the results of the correlation test between the Degree of Obstruction and comorbidities, including CHF, DM, GERD, HF, HT, lung tumor, Obesity, Rhinitis, and TB, it was found that the *p-values* were greater than 0.05 (p>0.05, accept Ho). Therefore, it can be concluded that there is no significant relationship between the Degree of Obstruction and the mentioned comorbidities, namely CHF, DM, GERD, HF, HT, lung tumor, Obesity, Rhinitis, and TB. In other words, patients with mild or moderate obstruction do not show a significant association with the presence or absence of these comorbidities.

Table 8 shows the correlation test results between Stable Asthma Diagnosis and comorbidities such as CHF, DM, GERD, HF, HT, lung tumor, obesity, rhinitis, and TB obtained a *p-value* greater than 0.05 (p>0.05, accept Ho). Therefore, it can be concluded that there is no significant relationship between Stable Asthma Diagnosis and comorbidities, namely CHF, DM, GERD, HF, HT, lung tumor, obesity, rhinitis, and TB. In other words, patients with a Stable Asthma Diagnosis, whether Fully Controlled, Partially Controlled, or Uncontrolled, do not have a significant correlation with the presence or absence of comorbidities such as CHF, DM, GERD, HF, HT, lung tumor, obesity, rhinitis, and TB.

Table 9 shows for the correlation test results between Restriction/Obstruction/Mixed patterns and patient comorbidities, including CHF, DM, GERD, HF, HT, lung tumor, Obesity, Rhinitis, and TB, the obtained *p-values* are greater than 0.05 (p>0.05, accept Ho). Therefore, it can be concluded that there is no significant relationship between Restriction/Obstruction/Mixed patterns and the mentioned patient comorbidities, namely CHF, DM, GERD, HF, HT, lung tumor, Obesity, Rhinitis, and TB. In other words, patients with Restriction/Obstruction/Mixed patterns are not associated with the presence or absence of these specific comorbidities.

DISCUSSION

This study offers a thorough analysis of the features of asthma patients and how they relate to risk factors, atopic history, family history, and spirometry parameters. According to the findings regarding smoking history and

stable asthma diagnosis, a sizable percentage of patients had smoked in the past, either actively (22.4%) or passively (52.3%). This study found no significant association between smoking history and asthma control levels, despite the fact that smoking history can have an impact on lung health. Given that active and passive smokers may have varying degrees of asthma control, this could point to the complexity of the factors affecting asthma management. These findings align with the research by Soraya,[19] confirming a significant correlation between atopic history and the severity of asthma in pediatric patients. Further identification and understanding of atopic history in children can be a crucial factor in determining appropriate management. Additionally, Cetemen found that obese children have a higher prevalence of asthma attacks.[20] Although this study did not find a significant correlation between smoking history (especially passive and active smoking) and asthma control, attention to the obesity risk factor remains relevant in evaluating asthma causative factors. The results from this study support previous findings by London,[21] indicating that a family history of asthma and allergies contributes to early-onset asthma in children. This study provides additional focus on the influence of maternal smoking during pregnancy, emphasizing that early-life environmental exposure can play a crucial role in the development of asthma at a young age. The lack of a significant association between smoking history and asthma control may be due to the heterogeneity of asthma phenotypes and biological adaptation. Asthma varies by endotype––such as allergic (Th2-high), non-allergic, or obesity-related forms––and smoking may impact some types more than others, with combined data potentially masking subtype-specific effects. Additionally, the age of asthma onset matters, as smoking may influence adult-onset asthma more strongly than early-onset forms. Biologically, a plateau effect may occur where further smoking doesn't worsen control beyond a certain inflammation threshold, and chronic smokers may develop antioxidant defenses that reduce oxidative damage, complicating the detection of a clear doseresponse relationship.

This study examined the diagnosis of asthma exacerbation and associated risk factors, finding that while certain triggers––such as dust and chilly air––were frequently reported, not all of them were significantly correlated with the severity of asthma flare-ups. This highlights the complexity of asthma exacerbations, where exposure alone may not predict the intensity of symptoms. The findings underscore the importance of identifying and managing specific risk factors on an individual basis, as well as the need for further research to better understand how these environmental and lifestyle triggers influence the course and severity of asthma exacerbations. These findings align with previous studies, including research by Soraya, which also found the complexity of the relationship between risk factors and the severity of asthma.[19] The study emphasizes the significant correlation between atopic history and the severity of asthma in children, while this research focuses on environmental risk factors. While this study shows inconsistency with some previous research indicating a correlation between specific risk factors and asthma exacerbation, such as the study by Yavuzyilmaz investigating the prevalence of asthma attacks in obese children, these findings highlight the complexity of understanding the impact of risk factors on asthma exacerbation.[22]

The spirometry results reveal that most patients exhibit reduced values, indicative of airflow obstruction or restriction. This pattern aligns with what is typically seen in asthma, where decreased spirometric measurements reflect impaired lung function. Overall, these findings corroborate existing literature demonstrating a clear association between asthma and diminished respiratory capacity. The spirometry analysis results, showing that most patients have low spirometry values, align with the findings of previous studies, including research by Soraya and Yavuzyilmaz.[19,22] Soraya found a relationship between the severity of asthma in children and low spirometry values, while Yavuzyilmaz found a higher prevalence of asthma attacks in obese children, which may also reflect impaired lung function.[19,22] Airflow obstruction and reduced spirometric values in asthma arise primarily from chronic airway inflammation, bronchial hyperresponsiveness, and smooth muscle constriction. Inflammatory cells––especially eosinophils and mast cells––release mediators that cause edema of the bronchial mucosa and increased mucus production, narrowing the airways and reducing FEV□ and FEV□/FVC ratios. During an exacerbation, smooth muscle contraction further limits airflow, while over time structural changes such as subepithelial fibrosis and airway wall thickening (airway remodeling) can lead to a more fixed component of obstruction. Together, these mechanisms explain why asthmatic patients consistently demonstrate lower spirometry values and reinforce the link between the pathophysiology of asthma and impaired lung function.

Correlation Between Degree of Obstruction and Spirometric Parameters: There is a significant correlation between the degree of obstruction and spirometric parameters, such as FEV1, FVC, FEV1/FVC, PEF, FEF 25, FEF 50, and FEF 75. This is in line with expectations because airway obstruction generally correlates with a decrease in lung function. Particularly, the strong relationship with FEV1/FVC indicates the presence of relevant airway obstruction in this study's population. The correlation results between the degree of obstruction and spirometric parameters, such as FEV1, FVC, and FEV1/FVC, consistently align with previous research, especially conducted by Soraya and Yavuzyilmaz.[19,22]

They found that the severity of asthma correlates with changes in spirometric values. This provides additional confirmation of the relationship between airway obstruction and lung function in asthma patients. The strong relationship with FEV1/FVC highlights the success of using spirometry as the primary diagnostic tool for assessing airway obstruction. These findings are in line with the GINA diagnostic guidelines (2021), emphasizing the importance of measuring FEV1/FVC in confirming an asthma diagnosis.

Relationship with Atopy History and Family History: There is no significant correlation between stable asthma diagnosis and the patient's atopic history or family history. This indicates that asthma control levels are not directly correlated with atopic history. This could be attributed to other factors influencing asthma control beyond atopic history. Correlation with Comorbidity Types: There is no significant relationship between the degree of obstruction or stable asthma diagnosis and comorbidities such as CHF, DM, GERD, HF, HT, lung tumor, obesity, rhinitis, and TB. This suggests the complexity of managing asthma patients with comorbidities, where these factors might require different management approaches.

CONCLUSION

Smoking history showed no significant association with asthma control levels, although a large proportion of patients reported both active and passive smoking exposure. Environmental triggers such as dust and cold air were common but only some--particularly dust--were linked to exacerbation severity, underscoring the need for targeted avoidance strategies. Spirometric analysis demonstrated that lower values across FEV□, FVC, FEV□/FVC, PEF, and FEF indices closely tracked with the clinician□graded degree of airway obstruction, confirming their utility in assessing airflow limitation.

Conversely, neither a personal nor family history of atopy correlated significantly with stable asthma diagnosis or with the pattern and severity of obstruction, nor did comorbidities or the broad categories of risk factors. Taken together, these findings directly address the study's objective by highlighting that while lung function indices reliably reflect obstruction, atopic predisposition and smoking exposure do not predict control or severity in this cohort. Clinically, this supports a multifactorial, personalized approach--prioritizing spirometric monitoring and individualized risk□factor management--while advocating further research into the mechanisms by which non□atopic, non□smoking factors drive asthma heterogeneity.

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Original Article

Effectiveness of Mar'ke Bilar Drink Formulation on Pro- Anti-Free Radicals in The Blood of Women of Childbearing Age Operators in Gas Stations

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Abstract

Background : Women of childbearing age who wortk at gas stations are vulnerable to pollution exposure due to fuel emissions. The workers are susceptible to heavy metal (Pb) poisoning in the fuel content. This can cause health problems such as reproductive disorders, impaired kidney function and can even cause anemia in women. The aims of this study was to determine the effectiveness of Mar'ke Bilar formulation on Pb, MDA, and SOD in blood.
Methods : The study was Quasi Experimental with a pre and post test group design that allows researchers to see the comparison of the control group with the experimental group. The sample in the study was Women of childbearing age who worked as operators at gas stations with a total of 60 people and were determined based on purposive sampling with inclusion criteria then divided into 4 treatment groups. Data analysis used the T-dependent test to see the differences in each group then continued with the One Way ANOVA and Post Hoc-Tukey tests.
Results : The results of the study showed that the three of Mar'ke Bilar formulations were effective in reducing Pb and MDA levels, as well as increasing SOD levels, which are endogenous antioxidant enzymes that are important in the body's defense against oxidative stress. While formula 1 proved to be the most effective in increasing SOD levels.
Conclusion : Mar'ke Bilar affects blood Pb, MDA, and SOD levels and formulation 1 is the most effective in increasing SOD levels. It is necessary to consume colored foods such as Marke'Bilar which are very good for avoiding diseases caused by free radicals by air and water pollution, especially those caused by heavy metals.

Keywords : Mar'ke Bilar, Pb, MDA, SOD, Free radicals

INTRODUCTION

Health problems in humans, which comes from air, water, soil pollution and motor vehicle smoke and also factory waste. Air pollution contributes to the health of workers and communities in the open air such as highways and their surroundings and in factory environments. Traffic police, street vendors, buskers at red lights, beggars and Public Fuel Station (SPBU) operators, are one of the groups of people who are most vulnerable to Pb exposure, where Pb is used as a mixture to improve fuel quality so that the combustion process becomes better.[1]

Operators at gas stations, especially women of childbearing age are vulnerable to exposure to Pb metals, especially when the vehicle is burning.[2] The location of the gas station which is also close to the highway, can increase the risk of exposure to heavy metals from vehicles traveling on the highway.[3]

The entry of Pb into the human body through the respiratory or digestive tract, then accumulates in the blood and through the reduction-oxidation cycle will produce free radicals in the form of *Reactive Oxygen Species* (ROS) including Hydrogen Peroxide ((H_2O_2), Nitrogen Oxide (NO), Oxygen Peroxide (O_2-).[4]

Several studies on women of childbearing age have found that Pb can cause reproductive disorders, increased cases of infertility, menstrual disorders, decreased sexual libido, toxicity to reproductive organs and can also cause disorders in Hb formation which causes decreased work productivity.[2,5]

Pb as a free radical trigger, can damage endogenous antioxidant enzymes such as GPx *(Glutathione Peroxidase)*, CAT *(Catalase)*, and SOD *(Superoxide dismutase)*. Other disorders resulting from the accumulation of Pb in the body in women of childbearing age can cause cervical cancer and breast cancer[6] due to disruption of the signal transduction process in DNA which can disrupt gene expression (DNA).

Moreover, the accumulation of Pb in the body can also cause gene mutations so that during the fertile period, if fertilization occurs, it can cause miscarriage or even initiation into the fetus, resulting in defects in the fetus and the baby being born and the worst prognosis is giving birth to a child with autism.[7]

An increase in *Malondialdehyde* (MDA) and a decrease in SOD *(Superoxide dismutase)* are markers of ongoing oxidative stress. SOD as an endogenous antioxidant and MDA as a marker of free radicals in the body are effects caused by exposure to Pb metal.[8] The application of the antioxidant system is a mechanism in self-defense against pollutant stress characterized by the loss or decrease in the content of antioxidant vitamins.[5]

Endogenous antioxidants obtained from the foods we consume daily are able to stimulate the antioxidant enzymatic system (SOD) and can reduce the formation of ROS and its effects on health. *Foodstuffs* containing vitamins A, and C and the mineral zinc (Zn) and bioactive substances can be found in colored foods and are found to have radicals scavenger ability.[9,10]

Persimmon *(Diospyros kki L.)* and purple passion fruit *(Ipomoea Batatas Var. antin 3)* are known to contain bioactive compounds and antioxidants such as carotenoids, anthocyanins, flavonoids, vitamin C, zinc, and Fe.[11] One hundred grams of persimmon contains vitamin A 81 IU, and vitamin C 7.5 mg, while purple passion fruit contains vitamin A 2410 IU and vitamin C 28mg. These components can help eliminate free radicals caused by heavy metal exposure by inhibiting free radicals' toxicity.[12] Persimmon and purple passion fruit are typical fruits of North Sumatra. Purple sweet potato is widely available in North Sumatra, especially in mountainous areas. Starch from purple sweet potato can be used as a sugar substitute, as well as a thickener and stabilizer for beverage products.[13] In 100 gr of purple sweet potato contains vitamin A 7700 IU and vitamin C 22 mg.

The three food ingredients derived from fruit and tubers have the potential to be made into a healthy drink called Mar'ke Bilar, through the process of sorting ingredients, steaming, heating, blending, filtering to become fruit juice that is ready to drink. The combination of these three ingredients has a sweet taste, a passion fruit aroma like citrus and a purplish pink color so that it increases the appetite for consumption. This drink has gone through an acceptance process with organoleptic tests and examination of nutritional and phytochemical content at the FMIPA and Agricultural Product Technology Laboratory, Brawijaya University.[13]

Mar'ke Bilar is a functional drink that combines fruits and tubers that are expected to complement each other, especially the bioactive and antioxidant components, so that it can be an option as a functional drink. The fruits and tubers used are local food products, easy to obtain so that the processing process becomes easier.

Based on the description above, it is necessary to conduct research that aims to determine the effectiveness of Mar'ke Bilar drink formulations on pro (Pb, MDA) and anti-free radicals (SOD) on Women of Childbearing age operators at gas stations.

METHODS

This type of research was a *quasi-experiment with pre- and Post Test Design with Control*.[14] The research was conducted for a month from February 10th to March 10th, 2023 at several gas stations that were willing to be used as research sites in Medan and surrounding areas. Only 8 gas stations were willing to be used as research locations, considering that due to their busy schedules, the time available was more limited. The research

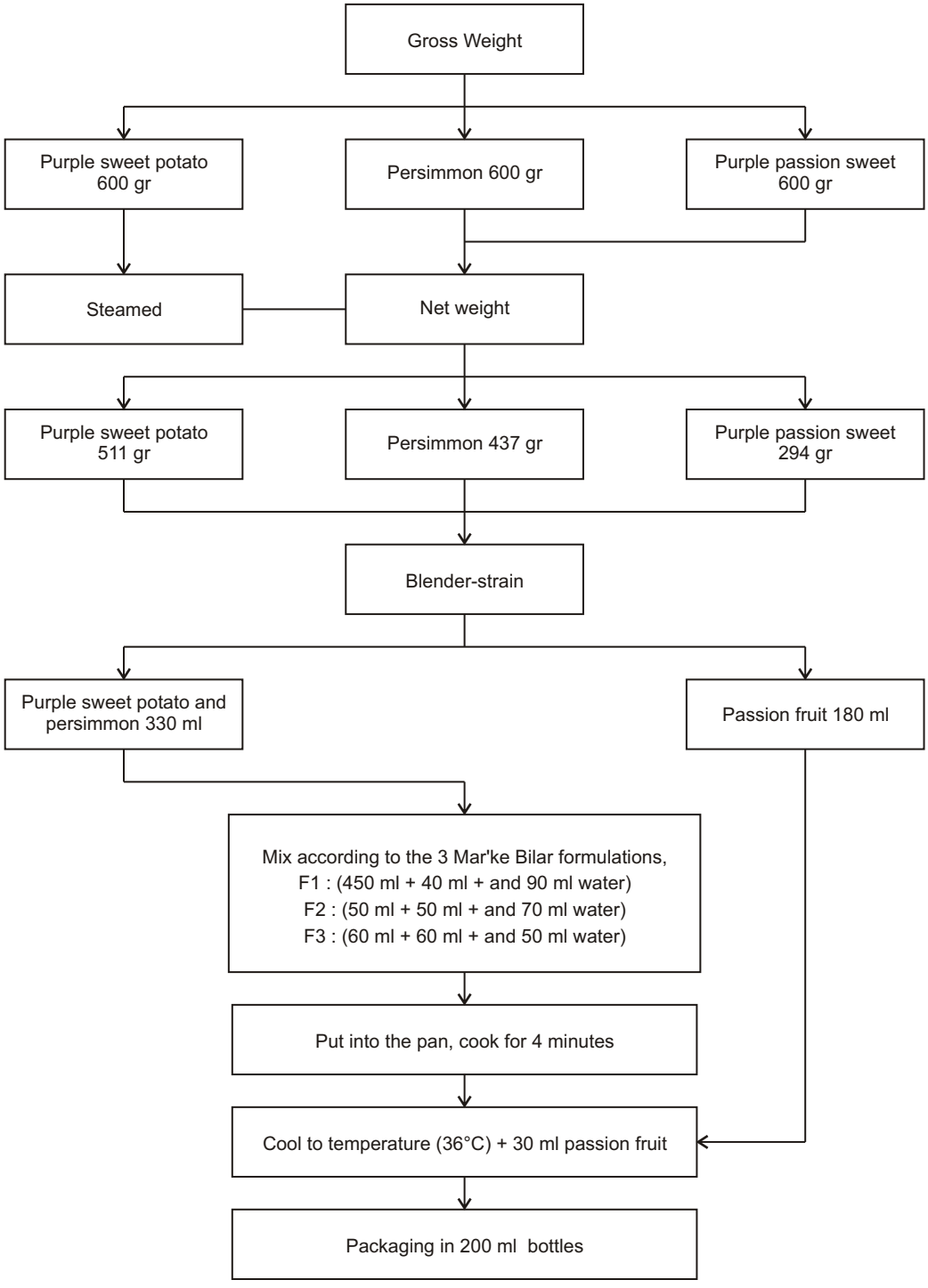

Figure 1. Procedure for Making Mar'ke Bilar Drink

population was Women of childbearing age as operators at gas stations in total 83 people The sample was determined by setting inclusion criteria: The sample was determined by purposive sampling, where researchers took samples based on the conditions and willingness to be sampled, so that researchers obtained samples combined with accidental sampling. The inclusion criteria were aged 20–35 years, still menstruating, not sick and actively working every day except holidays. Exclusion criteria: female gas station operators who are not pregnant because pregnant women are prone to initiation of heavy metal exposure to the fetus, Women of childbearing age in gas station operators who have worked ≤ 1 year, gas station operators who work part-

TABLE 1
Nutrient Content of Mar'ke Bilar with Various Formulations

Nutrients	Marke Bilar Beverage Formulation			Unit
	F1	F2	F3	
Vitamin C	51.73	56.81	65.87	mg/g
Beta carotene	2.79	4.49	5.1	µg/g
Zinc (Zn)	0.86	0.92	0.70	mg/kg
Iron (Fe)	3.93	4.68	4.42	mg/kg
Anthocyanins	170.99	233.5	268.51	ppm
Antioxidants	40.75	39.73	38.49	mg/ml
Phenol	0.38	0.57	0.91	%

Source: Brawijaya University Agricultural Products Laboratory in 2023

TABLE 2
Distribution based on sample characteristics

Characteristics Sample		Variable N	Frequency Percent %
Age	20–30 years old	30	50.0
	>30–35 years old	30	50.0
	Total	60	100
Education	Primary School	1	1.7
	Junior High School	2	3.3
	Senior High School	57	95.0
	Total	60	100
Length of Service	<13 months	14	23.3
	13–24 months	18	30.0
	>24 months	28	46.7
	Total	50	100

time, male gas station operators, gas station operators aged > 35 years and < 20 years. Based on the criteria, a sample of 60 people was obtained, obtained from the availability and willingness of the gas station management and woman of childbearing age operators by signing an informed consent. then divided into 4 groups based on the close distance between gas stations, made into 1 group to achieve a balanced division between the control group, formulation group 1, formulation group 2 and formulation group 3. Mar'ke Bilar was given once a day at shift change times (≤ 20 minutes of time provided by the manager) for 14 days, at 14.00 to 14.20 WIB. To avoid bias against the accuracy of the provision of Mar'ke Bilar drinks, the sample was also controlled for nutrient intake that could be a confounding factor in this study, such as protein, vitamin A, vitamin C, and zinc intake. The process of providing Mar'ke Bilar drinks was directly supervised by researchers and enumerators based on the formulation. Here are 4 groups based on the formulation:

1. The control group (15 people) was given red syrup (a syrup drink with a color similar to Mar'ke Bilar made from a mixture of sugar and fruit-flavored essence) 200 ml/day.
2. Formulation group 1 (15 people) was given 200 ml/day of Mar'ke Bilar consisting of: purple sweet potato 40 g, persimmon 40 g, purple passion fruit 30 g, sugar 15 g and water 90 ml.

TABLE 3
Nutrient Intake Before and After Mar'ke Bilar Administration

Indicator	Control	*p*	F1	*p*	F2	*p*	F3	*p*
	Before	After	Before	After	Before	After	Before	After
	Average ±SD	Average ±SD	Average ±SD	Mean ±SD	Average ±SD	Mean±SD	Average ±SD	Mean ±SD
Protein (gr)	57.95 ± 8.82	61.64 ± 12.77	0.194 72.56 ± 20.5	75.56 ± 15.17 0.609	9 55.2 ± 23.25	56.9 ± 23.46 0.218	67.35 ± 21.46	69.67 ± 23.28 0.339
Vitamin A (mg)	68.00 ± 13.08	75.74 ± 8.87	0.237 65.74 ± 13.95	67.13 ± 23.69 0.563	74.35 ± 12.65	72.62 ± 18.45 0.183	71.84 ± 15.08	72.56 ± 24.93 0.217
Vitamin C (mg)	51.89 ± 6.39	57.13 ± 8.46	0.212 66.67 ± 18.13	71.22 ± 20.23 0.500	75.56 ± 15.33	78.37 ± 11.05 0.101	81.88 ± 21.67	83.42 ± 26.84 0.264
Zinc (mg)	7.11 ± 20.22	7.08 ± 18.70	0.117 7.318 ± 15.81	7.54 ± 22.62 0.427	7.67 ± 11.38	7.71 ± 25.10 0.617	7.61 ± 18.17	7.85 ± 09.17 0.352

TABLE 4
Distribution of Mean, SD of Pb levels before and after Mar'ke Bilar administration

Group	Pb	n	Min	Max	Mean ± SD	*p Value*
Control	Before	15	0.001	9.160	1.978 ± 2.687	0.09*
	After	13	1.750	30.500	9.434 ± 8.971	
	Difference	–	24.4050	0.800	7.471 ± 7.795	
Formulation 1	Before	15	2.260	17.380	8.680 ± 4.939	0.002*
	After	12	0.001	6.910	2.443 ± 2.271	
	Difference	–	4.250	13.770	5.713 ± 5.130	
Formulation 2	Before	15	2.530	19.550	9.227 ± 5.139	0.001*
	After	15	0.001	11.170	4.529 ± 4.630	
	Difference	–	2.210	19.549	4.697 ± 4.840	
Formulation 3	Before	15	1.570	23.070	12.438 ± 7.064	0.001*
	After	15	0.001	8.870	3.391 ± 3.418	
	Difference	–	0.100	18.279	9.046 ± 5.682	

Notes: *Paired T Test

3. Formulation group 2 (15 people) was given 200 ml/day of Mar'ke Bilar consisting of: purple sweet potato 50 g, persimmon 50 g, purple passion fruit 30 g, sugar 15 g and water 70 ml.
4. Formulation group 3 (15 people) was given 200 ml/day of Mar'ke Bilar consisting of: purple sweet potato 60 g, persimmon 60 g, purple passion fruit 30 g, sugar 15 g and water 50 ml.

The nutrient content and procedure for making Mar'ke Bilar can be seen in Figure 1 and Table 1.

Blood samples were taken as much as 2cc from the left upper arm using a 2.5 cc syringe by a medical laboratory technology analyst. Then, the blood was put into a tube containing ethylenediaminetetraacetic acid (EDTA) solution and then separated into serum to be examined in the Molecular Laboratory of the Faculty of Medicine and in the FMIPA Laboratory of Brawijaya University Malang. Malondialdehyde and superoxide dismutase examinations were carried out using the enzyme-linked immunosorbent assay (ELISA) kit method with a spectrophotometer (ELx808 Ultra Microplate Reader) and Pb examinations were carried out using the Atomic Absorbance Spectrophotometry method (AAS, Shimadzu AA-6200 & AA6300). Before data analysis, the data normality was first tested using Kolmogrov-smirnov and was found $p>0.05$. Then the

TABLE 5
MDA Level Data Before and After Mar'ke Bilar Administration

Group	MDA	n	Min	Max	Mean ± SD	*p Value*
Control	Before	15	4.24	10.59	9.85 ± 1.74799	0,436*
	After	13	8.21	15.85	9.88 ± 1.980	
	Difference	–	6.35	10.59	1.29 ± 4.654	
Formulation 1	Before	15	4.30	12.30	7.53 ± 2.12947	0.001*
	After	12	4.20	7.75	6.23 ± 0.921	
	Difference	–	1.32	8.74	2.56 ± -3.155	
Formulation 2	Before	15	6.28	15.85	11.48 ± 2.96045	0.001*
	After	15	7.23	10.20	8.38 ± 0.767	
	Difference	–	1.54	6.76	3.22 ± -2.677	
Formulation 3	Before	15	3.51	13.11	9.15 ± 2.31775	0.001*
	After	15	2.80	7.88	6.83 ± 1.256	
	Difference	–	1.31	6.35	2.32 ± -1.817	

Notes: *Paired T Test

TABLE 6
SOD Level Data Before and After Mar'ke Bilar Administration

Group	SOD (ppm)	n	Min	Max	Mean ± SD	*p Value*
Control	Before	15	14.59	39.31	25.31 ± 7.519	0.327*
	After	13	16.48	42.61	25.97 ± 16.48	
	Difference	–	22.83	16.02	0.0349 ± 9.775	
Formulation 1	Before	15	16.02	45.03	29.56 ± 6.926	0.001*
	After	12	19.18	66.28	49.78 ± 19.18	
	Difference	–	9.89	31.90	11.11 ± 2.127	
Formulation 2	Before	15	16.22	34.81	24.47 ± .281	0.001*
	After	15	18.98	60.91	39.62 ± 18.98	
	Difference	–	6.26	40.61	8.358 ± 13.190	
Formulation 3	Before	15	16.73	69.55	28.67 ± 13.530	0.001*
	After	15	25.24	80.28	45.22 ± 25.24	
	Difference	–	1.61	44.00	9.4801 ± 13.283	

Notes: *Paired T Test

data was analyzed using the T-dependent test to see the differences before and after the levels of MDA, SOD and Pb in each treatment of different formulations. Meanwhile, to see the most effective changes, One Way Anova test was carried out, then continued with a Post Hoc-Tukey test. This study has obtained ethical approval from the Ethics Commiitte of the Poltekkes Kemenkes Medan No: 01.1447/KEPK/Poltekkes Kemenkes Medan/ 2023.

RESULTS

The distribution of sample characteristics, nutrient intake before and after Mar'ke Bilar, minimum, maximum,

TABLE 7
The mean difference in Pb, MDA, and SOD levels between groups after Mar'ke Bilar Drink Administration

Indicator	Control	Formulation 1	Formulation 2	Formulation 3
Pb	+7.471 ± 7.795 [a]	5.713 ± 5.130[b]	4.697 ± 4.840[b]	9.046 ± 5.682[b]
MDA	1.29 ± 4.654[a]	2.56 ± 3.155[b]	3.22 ± 2.677[b]	2.32 ± 1.817[b]
SOD	0.03 ± 9.775[a]	11.11 ± 12.127[c]	8.35 ± 13.190[b]	9.48 ± 13.28[b]

ANOVA Test and Post Hoc-Tukey

mean, and SD values as well as the difference in Pb, MDA, and SOD levels before and after Mar'ke Bilar drink administration can be seen in the figures and tables below.

Table 2 showed that 60 samples of women of childbearing age aged 20–30 years were found in as many as 30 people (50%). Most of the respondents had a high school education as many as 57 people (95%), samples with elementary education were 1 person (1.7%) and junior high school was 2 people (3.3%). Based on the length of work, the largest sample was > 24 months, namely 28 people (46.7%), <13 months of work for as many as 18 people (30%). Based on the length of employment, the largest sample was in the gas station operator group >24 months, namely 28 people (46.7%). The length of employment will affect the condition of Women of childbearing age gas station operators who get tired easily and sometimes have difficulty concentrating. This is in accordance with Stamara's 2020 research. Pb as a heavy metal produces toxic effects and free radicals enter the body only for a few months if exposed to Pb with a working duration of 8–9 hours.[15]

Table 3 showed that the intake of nutrients in Women of childbearing age working as gas station operators has no difference before and after the Mar'ke Bilar healthy drink treatment, which is > 0.05. Nutrient intake before and after treatment was tested using a *paired t-test* to control the effectiveness of the Mar'ke Bilar healthy drink treatment. However, when viewed from the average number in each treatment group there is an increase in the intake of protein, vitamin A, vitamin C and zinc in each group tends to increase but some have decreased. But when viewed from the adequacy of Women of childbearing age aged 20–35 years in the control group and formulation group 2 looks still below. As for the adequacy of vitamin A of 600 mg/day, the daily intake of vitamin C is still below normal, for vitamin C intake the adequacy is 75 mg/day, and only formulation groups 2 and 3 have met the recommended adequacy rate. As for the adequacy of zinc at the age of women of childbearing age of 8 mg/day, while what is obtained based on the recall results is still below the AKG 2019.

Table 4 Shows a decrease in Pb levels after being given Mar'ke Bilar in formula groups 1, 2, and 3. The results of the paired t-test showed that the administration of Mar'ke Bilar showed a significant difference in formulation groups 1, 2, and 3, p<0.05. While in the control group without Mar'ke Bilar administration, there was an increase in the average Pb level from 1.978 µg/dl to 9.434 µg/dl with a difference of .7.471 µg/dl. When tested based on the paired t-test before and after p>0.05.

Table 5 shows a decrease in MDA levels after being given Mar'ke Bilar in formula groups 1, 2, and 3. The results of the paired t-test showed that the administration of Mar'ke Bilar showed a significant difference in formula groups 1, 2, and 3, p<0.05. While in the control group without Mar'ke Bilar administration, there was an increase in the average MDA level from 9.85 nmol/mL to 9.88 nmol/mL with a difference of 1.29 nmol/mL. When tested based on the paired t-test before and after p> 0.05.

Table 6 shows a decrease in SOD levels after being given Mar'ke Bilar in formula groups 1, 2, and 3. The results of the paired t-test showed that the administration of Mar'ke Bilar showed a significant difference in formula groups 1, 2, and 3, p<0.05. While in the control group without the administration of Mar'ke Bilar, there was an increase in the average MDA level from 25.31 ppm to 25.97 ppm with a difference of 0.0349 ppm. When tested based on the paired t-test before and after p> 0.05.

Table 7 shows that after a one-way ANOVA statistical test was conducted to see the differences between treatment groups, then a post hoc-tukey test was continued to see which group had the most influence for each Pb, MDA, and SOD levels. In the Pb examination indicator, the notation shows that for the control group there was a difference towards an increase indicated by the notation a (Table 4). While after the administration of Mar'ke Bilar in formula groups 1, 2 and 3 showed a decrease indicated by the notation changing to b.

In the MDA examination indicator, the notation shows that for the control group there was a difference towards an increase indicated by the notation a (Table 5). While after the administration of Mar'ke Bilar in formula groups 1,2 and 3 showed a decrease indicated by the notation changing to b.

In the SOD examination indicator, the notation shows that for the control group there was a difference towards an increase indicated by the notation a (Table 6).

Meanwhile, after the provision of Mar'ke Bilar, it turns out that formula group 1 is significantly different from formula groups 2 and 3 which are shown with the notation c, while formulations 2 and 3 are in the notation b.

DISCUSSION

Sample Characteristics

The sample in this study amounted to 60 women of childbearing age. Based on the division of age groups, the percentage is divided into 2, namely 50% aged 20–30 years and the other 50% for the group >30–35 years. The selection of characteristics based on the age of Women of Childbearing Age is based on the fact that at the age of 20–29 years the process of maturation of a woman's reproductive cells is at its peak of maturity. Women in the age range of 20–29 years have a 95% chance of experiencing pregnancy, and will decrease to 90% if Women of Childbearing Age has reached the age of 30 years.[16]

If poisoning occurs due to Pb metal from exposure to motor vehicle fumes in women of childbearing age, it can cause reproductive system disorders in Women of Childbearing Age gas station operators by preventing infertility, decreased sex libido, and miscarriage, where according to Azami, *et al* (2022) states that the activity of biotransformation enzymes decreases and the resistance of certain organs to the effects of Pb decreases. This causes the older a person is, the higher the accumulation of Pb in body tissues will be.[17]

Based on the length of employment, 28 people (46.7%) were found to have worked for more than 24 months. Gas station employees who have worked for ≥4 years as operators have an average blood lead (Pb) level of 24.97 µg/dL. This is in line with the results of research by Stamara Ghina (2020) and Yeni (2021) which reported that women who work as gas station operators have blood Pb levels of 20.00 µg/dL.[15] According to the Centers for Disease Control and Prevention (CDC) in the United States and the Indonesian Ministry of Health, the normal threshold value is <10 µg/dL(1820), while the World Health Organization (WHO) sets an acceptable limit of ≤5 µg/dL.[19,21] Lead enters the body through the respiratory tract and then into the bloodstream, causing enzyme inactivation and acting as a highly reactive radical.[2]

Effect of Mar'ke Bilar on Pb levels

The results showed that there were differences before and after the administration of Mar'ke Bilar with various formulations (1, 2, and 3) on the blood Pb levels of Women of childbearing age working as gas station operators which tended to decrease. While in the control group, there was an increase in Pb levels without being given Mar'ke Bilar. The results of the ANOVA test were continued with the Post Hoc-Tukey Test, it turned out that the control group had different notations (a) with formulation groups 1, 2, and 3 (b). After being given the treatment, there was a decrease that could be seen from the difference in numbers that showed differences in notation. Mar'ke Bilar (Passion Fruit, Persimmon and Purple Sweet Potato) drink is one of the processed healthy drinks that contain antioxidants that can help prevent oxidative damage and prevent damage caused by Pb invasion into the blood including increasing free radicals, lysing erythrocytes and inactivating several enzymes in the body. Mar'ke Bilar contains vitamins that act as antioxidants such as Beta-carotene(pro-vitamin A)[22] and Vitamin C and the mineral Zinc. In addition, Mar'ke Bilar also contains bioactive substances anthocyanins and phenols that function to bind free radicals due to the accumulation of Pb in the blood.[23]

Vitamin A that functions as an antioxidant is beta-carotene (pro-vitamin A) obtained from colored foods that tend to be yellow and orange, such as food ingredients for making Mar'Ke bilar drinks. Beta-carotene antioxidants act as scavengers of free radical reactions and have a key role in preventing oxidative stress that occurs due to Lead in the body as antioxidants that act as scavengers that can collect free radical molecules in the body. Vitamin A is a natural antioxidant compound that fights free radical reactions and has a key role in preventing oxidative stress that occurs due to Lead in the body.[24]

Vitamin C can stimulate gelatin compounds that can reduce the availability of Pb metal in the blood. Vitamin C also has the function of translocating Pb metal to the disposal estuary either through the respiratory or urinary system. Vitamin C performs immobilization of heavy metals to reduce the absorption of Pb in the small intestine and vitamin C can inhibit the uptake of Pb at the cellular level so that the cytotoxicity process of Pb becomes decreased or even completely undetectable.[24–26]

Other antioxidants in the form of zinc minerals and bioactive substances such as anthocyanins and phenols in the Mar'ke Bilar content are able to suppress the movement of lead so that it does not interact with sulfhydryl groups or Pb metal cofactors in enzymes and molecules so that the metal prosthetic group cannot be replaced by Pb.[10,27]

This is in accordance with Sundari's 2017 study, where in this study, vitamin C given orally as much as 200 mg was found to be able to reduce Pb levels in female street sweepers in Denpasar, Bali.[25] Giving vitamin C and iron was able to neutralize Pb in the body so as to prevent the hemolytic process during the formation of Hb in adolescent girls.[28] While beta-carotene can reduce homocysteine in workers exposed to Pb.[19] Other studies of giving vitamin C to experimental animals, mice and

humans, the effectiveness of vitamin C in treating poisoning due to Pb metal, especially on neurotoxic effects.[29]

Effect of Mar'ke Bilar Administration on MDA Levels

The results showed that there were differences before and after the administration of Mar'ke Bilar with various formulations (1, 2, and 3) on the blood MDA levels of Women of childbearing age working as gas station operators which tended to decrease. While in the control group, there was an increase in MDA levels without being given Mar'ke Bilar.

Mar'ke Bilar has the special feature of being made from three food ingredients that are rich in antioxidants and bioactive substances that complement each other, this can be seen from the total antioxidant content in the three formulations (Formulation 1 is 40.75 mg/ml, formulation 2 is 39.73, formulation 3 is 38.49) which shows very strong activity.[11] The results of the ANOVA test were continued with the Tukey Post Hoc Test, it turned out that the control group had different notations (a) with formulation groups 1, 2, and 3 (b). After being given the treatment there was a decrease that could be seen from the difference in numbers that showed differences in notation.

Mar'ke Bilar drink can reduce MDA levels which are free radical biomarkers due to Pb metal induction. Excessive Pb increases that invade the blood, resulting in increased reactions between Pb and unsaturated fatty acids commonly called lipid peroxidation. The occurrence of lipid peroxidation causes increased MDA levels in the body. The content of vitamin C, beta-carotene, and zinc minerals as well as their bioactive content can reduce MDA by protecting PUFA phospholipid membranes by donating Hydrogen ions (H+) to lipid peroxyl radicals (LOO*).[30] Free radicals damage cell membranes, where the main component is unsaturated fatty acids (PUFA) which contain double bonds so that they are more susceptible to free radical attack and will cause oxidative destruction of lipids from fatty acids called lipid peroxides. (Fauziah 2018, and Dewangga 2022).[31,32]

Mar'ke Bilar drink is also able to maintain the stability of the cell membrane where lipid peroxidation occurs so that the intensity of MDA formation can be inhibited. Another way antioxidants can break the chain of peroxy radical bonds with other molecules. This event will inhibit the formation of other peroxy radical chains so that MDA levels are not formed.[33]

Another function of Mar'ke Bilar which contains several antioxidants such as vitamin C helps form active free radicals into ascorbyl radicals which have a low reactive level. Vitamin C and beta-carotene are able to act as free radical scavengers by changing free radicals into reactive ones by providing hydrogen ions, including neutralizing singlet oxygen part of ROS.

The antioxidant content in Mar'ke Bilar such as Vitamin C, Betacarotene, and zinc minerals can stimulate the antioxidant enzymatic system to reduce the formation and effects of ROS so that MDA levels in the blood can decrease.[9] Other abilities of exogenous antioxidants include being able to repair body cells, neutralize reactive free radicals, especially oxygen types, then biochemically able to remain in the body.[9] Lee's 2018 research examining zinc in foodstuffs turned out to have a function that is an antioxidant and regulates oxidation-reduction reactions to be conditioned to decrease.[10] This research is also supported by other studies that provide antioxidant content in the form of vitamin C, and zinc which can prevent lipid peroxides in the liver and tissues so that the formation of oxidative stress can be prevented.[24,27]

Effect of Mar'ke Bilar Administration on SOD Levels

The results showed that there were differences before and after the administration of Mar'ke Bilar with various formulations (1, 2, and 3) on the blood SOD levels of Women of childbearing age working as gas station operators which tended to increase. In the control group, there was an increase but very small where the average increase was 0.0349. The results of the ANOVA test continued with the Post Hoc-Tukey Test, it turned out that the control group had different notations (a) with the formulation groups 2 and 3 (b) and the most effective in increasing SOD was formulation 1. After treatment there was an increase that could be seen from the difference in numbers that showed differences in notations. Formulation 1 with a relatively lower content of antioxidants and bioactive substances compared to formulations 2 and 3 turned out to be the most effective formulation. This is because the high antioxidant content in a food can also interfere with the production of enzymatic oxidants and even excessive doses can cause cell death.[10]

Other studies have stated that giving high doses of antioxidants can also be toxic and can also affect the rate of oxidation as the task of antioxidants.[20]

Mar'ke Bilar drink is a drink that is high in exogenous antioxidants and bioactive substances that can stimulate enzyme activity including SOD.[34] Another mechanism in increasing SOD by Mar'ke Bilar drink is that antioxidants and bioactive substances directly donate hydrogen ions and can neutralize the toxic effects of Pb and suppress the formation of free radicals so that they can directly increase endogenous antioxidant gene expression through several mechanisms.[35] The mechanism of increasing endogenous antioxidant gene expression is through the activation pathway of *Nuclear Factor Erythroid 2 Related Factor 2* (NRF2). This gene is

involved in the synthesis of endogenous antioxidant enzymes including SOD.[36,37]

SOD enzyme is an endogenous antioxidant that works by regulating ROS, thus the role of molecules that have activity as exogenous antioxidants such as colored fruits and vegetables and other foodstuffs that are high in pigment content of bioactive substances are needed to be consumed in counteracting oxidative stress.[38]

Dewangga's research in 2022, using purple sweet potato as an antioxidant, was able to increase blood SOD as an endogenous antioxidant.[32] Research conducted by Mochida in 2022 by giving persimmon was able to increase SOD due to the potential antioxidant content of persimmon.[32,39]

Exogenous antioxidants are compounds or systems that can safely interact with free radicals and stop the chain reaction before important molecules are damaged. Antioxidants (eg, flavonoids phenolic acids, tannins, vitamin C, and vitamin E) have diverse biological properties, such as anti-inflammatory, anticarcinogenic and anti-atherosclerosis effects. Several types of vitamins are important antioxidants for the body. Antioxidants that come from food include vitamin C, vitamin E, vitamin A, selenium, and zinc.[40]

Vitamin C is one of the water-soluble vitamins that has reducing properties so that this vitamin has important properties as an antioxidant that affects the body's redox potential. The role of vitamin C is good in helping to neutralize antioxidants, especially lead (Pb), because Pb also has oxidative stress properties, vitamin C is also able to be a free radical scavenger and can react directly. The results of a study conducted by Fauzi Susilo (2008) also found the same thing that giving vitamin C to albino mice at a dose of 0.2 mg/g/day for 36 days can protect nerve cells from free radical compounds caused by exposure to the heavy metal Pb. Another study by Silitonga (2018) stated that vitamin C can ward off free radicals, bind and clean heavy metals due to exposure from the environment.

Antioxidants increase the function of immune cells, maintain the integrity and function of lipids in cell membranes so that they can protect cellular proteins and nucleic acids that function to regulate signal transduction and DNA expression by means of DNA repair so that cancer prevention can occur. [26,31]

The process of preventing cancer in Women of Childbearing Age can also be done through preventing free radical invasion through inhibiting NF-κβ activation, preventing apoptosis which ultimately prevents the target of cancer p<38 in activating cancer cells.[41]

Antioxidants can also prevent disorders in the reproductive system of Women of Childbearing Age gas station operators by preventing infertility, decreased sex libido, miscarriage, and also increasing the regulation of luteinizing hormone which will affect the gonadotropin hormone produced by the pituitary gland in the brain which is needed by Woman of Childbearing age in the ovulation process.[19,38]

The urgency of antioxidants in Woman of Childbearing age gas station operators is the binding of Pb due to exposure to motor vehicle fumes. This process can increase the amount of lead in blood plasma so that Pb toxicity occurs, where antioxidants prevent an increase in Zinc Protoporphyrin (ZPP) levels so that the heme formation process can take place properly. Antioxidants also work to inhibit the activity of deta aminolevulinic acid dehydratase (ALAD), extend the life of red blood cells, maintain membrane stability, so that anemia in Women of Childbearing Age can be prevented.[19,21,26]

The impact on Women of Childbearing Age gas station operators, if consuming antioxidants in sufficient quantities will increase fitness and concentration while working can run optimally.[19]

The limitation of this study, it should be tested first on experimental animals as a pre-clinical test, so that the accuracy of the sample homogeneity process can be maintained. The number of samples obtained is still not maximal, this is because many gas station owners do not allow the blood drawing process to be carried out during working hours so that the blood drawing seems to be taken in a hurry. A calm atmosphere when taking blood can affect the results of the variable examination.

CONCLUSION

Giving Mar'ke Bilar formula 1, 2, and 3 reduces Pb and MDA levels and increases SOD levels, where formula 1 is the most effective treatment in increasing SOD levels. It is necessary to consume colored foods such as Mar'ke Bilar which are very good for avoiding diseases caused by free radicals caused by air and water pollution, especially those caused by heavy metals.

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Original Article

Spatial and Temporal Analysis of Gallbladder Stone Cases at dr. Soekardjo Hospital, West Java: An Evidence-Based Study

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Abstract

Background : Gallbladder stone cases in East Priangan area have significantly increased due to diet, lifestyle, and stress levels of the community. However, studies on the seasonal variation of gallbladder stone cases remains limited, particularly in Indonesia.This study aims to investigate the occurrence of gallbladder stone cases in East Priangan, West Java, Indonesia, based on the medical reports in 2022–2023. Furthermore, the dispersal of gallbladder stone cases is mapped as spatial and temporal distributions.

Methods : This study employed an observational, cross-sectional design. Data was collected from the medical records at dr. Soekardjo General Hospital of Tasikmalaya between January 2022 and December 2023. Data used in this study were available data of 2,032 visit records, comprising 1,311 visits in 2022 and 991 visits in 2023, for both inpatient and outpatient care. Two types of statistical tests were used in this study: the Chi-Square test and Moran's I value.

Results : The Global Moran's I test revealed clusters in the spatial distribution of gallbladder stone for both 2022 (I = 0.434) and 2023 (I = 0.521). Additionally, monthly variations ($p = 0.001$), age ($p = 0.023$), and gender ($p = 0.001$) were found to be statistically significant factors influencing the risk of gallbladder stones.

Conclusion : The spatial distribution of gallbladder stone was concentrated in the certain clusters. Moreover, there were significant correlations between monthly variations, age, and gender with gallbladder stones.

Keywords : cholelithiasis, digestive health, evidence-based study, gallbladder stone, spatial analysis, temporal analysis.

INTRODUCTION

Gallbladder stones are among the most common gastroenterological disorders worldwide.[1] This disease could threaten a person's life due to an obstruction of the bile flow through the biliary system. Many experts on the digestive health system are still debating the causes of gallbladder stones, whether the bile contains excessive cholesterol and bilirubin, or is incorrectly emptying the gallbladder. Gallstones develop when certain substances in the bile, such as cholesterol and calcium bilirubinate, are present in concentrations that approach the limits of their solubility.[2] This supersaturation can occur due to several factors, including cholesterol supersaturation and bile concentration. Surgery is usually required to remove the gallbladder stones if the symptoms show in the patient. Gallbladder stones affect 6% of people worldwide, with an incidence greater in South America and among women.[3] In the US, the prevalence of gallbladder stone disease increases by 10–20% in adults, and more than 300,000 annually cost direct and indirect surgery, estimated to be 6.5 billion USD.[4,5] Medical records from 1995 show that as many as 5% to 15% of people in Europe suffered from gallbladder stone diseases.[6] Gallbladder stone occurrence varies from 3% to 10% in Asian countries; for instance, Taiwan, Mainland China, Japan, and Northern India are approximately 5.0%, 10.7%, 3.2%, and 7.1%, respectively.[7,8] The information on gallbladder stone cases in Indonesia is still limited and unclear which is considered comparable to that of other Asian countries.[9,10]

The risk factors linked with gallbladder stones vary between nations due to changes in genetic, nutritional, environmental, behavioral, and socio-economic conditions.[11] According to medical experts, the 4F (female, fat, forty, and fertile) is a predictive factor for gallbladder stone patients.[4] The prevalence of gallbladder stone diseases is roughly twice as high in females as it is in males, due to physiological differences, low nutritional status, rising biological stress, poor sanitation and hygiene, lack of access to healthcare, and inactive lifestyles.[12] Excess body weight (fat) can also induce a prolithogenic condition and increase the likelihood of either gallstone development or existing gallbladder stone becoming symptomatic.[13] Comparing slightly overweight women (24–25 kg/m^2) to people of normal weight, the risk factor for gallbladder stone disease was 1.7; however, in overweight women (32 kg/m^2), it rose rapidly by 6.0.[14]

Gallbladder stones are ten times more common in adults over 40 (Forty) years old due to a reduction in the activity of cholesterol 7 α-hydroxylase, the limiting enzyme for bile acid production.[15,16] As enzymatic activity decreases and biliary cholesterol accumulates, the aging person experiences cholesterol saturation, and decreases gallbladder emptying mobility.[17] Fertile women, who have one or more child(ren), are assumed to be at higher risk due to excessive estrogen levels and the link between gallbladder stones and pregnancy.[18] Biliary sludge (a precursor to gallbladder stones) and gallbladder stones occur at rates of up to 30% and 12%, respectively, throughout pregnancy and postpartum, and 1–3% of pregnant women have a cholecystectomy due to clinical symptoms or difficulties during the first year after birth.[19]

East Priangan is an area in West Java Province that includes four districts and two cities, namely the regencies of Garut, Tasikmalaya, Sumedang, and Ciamis; and the cities of Tasikmalaya and Banjar. The economic growth in this strategic region is advancing rapidly, with a positive trend of 4.80−5.20% in 2023.[20] Food consumption rates among consumers in East Priangan significantly increases, particularly using social media. For instance, based on data from Badan Pusat Statistik Tasikmalaya in 2023, household consumption in Tasikmalaya City was 69.84%, and the average food consumption per capita was 51.93 percent.[21] The rapid economic growth and culinary businesses offering fast food have allegedly escalated the digestive system disorder in East Priangan. Gallbladder stone cases are believed to be more common among people who prefer to eat low-fiber fast food.

This study aims to investigate the occurrence of gallbladder stone cases in East Priangan, West Java, Indonesia, in 2022−2023. Furthermore, the dispersal of gallbladder stone cases is mapped as spatial and temporal distributions.

METHODS

This analytic observational with a cross-sectional study was used in this research by using daily hospital admission data on gallbladder stone cases from the medical record unit of dr. Soekardjo General Hospital, Tasikmalaya. This referral general hospital provides follow-up medical treatment from the clinic and hospital in East Priangan. The data provided by the hospital consists of the number of admissions and diagnoses, with additional personal information such as age, gender, and original region (see Table 1). This study utilizes the available data of 2,032 visit records, comprising 1,311 visits in 2022 and 991 visits in 2023, for both inpatient and outpatient care at dr. Soekardjo General Hospital, Tasikmalaya. Ethical approval for this study was obtained from Medical College, Faculty of Medical and Health, Muhammadiyah University of Jakarta, and written informed consent was obtained from dr. Soekardjo General Hospital, Tasikmalaya, for patient information to be published in this article. All data included met the criteria of residency in East Priangan (covering Garut Regency, Tasikmalaya Regency, Ciamis Regency, Pangandaran Regency, Tasikmalaya City, and

TABLE 1
Total recorded data of gallbladder stone cases during 2022–2023 in East Priangan, West Java

Characteristics	Number of Admissions	
	2022	2023
Age Group, n (%)		
<5	13 (1)	8 (1)
5 – 14	12 (1)	5 (1)
15 – 24	57 (4)	72 (7)
25 – 34	184 (14)	179 (18)
35 – 44	305 (23)	173 (17)
45 – 54	310 (24)	248 (25)
55 – 64	275 (21)	194 (20)
65 – 74	120 (9)	84 (8)
75 – 84	35 (3)	25 (3)
85 – 94	0 (0)	3 (0)
Gender, n (%)		
Male	456 (35)	354 (36)
Female	855 (65)	637 (64)
Regional Origin, n (%)		
Garut Regency	27 (2)	13 (1)
Tasikmalaya Regency	506 (39)	423 (43)
Ciamis Regency	146 (11)	131 (13)
Pangandaran Regency	0 (0)	4 (0)
Tasikmalaya City	624 (48)	407 (41)
Banjar City	8 (1)	13 (1)

Banjar City). Subsequently, the data was processed and displayed in spatial and temporal distribution, visualizing the gallbladder stone cases in East Priangan.

This study employed two types of statistical tests: the Moran's I and Chi-square test. Spatial autocorrelation tests was employed to analyse the distribution of gallbladder stone diseases. The Moran's I method was used to quantify the spatial autocorrelation in this study by using GeoDa, an open-source software designed for spatial data analysis.[22] This method is a proven and one of the most popular methods to analyse spatial autocorrelation problems in the disease epidemiology studies.[23] The Global Moran's I statistic helps identify overall patterns in the data distribution and is visualized through a Moran scatter plot.[24] The Moran scatter plot classifies spatial autocorrelation into four quadrants. The upper-right and lower-left quadrants represent positive spatial autocorrelation, where similar values are clustered in neighbouring locations. These are referred to as high-high (hot-spot) and low-low spatial autocorrelation (cold-spot). Conversely, the lower-right and upper-left quadrants indicate negative spatial autocorrelation, where dissimilar values are adjacent. These are termed high-low and low-high spatial autocorrelation.[24]

Local clusters and outliers are identified using the Local Indicator of Spatial Association (LISA) method, which has two key features.[25] First, it offers a statistical value for each location along with an assessment of its significance. Second, it ensures that the sum of the local statistics is proportional to a corresponding global statistic. The LISA can be visualized in the significance and cluster map. The significance map highlights areas with a notable local statistic, using progressively darker shades of green to represent higher levels of significance. It begins with $p<0.05$ and displays all relevant

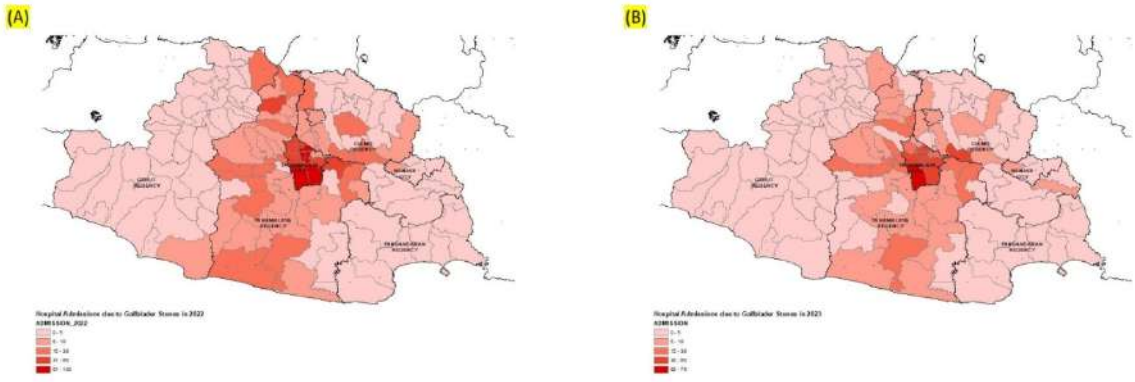

Figure 1. Spatial dispersal of gallblader stone cases in East Priangan, West Java in (a) 2022 and (b) 2023

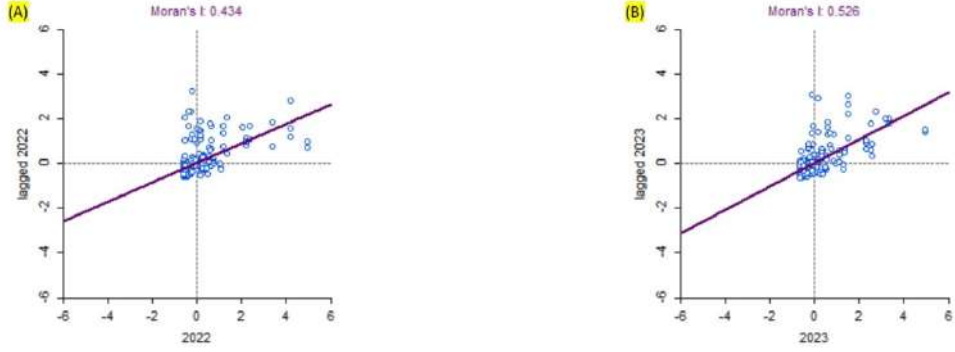

Figure 2. Univariate Global Moran's I value for gallbladder stone dispersal in (a) 2022 and (b) 2023

significance categories, determined by the number of permutations used. The cluster map further enhances the visualization by indicating the type of spatial association, which is derived from the value's position and its spatial lag on the Moran scatter plot.

The Chi-Square test was used to evaluate the following hypotheses: (a) whether age and gender influence the occurrence of gallbladder stone disease, and (b) whether seasonal variations affect the temporal distribution of gallbladder stone disease. Hypothesis (a) was tested using 96 randomly selected samples from each group (gallbladder stones and non-gallbladder stones admissions). The sample size was calculated using the cross-sectional study formula, assuming a type 1 error rate with a 95% confidence level, proportion in a population of 0.5 (for unknown proportions), and a tolerable error margin of 10%. If the *p-value* is less than 0.05, the hypothesis is accepted; otherwise, if the *p-value* exceeds 0.05, the hypothesis is rejected.[26]

Hypothesis (b) was tested using the full sample of 2,302 participants. The samples were categorized based on monthly distribution and divided into seasonal groupings for both tropical and non-tropical regions. The testing method followed the approach used in Khan *et al.*'s study, where Chi-square test was applied to analyze the seasonal variability of gallbladder stones.[27] The seasons were defined as spring (March−May), summer (June−August), fall (September−November), and winter (December−February). Since this study was conducted in a tropical region, seasonal effects were also examined based on the typical tropical seasonal patterns: dry season (March−August) and wet season (September−February). A Chi-square goodness-of-fit test was employed to evaluate the seasonality of acute gallbladder stone cases while accounting for variations in the number of days per season. The day counts used for each season were 92 for spring, 92 for summer, 91 for fall, and 90.25 for winter.

RESULTS AND DISCUSSIONS

Spatial Dispersal of Gallbladder Stone Cases

Spatial analysis was used to obtain the dispersal of gallbladder stone cases in East Priangan. Figure 1 indicates that Tasikmalaya City was recorded as the highest number of hospital admissions, with 624 (48%) in

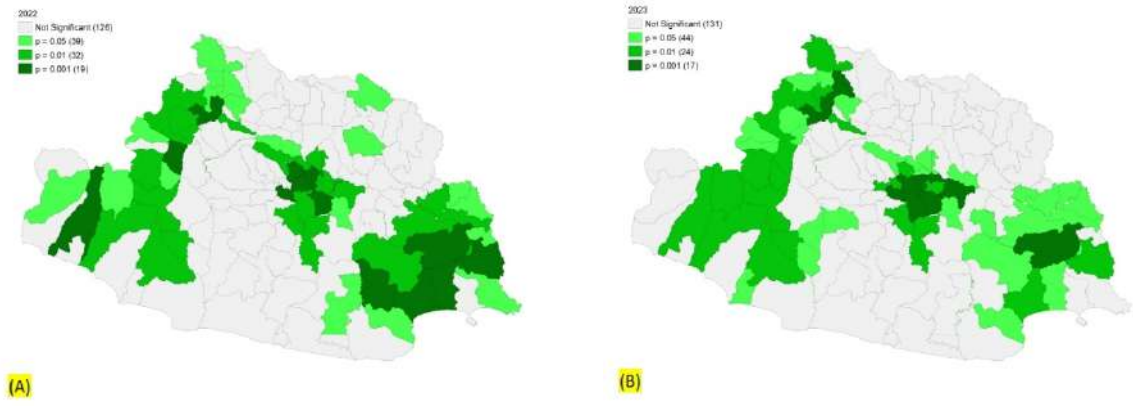

Figure 3. Significance map of gallbladder stones in (a) 2022 and (b) 2023

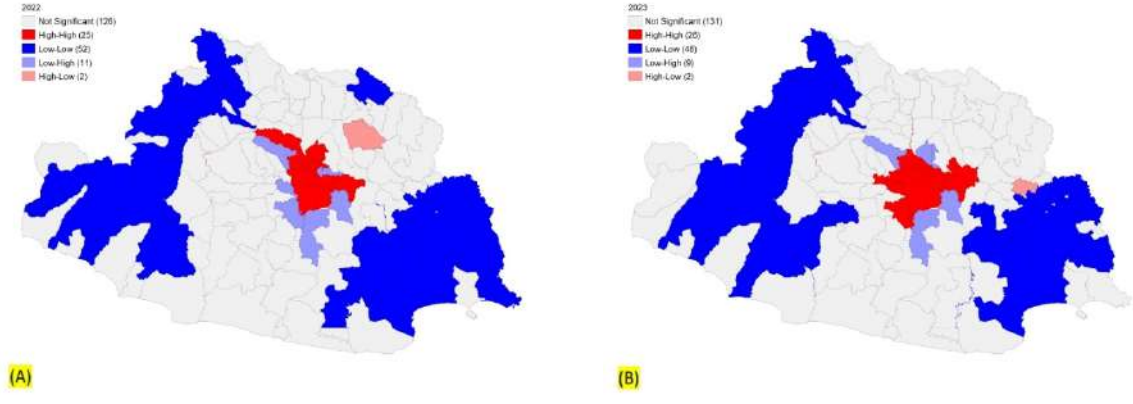

Figure 4. Cluster map of gallbladder stone dispersal in (a) 2022 and (b) 2023

2022 and 407 (41%) in 2023, followed by Tasikmalaya Regency with 506 (39%) visits in 2022 and 423 (43%) in 2023. Ciamis Regency also had a significant number of admissions with 146 (11%) cases in 2022 and 131 (13%) cases in 2023 (see also Table 1). Conversely, the gallbladder stone cases in Garut Regency, Pangandaran Regency, and Banjar City demonstrates fewer hospital admissions, likely due to the greater distance to dr. Soekardjo General Hospital of Tasikmalaya, making it less accessible compared to the nearby Tasikmalaya City and surrounding areas. Additionally, this may be due to the presence of referral general hospitals in both Garut Regency and Banjar City, specifically dr. Slamet General Hospital of Garut and Banjar City General Hospital.

Spatial autocorrelation testing shows positive Global Moran's I value for gallbladder stone case distributions in 2022 (0.434) and 2023 (0.526), indicating the presence of clusters across East Priangan (see Figure 2). The significance map highlights areas with high statistical significance (p<0.05), revealing a strong spatial connection between data points across sub-districts and helping to identify cluster locations (Figure 3). The cluster map (Figure 4) shows that the highest concentration of gallbladder stone cases, known as a hot-spot, is in Tasikmalaya City and its surroundings. In contrast, a cold-spot, or low case cluster, was observed in Garut Regency, Pangandaran Regency, and parts of Ciamis Regency and Banjar City. Areas like Tasikmalaya Regency and parts of Ciamis Regency, with lower significance, show weaker spatial connections between gallbladder stone case distributions across sub-districts.

In large regions, fast food often becomes popular due to busy lifestyles and irregular eating habits. People may not consume enough high-fiber foods, which can lead to health issues like gallbladder stones. DietR high in saturated fats––found in fried foods, red meats, and full-fat dairy products––raises the risk of gallbladder stones. Refined sugars and carbohydrates, such as those in pastries and sugary drinks, also contribute to this risk, with high fructose being particularly harmful. Low fiber intake intensifies the problem, as fiber helps manage bile acid and cholesterol, reducing gallbladder stone risk.

TABLE 2
Chi-square Test Result of Seasonal and Monthly Variability of Gallbladder Stones

Variable		Number of Days	Gallbladder Stones	*p*
Season (tropical)	Dry	184	1161	0.984
	Wet	181.25	1141	
Month	Jan	31	180	0.001
	Feb	28	217	
	Mar	31	248	
	Apr	30	99	
	May	31	109	
	Jun	30	144	
	Jul	31	242	
	Aug	31	319	
	Sep	30	194	
	Oct	31	173	
	Nov	30	192	
	Dec	31	185	

Therefore, regular meals are advised for maintaining gallbladder health.[28] Studies show that less physical activity is linked to a higher risk of symptomatic gallbladder stones and gallbladder surgery. For instance, men can reduce their risk of symptomatic gallbladder stone by 34% through 30 minutes of endurance exercise five times a week. Additionally, 2–3 hours of recreational exercise weekly might lower the need for gallbladder surgery by about 20%.[29]

Temporal Dispersal of Gallbladder Stones

The temporal analysis shows that hospital admission trends for gallbladder stones were similar in 2022 and 2023 (see Figure 5). After adjusting for the different number of days in each month, the monthly variation in gallbladder stone cases was statistically significant across the year ($P < 0.001$). However, seasonal variations were not statistically significant compared to monthly variations, with *p-values* of 0.984 (see Table 2).

At dr. Soekardjo General Hospital of Tasikmalaya, the number of hospital admissions for gallbladder stone case decreased during dry season in March to April and reached maximum in August (see Figure 5). This is slightly contrast with a review of 12 studies conducted between 1990 and 2017 across seven countries (Saudi Arabia, England, Pakistan, the United States, Taiwan, and Germany) which shows that gallbladder stone cases typically increase during the summer and decrease during winter.[30] Similarly, Khan *et al*.[27] found a rise in gallbladder stone cases during the summer months in Pakistan from 1988 to 2018, and this trend was also observed in England during the British summer from 2010 to 2019.[31]

Khan *et al*. have proposed that seasonal variations in gallstone-related infections may be linked to increased bacterial infections during warmer temperatures.[27] This could lead to more cases of acute gallbladder stones in the summer. However, seasonal variations in gallstone disease are a complex phenomenon with multiple contributing factors. While numerous studies have been conducted, the exact causes of these patterns remain unclear. Factors such as human behaviour, microbial virulence, foodborne illness rates, and immune responses are likely contributing to seasonal patterns in gallstone disease.[32–35] The combination of these factors likely explains the higher incidence of infectious diseases in the summer. A more comprehensive understanding of the underlying causes of seasonal infectious disease patterns could contribute to the development of more effective prevention and intervention strategies.

However, studies on the seasonal variation of gallbladder stone cases remains limited, particularly in Southeast Asia, and especially in Indonesia. Since most studies on the seasonal variability of gallbladder stones have been conducted in non-tropical regions, comparative studies on the effects of seasonal variability on gallbladder stone cases in Indonesia are scarce. There

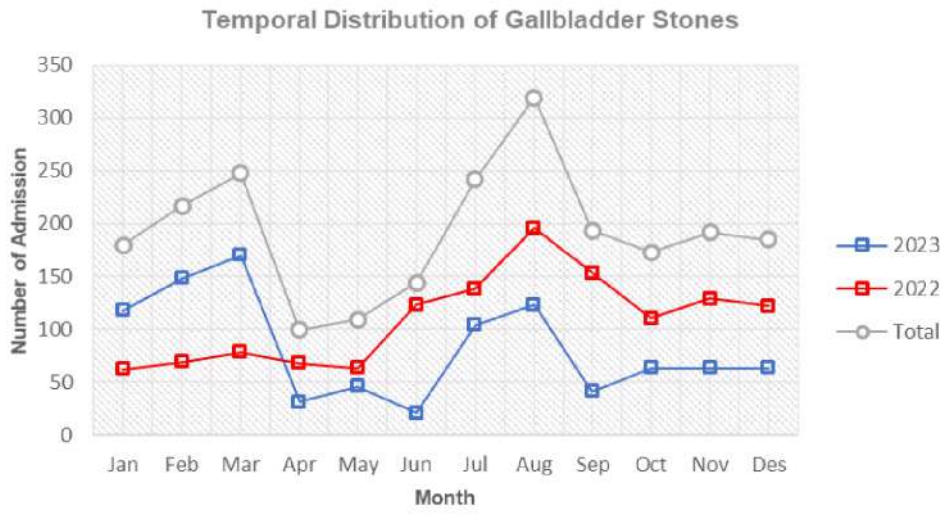

Figure 5. Temporal dispersal of gallblader stone cases in East Priangan, West Java in 2022 and 2023 (p<0.001)

TABLE 3
Chi-square test result of age and gender with gallbladder stone cases

Variable		Gallbladder Stones		Non-Gallbladder Stones		*p*
		n	%	n	%	
Age	5 – 14	1	1	1	1	0.023
	15 – 24	7	7	13	14	
	25 – 34	13	14	15	16	
	35 – 44	20	21	29	30	
	45 – 54	17	18	24	25	
	55 – 64	27	28	11	11	
	65 – 74	6	6	2	2	
	75 – 84	5	5	1	1	
65 – 74	Male	26	27	57	59	0.001
	Female	70	73	39	41	

is no study that directly explains about seasonal variability of gallbladder stone in Indonesia.

Age Disparity of Gallbladder Stone Cases

The Chi-square test demonstrated a significant association between age and the occurrence of gallbladder stones at Dr. Soekardjo General Hospital in Tasikmalaya (p = 0.023) (see Table 3), with an average age of 41 ±14 years old. The age group of 55–64 had the highest number of hospital admissions with 28% visits among all categories. This result aligns well with previous studies. Atsariyah *et al*. identified the significant relationship between age and gallbladder stones, with an average age of 50 ± 13.84 years. In 2017, the incidence of gallbladder stones in China increased from 7.4% among individuals under 30 years of age to 43.1% in those aged 50 to 70.[36] A similar trend was observed in Sevinc *et al*.'s study, where the prevalence of gallbladder stones rose with age, reaching 32.1% among 106 cases out of a total of 330.[37] These findings also support Ansari-Moghaddam's study, which indicates that patients over 45 years old are at a higher risk of developing gallbladder stones compared to those aged 30 to 44.[38]

Gender Disparity of Gallbladder Stones

In this study, the Chi-square test identifies a significant link between gender and the presence of gallbladder stones at dr. Soekardjo General Hospital of Tasikmalaya, with a *p-value* of 0.001 (see Table 3). The results show significant gender differences in gallstone risk: women account for 73% of all admissions, while men make up just 27%. These findings are consistent with previous research. For example, Atsariyah *et al*. reported a similar gender split, with 64% of gallstone patients being women and 36% being men, though the study found no significant effect of gender on gallstone formation (P<0.224).[39] In contrast, Song *et al*.'s research in China in 2017 revealed that gender significantly influenced gallstone prevalence (p<0.001), with 50.6% of cases (199,820) in women and 49.4% (194,827) in men.[36] Moreover, Ansari-Moghaddam *et al*. found that women were 2.73 times more likely to develop gallbladder stone than men (95% CI; 1.34–5.56).[38]

CONCLUSION

This study found that hospital admissions for gallbladder stones at dr. Soekardjo General Hospital of Tasikmalaya were spatially clustered (Global Moran's I = 0.434 in 2022 and I = 0.521 in 2023). Hot spot clusters were identified in Tasikmalaya City, while surrounding regions exhibited cold-spot clusters. In Tasikmalaya Regency, cases were randomly distributed with no significant clustering (p < 0.05). Additionally, monthly variations significantly affect the incidence of gallstone cases (p < 0.001), which decreased during dry season from March to April, but reached maximum in August. Furthermore, age (p < 0.05) and gender (p < 0.001) significantly influenced gallstone risk, with female sex and individuals aged 55–64 being more susceptible.

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Original Article

Comparison of Line Dance and *Senam Lansia* to Improve Cardiorespiratory Endurance in Elderly

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Abstract

Background : Physical activity in the elderly has a positive impact on promoting healthy aging. Senam lansia is used to be taught to healthy elderly in community however it is boring because of monotonous movement. Line dance is an alternative aerobic exercise that has been taught to elderly. However, there was not any research that compares the effectiveness of both exercises to cardiorespiratory endurance. This study come to verify that the increase of cardiorespiratory endurance in elderly after line dance was better than senam lansia.
Methods : This study was a quasi-experimental pre-test and post-test- controlled group design on 2 groups which was line dance group (n = 20) and senam lansia group (n = 16). The exercise was given based on each group 3 times a week for 6 weeks and VO_2max measurement were taken with 6-minute walking test before and after intervention in both groups.
Results : The increase of the mean difference/delta VO_2max between groups did not differ statistically between groups (line dance: 2,19 ± 3,54 vs senam lansia: 1,79 ± 1,68; p = 0.683). There was a statistically significant difference in the mean pre- and post-intervention VO_2max values at line dance group (32,20 ± 3,99; p = 0.013) and senam lansia group (27,03 ± 3,77; p = 0.001).
Conclusion : The increase of cardiorespiratory endurance in line dance group and senam lansia group did not differ statistically.

Keywords : elderly, line dance, senam lansia, cardiorespiratory endurance

INTRODUCTION

The elderly are increasing in number every year and cause a shift in population structure.[1] This condition occurs throughout the world and is expected to increase to 22% of the total population by 2050.[2] The healthy aging process is something that needs to be achieved by all elderly people, one of which is by regularly doing physical exercise.[2,3] The recommended physical exercise in the elderly consists of aerobic, strengthening, balance and flexibility exercises.[4] Physical exercise will maintain and improve the level of cardiorespiratory fitness of the elderly which tends to decrease due to reduced muscle mass and O_2 extraction.[5]

Light intensity aerobic exercise that is commonly given to the elderly in Indonesia is *senam lansia*. *Senam lansia* is proven to increase cardiopulmonary endurance, reduce blood pressure, and reduce body fat levels.[6,7] However, monotonous movements with the same music accompaniment make the elderly quickly become bored and lose interest in doing physical exercise. Line dance is an alternative aerobic exercise that focuses on the lower extremities so that it can improve walking ability and independence of the elderly.[8,9] Walking ability assessed by the 6-minute walk test can describe the prediction of a person's cardiorespiratory fitness, especially the elderly.[10]

Currently, research on line dance and *senam* in Indonesia is still limited. Therefore, this study aims to compare the effect of line dance and *senam lansia* on improving cardiorespiratory fitness in the elderly.

METHODS

This study was conducted at Mitra Graha line dance community, Semarang and Soegijopranoto Primary Clinic *senam lansia* community, Semarang in November – December 2023. These two groups were determined based on the cluster sampling method. This study is a quasi experimental pre-test and post-test-controlled group design. Participants in the line dance and *senam lansia* groups received exercise 3 times a week for 6 weeks.

The population in this study were elderly people aged 60–75 years. Inclusion criteria include: (1) ability to walk independently without assistive devices; (2) lower extremity muscle strength, MMT [Manual Muscle Testing] ≥4; (3) ability to do light activities for 60 minutes without complaints (PASE [Physical Activity for the Elderly] score >65); (4) Montreal Cognitive Assessment score ≥26; (5) Mini Nutritional Assessment score >7; (6) participated in line dance or *senam lansia* groups for the previous 6 months to 1 year, with a frequency of less than 8 times per month. Exclusion criteria included: (1) musculosceletal and neuromuscular problems that cause pain (NRS ≥4), impaired standing and walking; (2) leg length discrepancy >5 cm; (3) uncontrolled comorbidities (blood glucose <80 mg/dL or >250 mg/dL, blood pressure <100/80 mmHg or >180/100 mmHg, unstable angina); (4) fear of falling (fall efficacy scale >70). Drop out criteria included: (1) failure to following and complete the schedule for 2 consecutive times or 3 times during the study; (2) failure to show up to the initial or final examination of the study; (3) hemodynamic

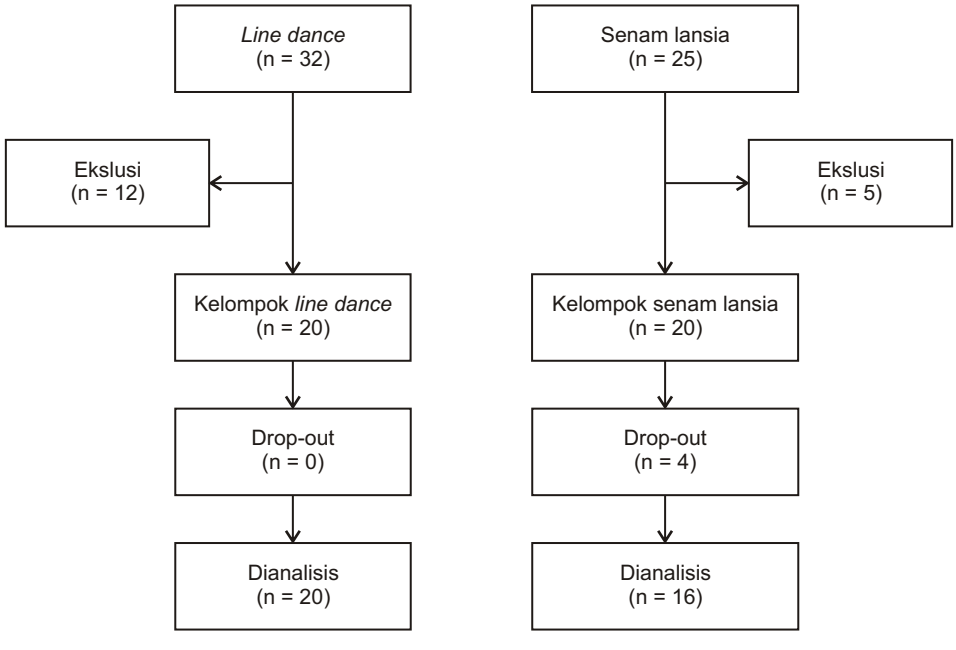

Figure 1. Diagram Consort

TABLE 1
The Baseline Characteristics of Data Subjects

Variabel	Group		*p*
	Line Dance (20)	Senam Lansia (16)	
Age	64.90 ± 5.45	68.75 ± 4.99	0.025‡*
Gender			
Male	0 (0)	4 (25)	0.031¥*
Female	20 (100)	12 (75)	
Education			
Elementary school	0 (0)	4 (25)	0.001¥*
Junior high school	1 (5)	7 (43.8)	
Senior high school	15 (75)	3 (18.8)	
Bachelor	4 (20)	2 (12.5)	
Comorbidities			
Hipertension	11 (55)	10 (62.5)	0.910¥
Diabetes Mellitus	1 (5)	2 (12.5)	0.574¥
Heart disease	2 (10)	0 (0)	0.492¥
Extremities weakness	0 (0)	0 (0)	–
Lung problems	0 (0)	1 (6.3)	0.910¥
Internal fixation	0 (0)	2 (12.5)	0.190¥
Body weight	53.08 ± 8.43	54.63 ± 8.15	0.581§
Body height	151.13 ± 7.14	149.88 ± 9.23	0.245‡

* Significant ($p < 0.05$); ¥Chi-Square; ‡Mann-Whitney; §Independent t

disturbances during exercise (shortness of breath, excessive fatigue, oxygen saturation <90% or decreased by 4%, blood pressure >200/100 mmHg); (4) subject is withdrawn from the study. Subjects were recruited by purposive sampling method and grouped into line dance and *senam lansia* groups.

The line dance group received a 60-minute workout consisting of 10 minutes of warm-up, 40 minutes of core movements, and 10 minutes of cool-down. The workout is given 3 times a week, for 6 weeks. The first 2 weeks they were given beginner movements (100 – 160 beats per minute, 32 – 48 counts), the next 3 weeks were improved movements (160 – 200 beats per minute, 64 counts), and in the last week an evaluation of all movements. The *senam lansia* group received the 2013 KEMENPORA *Senam Lansia* Bugar exercise which consisted of 10 minutes of warm-up, 40 minutes of core movements, and 10 minutes of cooling down. Both groups were guided by certified instructors. Participants of each group were reminded via whatasapp group, 1 day before the training schedule.

Cardiorespiratory fitness was assessed with a 6-minute walk test before and after the exercise. The mileage obtained was converted to predicted VO_2max (Volume Oxygen Maximum) using the Nury formula. The Nury formula is a predictive VO_2max calculation specific to the Indonesian population that takes into account the distanced walked for 6 minutes on a 15-meter track, age, height, weight, and gender.[11,12] Data analysis included descriptive analysis and hypothesis testing. Data normality test using Shapiro-Wilk test. A *P value* of ≥ 0.05 indicates normally distributed data.

Differences in predicted VO_2max values before and after training in each group were tested with a paired t-test if the data was normally distributed and the Wilcoxon test if the data was not normally distributed. The difference in cardiorespiratory fitness values between groups was tested by unpaired t-test if the data was normally distributed and Mann-Whitney test if the data was not normally distributed. All data was analyzed with SPSS ver 20.00 software. The significance value was *p value* <0.05.

TABLE 2
Analysis of 6 Minutes Walking Distance

6MWD	Group		*p*
	Line Dance (20)	Senam Lansia (16)	
Pre test (meter)	455.25 ± 64.25	344.06 ± 53.52	<0.001§*
Post test (meter)	496.50 ± 83.72	377.81 ± 42.15	<0.001§*
p	0.013¶*	0.001¶*	
Delta (meter)	41.25 ± 66.88	33.75 ± 31.70	0.683§

* Significant ($p < 0.05$); § Independent t; ¶ Paired t

TABLE 3
Analysis of VO_2max prediction

VO_2max prediction	Group		*p*
	Line Dance (20)	Senam Lansia (16)	
Pre test (mL/kg/min)	30.01 ± 2.95	25.24 ± 4.31	<0.001§*
Post test (mL/kg/min)	32.20 ± 3.99	27.03 ± 3.77	<0.001§*
p	0.013¶*	0.001¶*	
Delta (mL/kg/min)	2.19 ± 3.54	1.79 ± 1.68	0.683§

* Significant ($p < 0.05$); § Independent t; ¶ Paired t

This study was reviewed and accepted by the Health Research Ethics Commission (KEPK), Faculty of Medicine, Diponegoro University with Document No. 532/EC/KEPK/FK-UNDIP/X/2023.

RESULTS

There were 32 participants in the line dance group and 25 participants in the *senam lansia* group. A total of 12 participants in the line dance group were excluded because 11 participants did not meet the inclusion criteria and 1 participant refused to participate. In the *senam lansia* group, there were 5 people who did not meet the inclusion criteria. The data sample selection consort diagram is shown in Figure 1.

The data characteristics of both groups are shown in Table 1. The table shows the homogeneity test of the data characteristics of the two groups consisting of age, gender, education, comorbidities, weight, and height. There were significant differences in age ($p = 0.025$), gender ($p = 0.031$), and education level ($p = 0.001$) between the two groups.

Analysis of the increase in 6-minute walk distance before and after training in each group and between groups is shown in Table 2. The 6-minute walk distance before and after exercise increased significantly in the line dance ($p = 0.013$) and *senam lansia* ($p = 0.001$) groups. However, this increase in distance was not significantly different between the two groups ($p = 0.683$).

Analysis of the improvement of cardiorespiratory endurance as indicated by the predicted VO_2max value before and after exercise in each group and between groups is shown in Table 3. There was a significant increase in the predicted VO_2max value before and after exercise in each group (line dance, $p = 0.013$; *senam lansia*, $p = 0.001$). The increase in predicted VO_2max between groups was not significantly different ($p = 0.683$).

DISCUSSION

This study shows an increase in cardiorespiratory endurance as indicated by the predicted VO_2max value in the line dance and *senam lansia* groups before and after training. In the line dance group, the VO_2max value increased by 2.19 ± 3.54 mL / kg / min, and in the *senam lansia* group there was an increase in predicted VO_2max of 1.79 ± 1.68 mL / kg / min. Both line dancing and *senam lansia* are aerobic exercises that can improve cardiorespiratory fitness when given at the right intensity and duration. In this study, the exercises given adjusted the WHO recommendations regarding aerobic exercise, 3-5 times per week with a duration of 30–50 minutes per

session. Regular physical exercise in the elderly in accordance with the right dose is one of the health promotion and primary prevention efforts that can be carried out together in the community as part of community-based prevention rehabilitation.[13]

Line dance is an interval aerobic exercise due to fluctuations in intensity during exercise. Line dance causes peripheral adaptations characterized by arteriolar vasodilation and increased mitochondrial density as well as central adaptations such as increased maximal volume of the heart, cardiac output, and blood volume.[14,15] Line dance increases hip flexor and abductor muscle strength,[16] trains dynamic stability endurance due to changes in mediolateral CoM (Center of Mass), and improves balance and gait propulsion due to improvements in anteroposterior CoM.[17] Changes in GRF (Ground Reaction Force) while doing line dance will increase greater energy use, increase heart rate and VO_2max.[18] A decrease in VO_2peak (Volume Oxygen Peak) values with age will cause a relative increase in the intensity of a person's walking which is related to a decrease in muscle mass and strength, especially in the lower extremities.[19] *Senam lansia* has a mechanism similar to line dance in improving cardiorespiratory fitness. *Senam lansia* causes vasodilation, an increase in oxygen consumption by 10 - 20% and tidal volume by 2.5 – 3 L / min., cardiac output by 40 – 60%.[20,21] Arm movements in *senam lansia* will increase the sympathetic connection to the heart so that central adaptation occurs in a person's cardiorespiratory endurance.[15] *Senam lansia* increases oxygen demand and blood flow in the muscles.[22,23]

Line dance has an advantage when compared to *senam lansia*, of which it can be increase in intensity and difficulty of movement according to the ability of the participants. This increase in intensity needs to be done to be able to maintain optimal cardiorespiratory endurance of the elderly. Line dance in this study proved to be safe for the elderly group, as evidenced by the absence of injuries and severe symptoms such as shortness of breath and chest pain during exercise. Previous study conducted by Honglian showed a significant improvement in blood pressure and quality of life of elderly people with cardiovascular and cerebrovascular disease after receiving low and middle intensity line dance compared to those in control group who only get pharmacological therapy alone.[24] Another study comparing line dance and daily physical activity showed that line dance significantly improved knee muscle strength, lower extremities function, walking speed, endurance, and perceived mobility limitation.[25]

Comparison of delta VO_2max between the two groups did not differ significantly (p = 0.683). This is related to the difference in baseline values in the two groups before training. Individuals with low VO_2max values before exercise will obtain significant improvements in cardiorespiratory endurance with low exercise intensity (40 – 50% VO_2max) due to central adaptation, while individuals with high VO_2max values require higher exercise intensity (>70% VO_2max) to obtain similar results.[15] Gender will also affect the decline in VO_2peak, heart rate, ejection fraction, and cardiac index. Men have a greater VO_2max than women at various ages so that the fitness function of elderly men will tend to be better than elderly women.[26]

The *senam lansia* group had male participants although almost 80% of the participants were dominated by women, while the line dance group consisted entirely of female participants. This condition may be due to the lack of socialization and public understanding of line dance as a physical exercise not just as a dance exercise and the importance of physical exercise in the elderly as an effort to maintain health and prevent disease. A higher level of education was found in the line dance group. This suggests that a high level of education will help a person to get health information and the latest types of physical exercise that can maintain their health condition. The gender distribution and education level of the research subjects in both groups were able to provide a general picture of elderly participation in the community. This study had some limitations that might affect the study results. This study did not randomize the sample so that the distribution of age, gender, and education levels in the two groups were significantly different. The duration of exercise given is still unable to show the long-term effects of improving cardiorespiratory endurance.

CONCLUSION

The increase of cardiorespiratory endurance in line dance and *senam lansia* group did not differ statistically. Both line dance and *senam lansia* can significantly improve cardiorespiratory endurance in the elderly.

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Original Article

The Risk of Bag-Carrying Related to Musculoskeletal Pain in Medical Students

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Abstract

Background : Musculoskeletal discomfort affects medical students' neck, shoulders, and back. Musculoskeletal pain may be linked to bag-carrying behaviors, which encompass the manner of carrying, duration of use, and weight of the bag. This study aims to determine the relationship between bag-carrying habits and musculoskeletal pain.
Methods : This is an observational analytic study with a cross-sectional approach. From 2022–2023, the research subjects were 70 Sriwijaya University medical students who met the inclusion and exclusion criteria. The sampling method employed a simple random sampling technique. We analyzed the obtained data using the Chi-square test in SPSS software.
Results : The majority of the subject was 20 years old and female. Most patients with musculoskeletal pain experience mild complaints in the lower neck (47.14%), right shoulder (41.43%), and back (41.43%). All of the *p-values* are ≥0.05, including how to carry a bag, bag weight, and the duration of using a bag with neck, shoulder, and back pain among medical students of Sriwijaya University.
Conclusion : There is no significant relationship between bag-carrying habits, which include how to carry a bag, bag weight, and the duration of using a bag, with neck, shoulder, and back pain among medical students of Sriwijaya University.

Keywords : bag-carrying, musculoskeletal disorders, medical students

INTRODUCTION

Medical students often carry thick and heavy books as part of their learning requirements.[1] Technology advancements have replaced the physical burden of books with digital loads, substituting bulky books with electronic files, and filling students' bags with modern devices like laptops, iPads, and similar gadgets.[2] The appropriate use of bags is essential to facilitate the transportation of numerous learning materials, bags must be used appropriately. Various bags are available for carrying items according to needs and purposes.[3,4]

Backpacks are commonly chosen because they can carry heavier loads and distribute the weight across most of the user's body. However, some individuals, especially women, opt for shoulder bags for aesthetic reasons due to their diverse designs. These bags can increase pressure on muscles, ligaments, joints, and bones, leading to musculoskeletal pain.[5] In 2018, a study on female Oxford students from various faculties found a highly significant correlation between musculoskeletal pain and time spent carrying bags.[6] Research on medical students at the University of Sumatera Utara indicated that students carrying bags on both shoulders were more likely to experience neck pain than those using a single-shoulder bag.[7] Several studies conclude that the neck, shoulders, and lower back experience the most common musculoskeletal pain.[6,7]

Musculoskeletal pain is a prevalent issue among medical students.[7,8] A study on the prevalence of Musculoskeletal Disorders (MSDs) among medical students in Saudi Arabia found that 59.8% of medical students experienced pain in at least one location in the past year. The most frequently reported musculoskeletal regions were the neck (36.7%), followed by the lower back (33.3%) and shoulders (22.3%) in the past year and week, but reports in the last week were less common.[9] This is somewhat different from previous research reporting musculoskeletal pain incidents in medical students at a university, with the majority in the lower back (38.2%), followed by the neck (27.2%), and lastly, the shoulders (19.7%).[10]

The above description suggests that musculoskeletal pain in medical students may be associated with the habit of carrying bags, including the carrying method, the duration of use, and the weight of the bag. This research aims to identify the distribution of bag-carrying methods, bag weight, and duration of bag use among students in the Medical Education Programme at the Faculty of Medicine, University of Sriwijaya and analyse its relationship with musculoskeletal pain. The limited availability of data on musculoskeletal pain experienced by medical students at the Faculty of Medicine of Sriwijaya University has piqued the researchers' interest in studying the relationship between bag-carrying habits and musculoskeletal pain among medical students at Sriwijaya University.

METHODS

This study is an observational analytic study with a cross-sectional approach. The research subjects were 70 medical students of Sriwijaya University for the period of 2022–2023 who met the inclusion and exclusion criteria. The sampling method used a simple random sampling technique. The musculoskeletal pain collected by Numeric Rating Scale Pain (NRS) and Nordic Body Map by questionnaire and was analyzed using the Chi-square test with SPSS software. This research also has been confirmed by ethical clearance No 186-2023 declared by Ethics Committee from Faculty of Medicine Sriwijaya University, Palembang, Indonesia.

RESULTS

The respondents' ages were varied, with nearly half being 21 years old (48.57%), 24.29% being 20 years old, and the

TABLE 1
Distribution of Research Subjects Based on Bag Usage Habits

		n	%
Habits of bag-carrying	One side of shoulder	39	55.71
	Both shoulders	31	44.29
Bag weight	>10% BW	4	5.71
	<10% BW	66	94.29
Duration of bag carrying	>30 minutes	24	34.29
	15–30 minutes	14	20.00
	10–15 minutes	26	37.14
	<10 menit	6	8.57

TABLE 2
Frequency Distribution of Students Based on Musculoskeletal Pain

Pain Location	Pain Category								N
	No Pain		Mild		Moderate		Severe		
	n	%	n	%	n	%	n	%	
Upper neck	38	54.29	23	32.86	8	11.43	1	1,43	70
Lower Neck	26	37.14	33	47.14	10	14.29	1	1,43	70
Left Shoulder	32	45.71	24	34.29	12	17.14	2	2,86	70
Right Shoulder	21	30.00	29	41.43	20	28.57	0	0,00	70
Left Upper-arm	57	81.43	11	15.71	2	2.86	0	0,00	70
Upper Back	28	40.00	29	41.43	11	15.71	2	2,86	70
Right Upper Arm	49	70.00	17	24.29	4	5.71	0	0,00	70
Waist	29	41.43	27	38.57	13	18.57	1	1,43	70
Lower Back	46	65.71	18	25.71	6	8.57	0	0,00	70
Buttocks	58	82.86	10	14.29	2	2.86	0	0,00	70
Left Elbow	67	95.71	3	4.29	0	0.00	0	0.00	70
Right Elbow	65	92.86	5	7.14	0	0.00	0	0.00	70
Left Lower Arm	64	91.43	5	7.14	1	1.43	0	0,00	70
Right Lower Arm	60	85.71	9	12.86	1	1.43	0	0,00	70
Left Wrist hand	64	91.43	5	7.14	1	1.43	0	0,00	70
Right Wrist Hand	59	85.51	9	13.04	1	1.43	0	0,00	69
Left Hand	64	91.43	5	7.14	1	1.43	0	0,00	70
Right Hand	60	85.71	8	11.43	2	2.86	0	0,00	70
Left Thigh	65	92.86	5	7.14	0	0.00	0	0.00	70
Right Thigh	62	88.57	8	11.43	0	0.00	0	0.00	70
Left Knee	67	95.71	3	4.29	0	0.00	0	0.00	70
Right Knee	65	92.86	5	7.14	0	0.00	0	0.00	70
Left Calves	58	82.86	9	12.86	3	4.29	0	0,00	70
Right Calves	59	84.29	8	11.43	3	4.29	0	0,00	70
Left Ankle	64	91.43	5	7.14	1	1.43	0	0,00	70
Right Ankle	59	84.29	9	12.86	2	2.86	0	0,00	70
Left Foot	63	90.00	6	8.57	1	1.43	0	0,00	70
Right Foot	63	90.00	5	7.14	2	2.86	0	0,00	70

lowest proportion being 23 years old (1.43% of the total respondents). More than half of the respondents were female (65.71%), while 24 individuals were male, or 34.29% of the total number of respondents.

According to the student distribution based on bag use patterns, most research subjects (55.71%) carry their bags on one shoulder. The majority of respondents (94.29%) carry bags that are less than 10% BB, and the duration of bag use is primarily between 10–15 minutes (37.14%) (Table 1). Based on the frequency distribution of students with musculoskeletal pain shown in the table below, the three places where the research subjects most often felt mild pain were the lower neck (47.14%), the right shoulder (41.43%), and the back (41.43%), which

TABLE 3
Relationship Between Bag-Carrying Habits and Neck Pain

Category		Neck Pain				N	*PR*	*p-value*
		Pain		No Pain				
		n	%	n	%			
Bag-carrying habits	One side of shoulder	27	69.2	12	30.8	39	1.129	0.487
	Both shoulders	19	61.3	12	38.7	31		
Bag weight	>10% BW	3	75	1	25	4	1.150	1.000
	<10% BW	43	65.2	23	34.8	66		
Bag Usage Duration	>30 minutes	15	62.5	9	37.5	24	1.000	1.000
	15–30 minutes	10	71.4	4	28.6	14		
	10–15 minutes	16	61.5	10	38.5	26		
	<10 menit	5	83.3	1	16.7	6		
Total						70		

TABLE 4
Relationship Between Bag-Carrying Habits and Shoulder Pain

Category		Shoulder Pain				N	*PR*	*p-value*
		Pain		No Pain				
		n	%	n	%			
Bag-carrying habits	One side of shoulder	31	79.5	8	20,5	39	1.368	0.052
	Both shoulders	18	58.1	13	41,9	31		
Bag weight	>10% BW	2	50	2	50	4	0.702	0.578
	<10% BW	47	71.2	19	28,8	66		
Bag Usage Duration	>30 minutes	20	83.3	4	16,7	24	1.033	0.834
	15–30 minutes	7	50	7	50	14		
	10–15 minutes	16	61.5	10	38,5	26		
	<10 menit	6	100	0	0	6		

had the same percentage as the right shoulder (Table 2).

The research subjects most frequently experienced the right location when viewed in the context of the moderate pain category. The left shoulder had a proportion of 17.41%, followed by the waist (18.57%) and the shoulder (28.57%). The Nordic Body Map Questionnaire identifies only five locations in the severe pain category: the left shoulder (2.86%), back (2.86%), abdomen (1.43%), upper neck (1.43%), and lower neck (1.43%).

The right shoulder exhibited a higher pain level than the left shoulder, as indicated by the proportion of no pain. The same assessment also revealed a higher incidence of discomfort in the lower neck compared to the upper neck. According to the Nordic Body Map, the left elbow and left knee are the least painful body regions, with a combined percentage of 95.71%.

This study used the Chi-square test to conduct statistical tests on a sample size of 70. If the chi-square test did not meet the requirements, the *p-value* was determined using Fisher's exact test results. More than 50% of the subjects who carry bags with one shoulder (69.2%) or both shoulders (61.3%) experience neck discomfort when viewed from the carrying bag perspective (Table 3). In the Chi-square test results, the *p-value* for the variable of how to transport a bag is 0.487. This suggests that no significant relationship exists between how students carry their bags and their neck pain at PSPD FK Unsri. In addition, the Chi-square test results for the bag weight variable did not indicate a

TABLE 5
Relationship Between Bag-Carrying Habits and Back Pain

Category		Back Pain				N	*PR*	*p-value*
		Pain		No Pain				
		n	%	n	%			
Bag-carrying habits	One side of shoulder	21	53.8	18	46.2	39	0.926	0.724
	Both shoulders	18	58.1	13	41.9	31		
Bag weight	>10% BW	3	75	1	25	4	1.376	0.624
	<10% BW	36	54.5	30	45.5	66		
Bag Usage Duration	>30 minutes	15	62.5	9	37.5	24	1.348	0.172
	15–30 minutes	9	64.3	5	35.7	14		
	10–15 minutes	10	38.5	16	61.5	26		
	<10 menit	5	83.3	1	16.7	6		

significant relationship with neck discomfort in PSPD FK Unsri students, with a *p-value* of 1.000. Musculoskeletal pain was experienced by over half of the research subjects who carried bags exceeding 10% of their body weight (75%), while 65.2% of the total group who carried bags below 10% experienced neck pain.

Based on the duration of bag use, the research subject who carried the bag for less than 10 minutes had the highest percentage of neck pain (83.3%). However, considering the number of students who experienced neck pain, the group that carried bags for 10–15 minutes (61.5%), comprising as many as 16 individuals, was the most affected. The relationship between the duration of bag use and neck pain was insignificant, as indicated by a *p value* of 1.000. Table 4 illustrates an analysis of the relationship between bag use habits and shoulder pain. The analysis results differ slightly from those of the study on neck pain. In the bag-carrying category, the group experiencing shoulder pain was more likely to carry the bag on one shoulder (79.5%). While in the category of bag weight, the group that carried a bag weight <10% of body weight (71.2%) showed a greater percentage than the other groups.

Based on the duration of bag use, the group that experienced shoulder pain with the highest percentage was the research subject who carried the bag for <10 minutes (100%). Meanwhile, when viewed from the number of students who experience shoulder pain, the most was the group who carrier bags with a duration of >30 minutes (83.3%), as many as 20 people. The relationship between the way of carrying a bag (p=0.052), the weight of the bag (p=0.578), and the duration of bag use (p=0.834) with shoulder pain is not significant based on the analysis results in Table 4.

Table 5 shows the results of the analysis of the relationship between bag use habits and back pain. Different results can be seen in the category of how to carry a bag, where the group with the highest percentage of pain is the group that carried the bag with both shoulders (58.1%). In addition, in the category of bag weight, the highest percentage is the group that carried a bag weight of more than 10% BW. However, in the category of duration of bag use, the highest percentage remained in the less than 10 minutes group (83.3%). Based on the *p-value* results, no significant relationship was found between the three variables of bag use habits and back pain experienced by the research subjects.

DISCUSSION

The research subjects in this study included all active PSPD students for the 2022–2023 period which included the Class of 2020, 2021, and 2022. Research sampling was carried out using a simple random sampling technique where respondents were randomly selected at each data collection time. The questionnaire was filled out voluntarily by students willing to become research samples.

The data collection results indicated that 73 students were willing to participate as research subjects in the study. Of this total number, it was found that three people failed to complete the questionnaire. Based on this, the final number of research samples in data processing amounted to 70 people. The majority of research subjects are female; this is also in line with the research on FK USU students in 2019.[7]

Based on the results of the distribution of research subjects according to bag usage habits, most students carry bags on one shoulder. When conducting the study, it was known that female respondents used more bags with one shoulder. These results show the same thing as the results of research on FK students at the University of

North Sumatra.[7] The study showed that female respondents were more likely to use bags with one shoulder, while men were more likely to use backpacks carried over both shoulders.[7] This is supported by a study that women prefer shoulder bags for appearance.[4] Most of the subjects in this study carried a burden of less than 10% of their body weight in their bags. The same phenomenon was demonstrated in a study of medical students in North Sumatra, where 149 out of 170 respondents carried less than 10% BW in their daily packs.[7]

The duration of bag use among respondents ranged from 10 to 15 minutes, influenced by their mode of transportation. Students living in boarding houses near campus tended to walk, resulting in longer bag-carrying times, whereas students commuting by vehicle reported shorter durations. Interestingly, the most common pain locations reported were the lower neck, right shoulder, and back. This pattern differs from earlier studies, such as research on musculoskeletal pain among medical students that identified the lower back as the most affected area (38.2%), followed by the neck (27.2%) and shoulders (19.7%).[1] Similarly, a study in Saudi Arabia reported the neck as the most frequent pain site, followed by the lower back and shoulders. This variation may be influenced by differences in lifestyle, posture, or cultural habits related to bag use and daily activities.

This study aimed to investigate the relationship between bag use habits and musculoskeletal pain. The findings revealed no significant association between how students carried their bags, bag weight, or duration of use with neck, shoulder, or back pain. These results are consistent with prior research among FK USU students, which also found no significant relationship between bag use patterns and musculoskeletal complaints. Similar conclusions were reported in other studies, which suggested that factors such as bag weight and carrying duration may not directly contribute to pain unless combined with other ergonomic or individual risk factors.[6]

The absence of a significant relationship in this study could be due to the relatively light bag weights and short carrying durations among participants. It is also possible that students adjusted their posture or load distribution to minimize discomfort, mitigating the potential impact of bag use on musculoskeletal pain. Additionally, other factors not assessed in this study, such as physical activity levels, pre-existing musculoskeletal conditions, or seating ergonomics during the study, may play a more prominent role in the development of pain.

CONCLUSION

The majority of medical students at Sriwijaya University carry packs with a weight of less than 10% BW on one shoulder for 10–15 minutes. The relationship between musculoskeletal discomfort and the habit of using bags among medical students at Sriwijaya University was not statistically significant.

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Original Article

The Correlation of Vitamin D Levels and Ghrelin, Adiponectin, and Resistin Levels Among Post-Ischemic Stroke Patients

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Abstract

Background : Dyslipidemia is one of the modifiable risk factors for stroke. The pathogenesis of dyslipidemia is through several mechanisms including adipose tissue-mediated adiponectin, resistin, and leptin secretion. Vitamin D is correlated with lipid profiles, but there is no evidence of the role of vitamin D in ghrelin, adiponectin, and resistin secretion, which may contribute to the mechanisms of the impairment of lipid profile among post-ischemic stroke. The aims of this study was to determine the correlation between vitamin D and ghrelin, adiponectin, and resistin among post-ischemic stroke.

Methods : An observational analytic study with a cross-sectional approach was conducted among forty post-ischemic stroke patients which was obtained consecutively. The serum vitamin D, ghrelin, adiponectin, and resistin levels were measured using the venous blood obtained from the median cubital vein. Data analysis was performed using the Spearman correlation test to determine the correlation between the serum vitamin D levels, ghrelin, adiponectin, and resistin levels.

Results : The age of our subjects is approximate 57 years old with the proportion of males being more than female subjects. There is a significant association between serum vitamin D levels and gender, total energy as well carbohydrate diet but no significant association between hormonal status and the characteristics of subjects was revealed. Furthermore, there is no significant correlation between serum vitamin D levels with the hormonal status of ghrelin, adiponectin, or resistin levels (p=0.994; 0.395; 0.858, respectively).

Conclusion : There is no significant correlation between serum vitamin D levels and serum ghrelin, adiponectin, or resistin levels among post-ischemic stroke patients

Keywords : Dyslipidemia, Vitamin D, Ghrelin, Adiponectin, Resistin

INTRODUCTION

Stroke is the second rank of neurological disorders leading to a high annual mortality index and morbidity with the consequences of disability among 50% of survivors with the consequences on social and economic aspects.[1]

Dyslipidemia is one of the modifiable risk factors for stroke and also a predictor for the outcome of stroke.[2,3] Previous studies revealed that high levels of serum triglyceride and LDL cholesterol and low levels of serum HDL cholesterol cause atherosclerosis, resulting in several vascular diseases.[3]

Dyslipidemia is one of the consequences of several mechanisms including the role of adipose tissue-mediated process.[5,6] Mature adipocytes can perform many functions, including synthesis of lipids, traffic of fatty acids across the membrane, and response to insulin signaling and adipokine secretion such as adiponectin, resistin, and leptin.[7] Adiponectin is a serum protein produced specifically by differentiated adipocytes. It affects the regulation of energy metabolism including lipid metabolism. Adiponectin has multidirectional biological action. It inhibits hepatic gluconeogenesis, reduces hepatic glucose output, and decreases the level of free fatty acids due to their oxidation.[8] Resistin is an adipokine that has been linked to the development of T2DM in rodent models. The overexpression of resistin from adipocytes induced the development of insulin resistance and dyslipidemia in healthy mice.[9]

Another hormone that can affect lipid metabolism is ghrelin. Ghrelin is a peptide hormone secreted by the stomach and duodenum and is involved in the short-term regulation of appetite as well as in the storage processes of lipids within white adipose tissue (WAT).[10] Ghrelin also affects the lipid metabolisms in the liver and promotes lipogenesis through receptor-mediated direct activation in hepatocytes.[11] Previous studies demonstrated that there was a correlation between ghrelin and LDL-cholesterol, as well as HDL-cholesterol.[12]

Vitamin D3 is one of the cholesterol derivates synthesized from 7-dehydrocholesterol in the skin upon ultraviolet irradiation. Its metabolism and function are still in debate. The classical function of 1a,25(OH)2-D3 is the regulation of calcium homeostasis. However, vitamin D receptor (VDR) is expressed in tissues not involved in calcium metabolism indicating that vitamin D may be implicated in the regulation of many non-calcemic functions including lipid metabolisms.[5,6]

Previous studies demonstrated that there is an association between Vitamin D and lipid profiles. Vitamin D deficiency is one of the risk factors for the occurrence of dyslipidemia.[5] Level of 25(OH)D3 negatively correlates with total cholesterol, LDL cholesterol, and triglyceride levels, and has a positive correlation with HDL cholesterol levels.[6] Furthermore, vitamin D supplementation resulted in a decrease of triglycerides, LDL cholesterol, and total cholesterol levels and an increase of HDL cholesterol level.[7]

The mechanisms of vitamin D affect metabolisms of lipids and cholesterol are still unknown. One of its mechanical hypotheses through the adipose mediated mechanisms resulted in the changes of lipid profile.[6] Furthermore, vitamin D3 also contributes to the absorption of calcium and inhibits lipid deposition.[5,6] through its interaction with membrane receptors, adaptor molecules, phosphatases, and nuclear coregulator proteins which contribute to the control of gene expression as well as cell signaling.[5,6]

Even though there is some evidence of the role of vitamin D in adipokine secretion especially for the pathogenesis of obesity, there is little evidence showing the relationship between vitamin D and ghrelin, adiponectin, and resistin, which may contribute to the mechanisms of the impairment of lipid profile among post-ischemic stroke, so herewith we determine their correlation to get a better understanding of the role of vitamin D on the lipid metabolism.

METHODS

This is an observational analytic study with a cross-sectional approach conducted in forty post-ischemic stroke patients in the outpatient Department of Neurology RSUP Dr. Kariadi Semarang from July to September 2022 obtained consecutively with the inclusion criterion 1) diagnosed with a post-ischemic stroke, 2) aged between 18–60 years old and consented to be involved in this study, and exclusion criterion of 1) history of vitamin D supplementation, 2) history of parathyroid hormone disorders, 3) history of dyslipidemic or cholesterol-lowering drugs consumption. Sample size estimation was measured based on the formula for the minimum sample for correlation study.

Demographic characteristics such as age, gender, and history of prior diseases including diabetes mellitus (DM), hypertension, and cardiovascular disorders were recorded from medical records, and the intake of energy, carbohydrate, protein, lipids, and cholesterol was assessed using 24-hour diet recall methods.

Vitamin D and serum ghrelin, adiponectin, and resistin levels were measured using the venous blood obtained from the median cubital vein. The measurement was performed in the *Gangguan Akibat Kekurangan Yodium* (GAKY) Laboratory Faculty of Medicine Universitas Diponegoro Semarang Indonesia employing ELISA methods. The protocol of examination was performed according to the manufacturer's sheet.

Data analysis was performed with *SPSS for Windows version 23*, which is divided into two phases. First, descriptive analyses was done to describe the

characteristics of the subjects. Categorical data was presented in frequency with percentage and numerical data was presented in the median, minimal, and maximal values because of abnormal data distribution. Then, analysis was continued using the Spearman correlation test to determine the correlation between the characteristics of subjects, serum vitamin D levels, and ghrelin, adiponectin, and resistin levels.

Study protocols were approved by The Local Research Ethics Committee and the ethical clearance was obtained from the Health Research Ethical Committee RSUP Dr. Kariadi Semarang Indonesia with the number of ethical clearance No 993-1/EC/KEPK-RSDK/2022.

RESULTS

Characteristics of Subjects

The demographic, recall of diets, and laboratory findings data of the subjects are demonstrated in Table 1.

Table 1 reveals that the age of our subjects is approximately 57 years old with 32 being the youngest and 71 being the oldest age with the proportion of male being more than female subjects. Regarding the vascular risks of stroke, hypertension is the most often with or without diabetes Mellitus and cardiac problems.

TABLE 1
Demographic Characteristics of Subjects

Variable	n	%	Median (min – max)
Age			57.5 (32 – 71)
Gender			
Male	23	57.5	
Female	17	42.5	
History of hypertension			
No	8	20.0	
Yes	32	80.0	
History of diabetes mellitus			
No	26	65.0	
Yes	14	35.0	
History of cardiac abnormality			
No	25	62.5	
Yes	15	37.5	
Energy recall diet (Kcal/day)			1482.3 (869.7 – 1921.3)
Protein recall diet (Kcal/day)			62.35 (33.20 – 156.80)
Lipid recall diet (Kcal/day)			41.75 (16.90 – 62.10)
Carbohydrate recall diet (Kcal/day)			221.6 (115.5 – 347.9)
Cholesterol recall diet (Kcal/day)			175.9 (66.1 – 400.1)
Total Cholesterol (mg/dL)			207.70 ± 40.81
LDL Cholesterol (mg/dL)			145.35 ± 46.47
Triglycerides (mg/dL)			159.50 ± 85.43
HDL Cholesterol (mg/dL)			47.47 ± 15.272
Vitamin D (ng/dL)			21.72 ± 9.54
Ghrelin (ng/dL)			223.83 ± 85.00
Adiponectin (ng/dL)			4366.33 ± 548.36
Resitin (ng/dL)			14.17 ± 1.12

TABLE 2
Correlation of Serum Vitamin D Levels and Characteristics of Subjects

Variable	Mean ± SD	*p*
Age		0.638
Gender		0.002*
Male	24.49 ± 8.56	
Female	17.97 ± 9.76	
History of hypertension		0.624
No	22.05 ± 7.56	
Yes	21.63 ± 10.08	
History of diabetes mellitus		0.288
No	23.32 ± 10.90	
Yes	18.74 ± 5.50	
History of cardiac abnormality		0.759
No	21.30 ± 7.67	
Yes	22.42 ± 12.32	
Energy recall diet (Kcal/day)		0.001*
Protein recall diet (Kcal/day)		0.076
Lipid recall diet (Kcal/day)		0.397
Carbohydrate recall diet (Kcal/day)		<0.001*
Cholesterol recall diet (Kcal/day)		0.444

*Significant (p<0.005)

TABLE 3
Correlation of Serum Ghrelin Levels and Characteristics of Subjects

Variable	Mean ± SD	*p*
Age		0.650
Gender		0.494
Male	226.22 ± 86.44	
Female	220.59 ± 85.54	
History of hypertension		0.735
No	224.13 ± 90.62	
Yes	223.75 ± 85.06	
History of diabetes mellitus		0.387
No	208.19 ± 72.58	
Yes	252.86 ± 100.74	
History of cardiac abnormality		0.944
No	219.08 ± 81.80	
Yes	231.73 ± 92.46	

TABLE 3. Continued.

Variable	Mean ± SD	*p*
Energy recall diet (Kcal/day)		0.815
Protein recall diet (Kcal/day)		0.323
Lipid recall diet (Kcal/day)		0.574
Carbohydrate recall diet (Kcal/day)		0.883
Cholesterol recall diet (Kcal/day)		0.634

TABLE 4
Correlation of Serum Adiponectin Levels and Characteristics of Subjects

Variable	Mean ± SD	*p*
Age		0.288
Gender		0.397
Male	4302.26 ± 492.00	
Female	4453.00 ± 621.48	
History of hypertension		0.204
No	4143.88 ± 729.54	
Yes	4421.94 ± 492.10	
History of diabetes mellitus		0.843
No	4363.69 ± 461.87	
Yes	4371.21 ± 701.31	
History of cardiac abnormality		0.706
No	4313.16 ± 511.31	
Yes	4454.93 ± 613.22	
Energy recall diet (Kcal/day)		0.955
Protein recall diet (Kcal/day)		0.980
Lipid recall diet (Kcal/day)		0.780
Carbohydrate recall diet (Kcal/day)		0.930
Cholesterol recall diet (Kcal/day)		0.653

Correlation of Serum Vitamin D Levels and Characteristics of Subjects

Initially, we determined the correlation between serum vitamin D levels and the characteristics of subjects. The results of its data analysis are depicted in Table 2.

Table 2. revealed that there is a significant association between serum vitamin D levels and gender, total energy as well carbohydrate diet, but none for other characteristics including age.

Correlation of Serum Hormonal Levels and the Characteristics of Subjects

The data analysis was continued for the hormonal status including ghrelin, adiponectin, and resistin levels and characteristics of subjects which are displayed in Table 3, 4, and 5.

Tables 3, 4, and 5 demonstrated that there is no significant association of hormonal status including the ghrelin, adiponectin, and resistin levels, and the characteristics of subjects including age.

TABLE 5
Correlation of Serum Resistin Levels and Characteristics of Subjects

Variable	Mean ± SD	*p*
Age		0.315
Gender		0.554
Male	14.08 ± 1.24	
Female	14.29 ± 0.96	
History of hypertension		0.542
No	13.95 ± 1.16	
Yes	14.23 ± 1.12	
History of diabetes mellitus		0.402
No	14.28 ± 1.20	
Yes	13.96 ± 0.96	
History of cardiac abnormality		0.786
No	14.21 ± 1.20	
Yes	14.11 ± 1.02	
Energy recall diet (Kcal/day)		0.467
Protein recall diet (Kcal/day)		0.948
Lipid recall diet (Kcal/day)		0.346
Carbohydrate recall diet (Kcal/day)		0.428
Cholesterol recall diet (Kcal/day)		0.311

TABLE 6
Correlation of Serum Vitamin D, Ghrelin, Adiponectin and Resistin Levels

Variable	*p*	Median (Min–Max) (ng/dL)	r
Ghrelin	0.994	192 (114 – 445)	-0.001
Adiponectin	0.395	4426.5 (2934 – 5738)	-0.138
Resitin	0.858	14.4 (11.5 – 16)	-0.029

Correlation of Serum Vitamin D, Ghrelin, Adiponectin and Resistin Levels

As our main outcome, the result of data analysis of correlation of correlation of serum vitamin D, ghrelin, adiponectin, and resistin Levels was provided in Table 6.

Table 6 provides the information that there is no significant correlation between serum vitamin D levels with the hormonal status of ghrelin, adiponectin, or resistin levels.

DISCUSSION

Dyslipidemia is one of the modifiable risk factors for cardiovascular events including stroke and also a predictor for the outcome of stroke.[2,4,13] Dyslipidemia is one of the consequences of several mechanisms such as the fatty tissue-mediated processes including synthesis of lipids, traffic of fatty acids across the membrane, and response to insulin signaling and adipokine secretion such as adiponectin, resistin, leptin, and ghrelin.[5,6]

Previous studies demonstrated that there is an association between Vitamin D and lipid metabolisms

resulting in the impairment of lipid profiles.[5] Level of 25(OH)D3 negatively correlates with total cholesterol, LDL cholesterol, and triglyceride levels, and has a positive correlation with HDL cholesterol levels.[6,14]

Several factors influence the photosynthesis and bioavailability of vitamin D and contribute to the risk of impaired vitamin D status starting from factors that impact the exposure to ultraviolet radiation, absorption of vitamin D, and metabolism of vitamin D.[15,16] Furthermore, several diseases affect vitamin D statuses such as kidney disease, liver disease, and malignancies.[15] Our results provide the evidence that there is a significant association between serum vitamin D levels and gender, total energy as well carbohydrate diet. These results align with a previous study that revealed higher intake of carbohydrates might reduce vitamin D levels and also the level of vitamin D is affected by gender.[17,18]

The relationship between serum vitamin D levels with the hormonal status of ghrelin, adiponectin, or resistin levels is still in debate. Some evidence provides that vitamin D modulates the secretion of many adipokines.[19] On the other hand, some studies showed that there is no effect of vitamin D on the hormonal status related to lipid metabolism.[20] Our results show that there is no significant correlation between serum vitamin D levels with the hormonal status of ghrelin, adiponectin, or resistin levels. These results are different from previous studies that demonstrated vitamin D is associated with resistin or adiponectin.[21–23] There is no evidence among post-stroke patients, previous studies using different comorbidities such as patients with inflammatory bowel diseases, post-menopause women, and patients with type II diabetes mellitus. Our findings also suggest that dyslipidemia among post-stroke patients has resulted from different mechanisms compared with other diseases that need to be studied further.[24]

Several factors contribute to vitamin D availability in the human body such as age, gender, or maybe genetics.[15–17] Even though our results provide evidence that there are several confounding factors associated with the particular vitamin D levels in the bivariate analysis such as gender, total energy, and carbohydrate diet, we did not continue the analysis to the multivariate. Furthermore, from our findings we did not classify the level of vitamin D according to the clinical approach, which may be useful in the management of patient related to vitamin D status. Thus our study only presents the statistical importance of these findings.

CONCLUSION

There is no significant correlation between serum vitamin D levels and serum ghrelin, adiponectin, or resistin levels among post-ischemic stroke patients.

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CONFLICT OF INTEREST

There is no conflict of interest.

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Original Article

Correlation of Single Breath Count Test with Peak Expiratory Flow Rate in Adult Patients Post COVID-19 Infection

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Abstract

Background : The utilization of spirometry has garnered significant attention recently due to its potential role in transmitting COVID-19. There is a critical need for a straightforward bedside assessment capable of accurately measuring respiratory function without relying on equipment and minimizing the risk of airborne transmission. This study was aimed to know the correlation between Single Breath Count Test (SBCT) values and Peak Expiratory Flow Rate (PEFR) values in adult patients recovering from COVID-19.

Methods : This study presents a cross-sectional design with consecutive subject recruitment. The SBCT was administered using a metronome, while PEFR was measured using a Mini Wright Peak Flow Meter on a sample of 38 participants, comprising 18 men and 20 women.

Results : Statistical analysis using Pearson's parametric correlation test revealed a significant relationship between SBCT and PEFR ($p < 0.05$), with a moderate positive correlation observed ($r = 0.516$).

Conclusion : This research demonstrates a significant, moderate positive correlation between SBCT and PEFR in adult post-COVID-19 patients, indicating that higher SBCT values correspond to higher PEFR measurements.

Keywords : Post COVID-19, Single Breath Count Test (SBCT), Peak Expiratory Flow Rate (PEFR).

INTRODUCTION

On March 11, 2020, the World Health Organization (WHO) announced that Coronavirus Disease 2019 (COVID-19) had become a global pandemic.[1] The data from the distribution map of confirmed COVID-19 cases in Indonesia indicate that the highest prevalence is observed among adults aged 31 to 45.[2] Various studies have demonstrated that the lungs are the organs most significantly affected by COVID-19.3 A systematic review and meta-analysis by Torres *et al*. identified various pulmonary function impairments in patients recovering from COVID-19. Notably, diffusion capacity abnormalities were observed in 39% of patients, while restrictive and obstructive patterns were found in 15% and 7% of cases, respectively.[3] The limited use of the gold standard for inspiratory function testing during the pandemic has hindered the objective assessment of these pulmonary function impairments. This limitation arises from the need to prevent airborne transmission, as procedures capable of generating droplets or aerosols in suspected COVID-19 patients, referred to as aerosol-generating procedures (AGPs), have been restricted.[4] Therefore, there is a need for a simple bedside examination that can accurately measure respiratory function without the use of equipment and with minimal potential for airborne transmission.[5]

The Single Breath Count Test (SBCT) serves as an alternative method for assessing respiratory function, offering several advantages. It is cost-effective, easy for patients to perform, and does not require the use of a mouthpiece. Consequently, it eliminates the risk of equipment contamination or the transmission of infectious diseases.[6] The SBCT has been shown to exhibit a strong correlation with forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1) values obtained from spirometry, as well as with Peak Expiratory Flow Rate (PEFR) measurements.[7] This study was conducted to investigate whether there is a correlation between the results of the SBCT and PEFR values in adult patients following COVID-19 infection.

METHODS

This study employed a cross-sectional design to evaluate the SBCT and PEFR in adult patients who had recovered from COVID-19. Subject recruitment was conducted at Dr. Cipto Mangunkusumo General Hospital and Persahabatan General Hospital between June 2023 and July 2023. Data were collected using consecutive sampling after receiving ethical clearance from the Ethics Commission of the Faculty of Medicine, University of Indonesia, and the Ethics Commission of Persahabatan General Hospital Number 32/KEPK-RSUPP/03/2023. The study included adult participants diagnosed with COVID-19 who had recovered, as well as controls from RSUPN Cipto Mangunkusumo and RSUP Persahabatan, provided they met the inclusion criteria, did not meet the exclusion criteria, and consented to participate by signing an informed consent form. Sampling continued until the required sample size was achieved.

The inclusion criteria for participation were: age between 18 and 60 years, a history of COVID-19 infection confirmed by PCR swab testing more than six months prior, classification as having mild, moderate, or severe symptoms during the infection, and fulfillment of recovery criteria, including completion of isolation and issuance of a monitoring completion statement (verified through healthcare facility records or the PeduliLindungi application). Recovery was confirmed by a physician's assessment at the healthcare facility or by the attending specialist. Additionally, participants were required to test negative for COVID-19 antigen swabs within 24 hours prior to the study, provide informed consent, and demonstrate normal cognitive function (MoCA-INA score ≥ 26).

Exclusion criteria encompassed individuals unable to comprehend or follow research instructions, those with contraindications for peak expiratory flow rate testing (e.g., myocardial infarction within the past week, abdominal or thoracic surgery within the past month, head surgery within the past three months, pneumothorax within the past two weeks, hemoptysis, respiratory tract infections, or dementia), and those undergoing active tuberculosis treatment. Individuals with a history of severe or unstable chronic obstructive pulmonary disease (COPD), severe or uncontrolled asthma, uncontrolled chronic cardiorespiratory conditions, or a Brinkman smoking index >600 were also excluded.

The researchers provided participants with detailed explanations of the study's objectives and potential benefits. Willing participants signed an informed consent form, and the researchers completed a research status form based on anamnesis and physical examination. Cognitive assessments using the MoCA-INA and COVID-19 antigen swab tests were performed to confirm eligibility. Subsequently, SBCT and PEFR measurements were conducted on eligible participants. All collected data were subjected to statistical analysis.

Data analysis was performed using IBM SPSS (Statistical Package for the Social Sciences) for Apple, version 20.0. The analysis included univariate, bivariate, and multivariate approaches. Univariate analysis was used to describe the characteristics of the studied variables and their distribution patterns. Bivariate analysis assessed the relationship between independent and dependent variables. The normality of data distribution was evaluated using the Kolmogorov-Smirnov or Shapiro-Wilk test, with normality assumed if $p > 0.05$. For numeric variables, Pearson correlation was applied to normally distributed data, while Spearman

correlation was used for non-normal distributions. When the independent variable was categorical and the dependent variable numeric, an independent t-test was used for normally distributed data, and the Mann-Whitney U test was applied for non-normal distributions. Statistical significance was defined as $p < 0.05$.

Multivariate analysis was conducted to identify factors most strongly associated with the dependent variable. Variables with $p < 0.25$ in the bivariate analysis were selected and further analyzed for their association with PEFR values. Results were considered statistically significant if $p < 0.05$. Hypothesis testing included significance values (p), correlation strength (r), and confidence intervals (CI). A significance level of < 0.05 and a 95% confidence interval were applied for hypothesis testing.

RESULTS

The study included 38 participants, 18 males (47.4%) and

TABLE 1
Characteristics of the Study Subjects

Variable		Frequency
Age (years), median		37.16 (23–57)
Sex, n (%)	Male	18 (47.4)
	Female	20 (52.6)
Body weight (kg), median		67.5 (48–107)
Body height (cm), median		162 (153–182)
Body mass index (kg/m^2), n (%)	Underweight	1 (2.6)
	Normal	11 (28.9)
	Overweight	8 (21.1)
Risk of Obesity	Obesity I	11 (28.9)
	Obesity II	7 (18.4)
Education level, n (%)	Senior high school	17 (44.7)
	Undergraduate	21 (55.3)
Job, n (%)	Laborer/employee/staff	37 (97.4)
	Family worker/volunteer worker	1 (2.6)
Annual household income, n (%)	High	37 (97.4)
	Low	1 (2.6)
Physical activity, n (%)	Light	32 (84.2)
	Moderate	6 (15.8)
The onset of COVID-19, n (%)	6 months – 1 year	8 (21.1)
	1 year – 2 years	22 (57.9)
	>2 year	8 (21.1)
Severity of COVID-19 infection, n (%)	Low	32 (84.2)
	Moderate	6 (15.8)
Brinkman Index, n (%)	Non-smoker	30 (78.9)
	Mild smoker	6 (15.8)
	Heavy smoker	2 (5.3)
Comorbid, n (%)	Hypertension	2 (5.3)
	None	36 (94.7)

TABLE 2
Results of SBCT Measurements

Variable		Frequency
Sex	Male	50 (±11)
	Female	40 (±11)
The onset of COVID-19	6 months – 1 year	47 (±13)
	1 year – 2 years	44 (±12)
	>2 years	44 (±10)
Severity of COVID-19 infection	Mild	45 (±11)
	Severe	44 (±15)
Brinkman Index	Non-smoker	–
	Mild smoker	43 (±11)
	Heavy smoker	52 (±14)
	Physical activity	47 (±14)
	Light	45 (±12)
	Moderate	43 (±14)

TABLE 3
Results of PEFR Measurements

Variable		PEFR (L/minute) (n=38)
Sex	Male	507.22 (±87.84)
	Female	372.50 (±67.81)
The onset of COVID-19	6 months – 1 year	412.50 (±95.43)
	1 year – 2 years	440.00 (±109.63)
	>2 years	450.00 (±98.56)
Severity of COVID-19 infection	Mild	433.75 (±94.49)
	Moderate	450.00 (±149.93)
Brinkman Index	Non-smoker	–
	Mild smoker	413.00 (±92.48)
	Heavy smoker	533.33 (±112.01)
	Physical activity	495.00 (±7.07)
	Light	435.62 (±104.20)
	Moderate	440.00 (±103.73)

20 females (52.6%). The demographic characteristics of subjects are summarized in Table 1.

The mean SBCT measurement for male subjects was reported to be 50, with the lowest value recorded at 27 and the highest at 70. For female subjects, the mean SBCT measurement was observed to be 40, with the lowest and highest values being 22 and 60, respectively. Regarding the time since COVID-19 onset, the group with an onset of 6 months to 1 year demonstrated a higher mean SBCT value (47) compared to other groups. In terms of disease severity, the mean SBCT values for mild and moderate COVID-19 cases were found to be similar, with

TABLE 4
Correlation between SBCT and PEFR

Variable	Mean (SB)	Correlation Coefficient (r)	*p-value*
SBCT	44.97 (±11.87)	0.516	0.001
PEFR	436.32 (±102.73)		

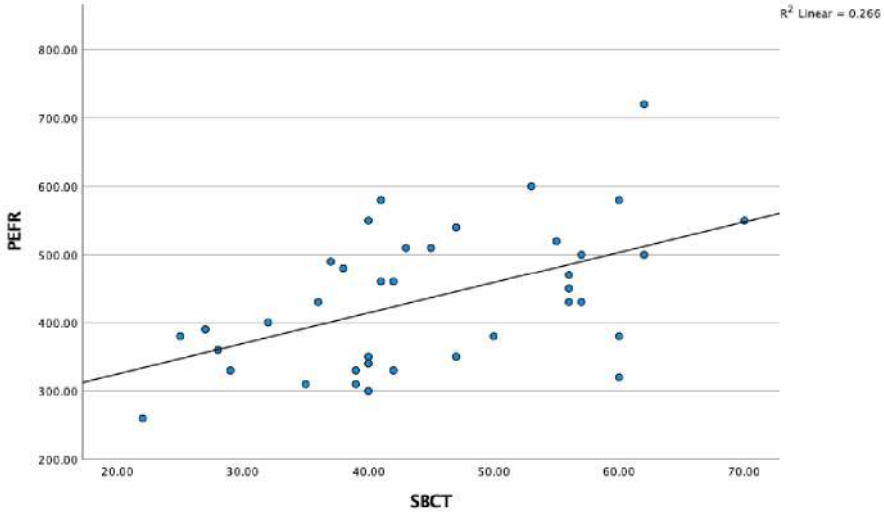

Figure 1. Correlation between SBCT and PEFR

averages of 45 and 44, respectively. When categorized by the Brinkman Index, the highest mean SBCT value was observed in the light smoker group, with a mean of 52. In relation to physical activity levels, the mean SBCT value for the light physical activity group was reported to be 45, while for the moderate physical activity group, it was slightly lower at 43. The SBCT measurement results are summarized in Table 2.

The mean PEFR for male subjects was reported to be 507.22 L/min, with the minimum and maximum values recorded as 350 L/min and 720 L/min, respectively. For female subjects, the mean PEFR was observed to be 372.5 L/min, with the lowest and highest values being 260 L/min and 520 L/min, respectively. In terms of the time since COVID-19 onset, the group with an onset exceeding two years was found to have a higher mean PEFR value (450 L/min) compared to other groups. Regarding the severity of COVID-19, the moderate severity group demonstrated a slightly higher mean PEFR (450 L/min) compared to the mild severity group. When analyzed by the Brinkman Index, the highest mean PEFR was observed in the light smoker category, with a value of 533 L/min. Concerning physical activity levels, the mean PEFR for the light physical activity group was nearly identical to that of the moderate physical activity group, at 435 L/min and 440 L/min, respectively.

The Pearson parametric correlation analysis conducted between SBCT and PEFR revealed a statistically significant relationship between the two variables ($p < 0.05$). This suggests that higher SBCT values are associated with higher PEFR values, demonstrating a moderate positive correlation (r = 0.516).

DISCUSSION

This study identified a statistically significant moderate positive correlation between the Single Breath Count Test (SBCT) and Peak Expiratory Flow Rate (PEFR) in adult patients recovering from COVID-19 (r = 0.516, $p < 0.05$), indicating that as SBCT values increased, PEFR values also tended to increase. Notably, male patients demonstrated higher mean SBCT and PEFR values compared to females, which may reflect sex-based differences in lung function and post-infection recovery.

These findings are consistent with previous studies. Bhandare *et al*. reported a stronger correlation (r = 0.7048) between SBCT and PEFR in healthy adults, suggesting a robust association. Bartfield *et al*. also found SBCT to be moderately correlated with both PEFR (r = 0.68) and FEV□ (r = 0.68), proposing it as a viable alternative to traditional spirometry. Escossio *et al*. demonstrated high sensitivity (94.44%) and specificity

(76.62%) of SBCT in hospitalized patients, while also noting excellent intra-rater reliability (ICC = 0.976). Other studies by Ali *et al*., Kalita *et al*., and Elsheikh *et al*. have further validated SBCT's utility across a variety of respiratory conditions, including asthma, Guillain-Barré Syndrome, and myasthenia gravis, showing moderate to strong correlations with various spirometry indices and respiratory measures.

This study enhances the evidence supporting SBCT as a simple, safe, and effective approach to evaluate ventilatory capacity. Its benefits include ease of use, cost-effectiveness, and low risk of cross-infection, which is especially pertinent in post-pandemic clinical environments. Additionally, it doesn't necessitate specialized equipment and is generally well-received by patients, making it particularly appropriate for resource-limited settings or during outbreaks.

Notably, this is the first study to investigate SBCT in adult patients recovering from COVID-19, providing new insights into post-viral pulmonary evaluation. A significant strength of the methodology was the consistent administration of both tests by the same examiner, which minimized variability.

The Limitation of this study is distribution of sex among smokers and non-smokers was not even, potentially introducing bias. Moreover, the number of underweight participants was small, and a handful of subjects initially had trouble with the testing technique; however, all completed the assessments after receiving guidance.

Overall, these findings reinforce the role of SBCT as a practical screening tool for post-COVID-19 pulmonary function and highlight the need for further studies to explore its applicability across broader patient populations and pulmonary conditions.

CONCLUSION

A moderate positive correlation was found between SBCT and PEFR in post-COVID-19 adults, with higher values observed in male patients. SBCT offers a safe, simple, and practical method for lung function assessment in post-pandemic care. Further studies are needed to validate its use across broader pulmonary conditions.

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Original Article

The Association between Monocyte-derived Macrophages TNF-α Expression and Anthropometrics Measures in Polycystic Ovary Syndrome

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Abstract

Background : Polycystic ovary syndrome (PCOS) is a female infertility disease that is often associated with low-grade chronic inflammation. This inflammation was suspected to correlate with certain body conditions, especially physical lipid composition that can be obtained under anthropometrics measurements. Monocytes and macrophages of PCOS patients were more susceptible to inflammation and contributed to this condition. Tumor Necrosis Factor-alpha (TNF-α), as one of the major inflammatory cytokines, holds an important role in the chronic inflammation of PCOS. This study aimed to evaluate the association of inflammatory cellular levels TNF-α in macrophage cells of women diagnosed with PCOS with physical anthropometrics.
Methods : Twenty PCOS women and ten healthy women as the control group were recruited in this study. Anthropometric data were measured. Peripheral blood was collected, mononuclear cells were isolated, and then cultured. Monocytes were selected and differentiated into macrophages. Macrophages were then exposed to oxidized Low-Density Lipoprotein (ox-LDL) and underwent flow cytometry to examine the level of TNF-α. Anthropometric data and TNF-α level were analyzed using Spearman-Rho Correlation test followed by a linear regression test.
Results : TNF-α expression was found to be statistically correlated with Body Mass Index (BMI), waist circumference (WC), waist-to-height ratio, visceral fat level, and muscle mass of arm (MMA) (p<0.05). However, only WC and MMA gave significant impact to macrophage TNF-α expression based on linear regression association test with equation -96.971 + 0.601*waist circumference + 2.665*MMA.
Conclusion : TNF-α was strongly associated with waist circumference and muscle mass percentage of arm in PCOS cases.

Keywords : Anthropometrics; Low-Grade Chronic Inflammation; PCOS; TNF-α.

INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is a multifactorial endocrine disorder that affects fertility in reproductive age women. The prevalence of women with PCOS in the world varies, which can reach 7–15%.[1] Based on the Rotterdam criteria, the occurrence of anovulatory disorders, the appearance of cysts in the ovarian morphology and hyperandrogenism both clinically and in biochemistry were three main symptoms in PCOS.[2] PCOS also has clinical implications from the accumulation of fatty tissue, such as type 2 diabetes, insulin resistance, and obesity.[1–3]

Several studies that have been conducted showed a link between cystic ovarian syndrome and low-grade chronic inflammation.[3–6] These studies found that there is not only a correlation but also a cause-and-effect relationship between chronic inflammation, obesity, and hyperandrogenism, which are the clinical manifestations of PCOS. Several inflammatory mediators, such as C Reactive Protein (CRP), Interleukin 1-β (IL-1β), Interleukin 6 (IL-6), and Tumor Necrosis Factor-α (TNF-α), were found to have higher levels in women with PCOS symptoms.[5] However, the chronic inflammation that occurs often only releases significant and permanent pro-inflammatory cytokines, but in low amounts that are difficult to detect in the blood circulation system.[7]

The exact pathophysiology and pathomechanism of the causal interaction between chronic low-grade inflammation and PCOS still need to be fully understood. Obesity and increased fat levels are thought to be one of the primary triggers for this condition.[8] An increase in fat levels will cause adipocyte cells in adipose tissue to increase size to accommodate and compensate for increased fat levels. The event of increasing the size of adipocyte cells is called hypertrophy.[9] When experiencing hypertrophy, adipocyte cells will experience hypoxia and release Nuclear Factor kappa B (NF-κB), which causes adipocytes to become more susceptible to inflammation, cell death by apoptosis mechanism, and release more fatty acids. The release of NF-κB, a transcription factor, will also cause an increase expression in MCP1 (Monocyte Chemoattractant Protein-1).[4,10]

MCP1 is a chemokine that attracts the chemotaxis of monocytes, neutrophils, and lymphocytes. MCP1 is also the main factor that regulates the migration and infiltration of monocytes from the endothelial tissue of blood vessels.[11] Increased MCP1 expression in PCOS cases causes the recruitment of more monocytes in adipose tissue. Meanwhile, immune cells in PCOS women, including monocytes, are thought to be more susceptible to inflammation and secrete more significant amounts of inflammatory cytokines.[12,13]

PCOS is strongly associated with obesity and therefore is also often linked to adipose tissue dysfunction. About 30–60% of PCOS women showed obesity characteristics, which could lead to metabolic complications.[9] Obesity is a surplus condition of energy uptake compared to metabolic needs. This condition might cause an imbalance in body fat and adipose tissue distribution inside the body. Adipose tissue is a dynamic organ that secretes adipokines, chemical molecules secreted by adipose, such as adiponectin, visfatin, and TNF-α.[8]

TNF-α is a member of the TNF superfamily.[14] This protein plays a vital role in inflammation reactions. Related to PCOS, TNF-α might induce high androgen secretion while decreasing SHBG (Sex Hormones Binding Globulin), thus manifesting hyperandrogenism. TNF-α is also suggested to have an association with obesity, infertility implications in PCOS, and other metabolic syndromes.[14,15]

Numerous studies found that there might be a mutual effect of obesity and increased inflammation condition in women suffering from PCOS. However, the exact pathomechanism is not fully understood. Fat and adipose tissue distribution might differ between lean, overweight, and obese individuals. Fat accumulation in certain parts of the body might cause some complications. This study wanted to examine the association between inflammation occurred in PCOS women through the level of TNF-α correlated with body fat distribution and presentation. TNF-α would be measured from monocyte cells as it was suspected that monocytes and macrophages in PCOS women were susceptible to inflammation compared to healthy women.

METHODS

Study design and subjects

This was a cross-sectional study. Samples were collected between February to May 2024 at Human Reproduction, Infertility, and Family Planning (HRIFP) Research Centre, IMERI FM UI. Twenty PCOS women were drawn as the case group, and ten healthy women were included as a control group. Purposive sampling technique was done under several inclusion criteria: Suspected PCOS and fulfilled 2 out of 3 Rotterdam criteria, irregular menstrual cycle, not a pregnant nor breast-feeding woman, in reproductive age (18–35 years old) and consented to participate in the research. Samples failed to blood collection, under PCOS medical treatment, or diagnosed with Atherosclerosis or type 2 Diabetes would be excluded. Subjects fasted for 8–10 hours prior to blood collection to obtain fasting glucose levels. After filling out the consent form, subjects were measured for their demographic and anthropometric data, and peripheral blood collection was performed. Seven milliliters of blood were collected in an anti-coagulant heparin tube. This study has passed ethical approval from The Ethics

TABLE 1
Anthropometric measurements and mean levels of TNF-α expression

Parameter	PCOS (n=20)	Control (n=10)	*p* value
Age (years)	31.1 ± 4.6	27.5 ± 2.1	0.053
Body height (m)	1.5 ± 0.1	1.6 ± 0.1	0.012*
Body weight (kg)	68.3 ± 14.1	54.7 ± 5.1	0.819
BMI (kg/m^2)	27.7 ± 5.2	22.3 ± 0.6	0.000*
Waist circumference (cm)	89.7 ± 10.3	78.4 ± 3.7	0.009*
Hip circumference (cm)	103.3 ± 10.4	95.7 ± 4.1	0.000*
Waist to Hip Ratio	0.87 ± 0.0	0.82 ± 0.0	0.993
Waist to Height Ratio	0.57 ± 0.0	0.50 ± 0.0	0.009*
Whole body fat (%)	34.4 ± 4.5	31.3 ± 2.0	0.011*
Resting metabolism (kcal)	1356 ± 187.4	1120 ± 92.5	0.091
Visceral fat level (cm^2)	8.2 ± 4.1	4.1 ± 0.9	0.000*
Whole body subcutaneous fat (WBSF) (%)	31.2 ± 5.3	26.5 ± 1.7	0.001*
Subcutaneous fat of Trunk (SCFT) (%)	27.1 ± 5.1	21.4 ± 1.8	0.000*
Subcutaneous fat of Arm (SCFA) (%)	47.5 ± 6.0	44.0 ± 2.5	0.000*
Subcutaneous fat of Leg (SCFL) (%)	44.1 ± 7.2	39.3 ± 2.4	0.001*
Whole body muscle mass (WMM) (%)	39.2 ± 2.4	24.1 ± 1.8	0.037*
Muscle mass of Trunk (MMT) (%)	18.2 ± 2.1	19.4 ± 0.8	0.000*
Muscle mass of Arm (MMA) (%)	24.0 ± 4.3	27.5 ± 1.3	0.000*
Muscle mass of Leg (MML) (%)	37.2 ± 1.8	37.3 ± 1.2	0.163
TNF-α Expression (%)	4.4 (1.0–27.7)	0.5 (0.1–3.1)	0.000*

Data are expressed as mean ± SD or median (minimum–maximum).
*p<0.05 significant difference among the groups using independent T-test for normally distributed data and Mann-Whitney test for non-normally distributed data.

Committee of the Faculty of Medicine, Universitas Indonesia-Cipto Mangunkusumo Hospital with approval number KET-205/UN.2.F1/ETIK/PPM.00.02/2024.

Demographic and anthropometric data collections

Subjects who agreed to participate in the study were measured for demographic data and body composition analysis. This analysis was performed using Omron Karada Scan (Production Facility Omron Dalian Co., Ltd., Shanghai, China). Fat body composition, basal metabolic rate, visceral fat, and waist-hip circumference were obtained from the measurements. Fat body composition measured in this study were Whole Body Fat Percentage (WFP), Whole Body Subcutaneous Fat (WBSF), Subcutaneous Fat of Arm (SCFA), Subcutaneous Fat of Trunk (SCFT), Subcutaneous Fat of Leg (SCFL), Whole Body Muscle Mass (WMM), Muscle Mass of Arm (MMA), Muscle Mass of Trunk (MMT), and Muscle Mass of Leg (MML). This study also measured subjects' body weight and height then converted them to Body Mass Index (BMI). The waist and hip circumferences were also measured and calculated using the waist-to-hip and waist-to-height ratios.

Macrophages culture

Eight milliliters of peripheral blood was collected in a heparin tube. White blood cells containing monocytes were separated from other blood components using Ficoll-Paque (Cytiva, Uppsala, Sweden) gradient centrifugation. Isolated monocytes from buffy coat were then cultured using 1 mL complete medium (RPMI [Sigma Aldrich, MO, USA], FBS 10% [Gibco, PRF, USA], Pen-Strep 1% [Gibco, NJ, USA], Amphotericin-B 1%

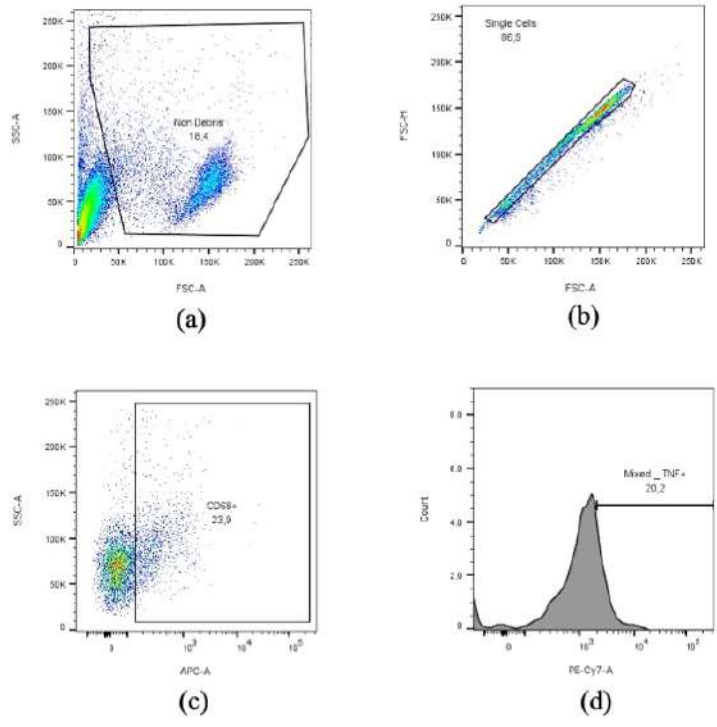

Figure 1. Flow cytometry to measure the levels of monocyte-derived macrophages TNF-α expression. (a) Non-Debris selection. (b) Single cells selection. (c) Macrophage selection with CD68+. (d) TNF-α expression.

[Gibco, NJ, USA]) under the influence of 50 ng/ml M-CSF growth factor (Sigma Aldrich, MO, USA) for 2 x 72 hours to differentiate into M0 macrophages. Suspected monocyte-derived macrophage was then exposed to oxidized LDL (Invitrogen, OR, USA) for 24 hours. After 24 hours, the macrophages were harvested using Trypsin EDTA (Sigma Aldrich, MO, USA) for 2 x 3 minutes.

TNF-α flow cytometry measurement

Harvested culture cells were washed using PBS (Gibco, NJ, USA) to remove the remaining culture media. After the wash, the cells were stained with allophycocyanin (APC)-conjugated CD-68 monoclonal surface antibody (Thermo Scientific, MA, USA) diluted in stain buffer to distinguish macrophage cells. Staining was then continued with phycoeritrin-Cyanine7 (PE-Cy7)-conjugated TNF-α monoclonal intracellular antibody (Thermo Scientific, MA, USA) staining to mark the TNF-α protein. Cell samples were then read using a BD FACS Canto II Flow Cytometry instrument (BD Biosciences, NJ, USA).

Statisical analysis

Statistical analysis in this study was performed with SPSS version 26 software. All tests were considered as two-sided, and a *p-value* < 0.05 was considered statistically significant. Variables were summarized as mean ± SD or median (minimum–maximum), according to the normality of data. The obtained TNF-α data was then tested for correlation with anthropometric data. Parameters that were proven to be statistically correlated were then tested with linear regression to determine the parameters that had the most influence on TNF-α.

RESULTS

In this study, we collected twenty women diagnosed with PCOS and ten healthy subjects as a control group. TNF-α expression from monocyte-derived macrophages with CD68+ was also examined using BD FACS Canto II Flow Cytometry system (Figure 1). TNF-α and other anthropometric variables showed significant differences between PCOS group and the control group, except for age, body weight, waist-to-hip ratio, and Muscle Mass of leg (MML) (Table 1).

The Spearman test revealed statistical correlations between TNF-α expression to BMI, waist circumference, waist-to-height ratio, visceral fat level, and muscle mass of arm (MMA) (Table 2). The five correlations gave *p-values* under 0.05 with r 0.431, 0.459, 0.451, 0.431 and -0,271 respectively. From the correlation test, the statistic continued with multivariate linear regression to evaluate which factor(s) influence the change of TNF-α expression.

Multivariate linear regression analysis was carried out using the backward method. In this test, TNF-α acted as the dependent variable. In contrast, the independent

TABLE 2
Correlation analysis summary of TNF-α expression as a dependent variable with anthropometrics data

TNF-α Expression to	r	*p* value
Age	0.101	0.952
Body weight	0.368	0.064
Body height	0.001	0.981
BMI	0.431	0.019*
Waist circumference	0.459	0.012*
Hip circumference	0.311	0.111
Waist-to-hip ratio	0.363	0.631
Waist-to-height ratio	0.451	0.010*
Whole body fat	0.219	0.262
Resting metabolism	0.073	0.337
Visceral fat	0.431	0.021*
Whole body subcutaneous fat (WBSF)	0.350	0.073
Subcutaneous fat of Trunk (SCFT)	0.297	0.119
Subcutaneous fat of Arm (SCFA)	0.222	0.274
Subcutaneous fat of Leg (SCFL)	0.271	0.155
Whole body muscle mass (WMM)	-0.110	0.560
Muscle mass of Trunk (MMT)	-0.238	0.222
Muscle mass of Arm (MMA)	-0.271	0.043*
Muscle mass of Leg (MML)	0.069	0.721

*$p<0.05$ significant correlation Spearman test

variables were 11 factors that yielded p values under 0.25 in the Spearman test (Table 2), including body weight, BMI, waist and hip circumference, waist-to-height ratio, visceral fat level, whole body subcutaneous fat, Subcutaneous Fat of Trunk (SCFT), Subcutaneous Fat of Leg (SCFL), Muscle Mass of Trunk (MMT), and Muscle Mass of Arm (MMA). The regression test showed two variables significantly associated with TNF-α: waist circumference and MMA (Table 3). Thus, the linear regression equation for this model to predict TNF-α expression was -96.971 + 0.601*waist circumference + 2.665*MMA with *p value* 0.02. This equation can describe macrophage's TNF-α expression in 16.4%.

DISCUSSION

TNF-α is a cytokine that holds a significant role in inflammation and acts as a pro-inflammatory messenger.[15] TNF-α, dominantly produced by monocytes and macrophages, regulates the transcription of other proteins, such as other cytokines, growth factors, and cell adhesion molecules.[16] Although its famous role as an inflammatory cytokine, TNF-α was not significantly higher in circulating serum of women with PCOS when compared to those of healthy women, conflicting with their low-grade chronic inflammation.[14]

This research sought to assess the expression of TNF-α in cellular macrophages among women with PCOS compared to healthy ones. Statistical tests revealed that TNF-α expression of monocyte-derived macrophage was higher in PCOS women. This result proved that at cellular levels, TNF-α as an inflammatory cytokine was produced higher by the immune cells, in this case, macrophage cells. This finding might explain chronic low-grade inflammatory conditions in women suffering from PCOS that were unable to be detected in circulating serum. A past study stated that the different levels of TNF-α expression might also be caused by a polymorphism in the gene promoter. The presence of GGCCT haplotype in the promoter region changed the susceptibility of the macrophages and monocytes that produce more inflammatory cytokines than anti-inflammatory mediators. This GGCCT haplotype was found to have a higher frequency in PCOS women,

TABLE 3
A multiple linear regression analysis utilizing the backward method was conducted to examine TNF-α expression as the dependent variable, with independent variables including weight, BMI, waist circumference, hip circumference, waist-to-height ratio, visceral fat, total body subcutaneous fat, SCFT, SCFL, MMT, and MMA.

Model	Unstandardized coefficients		Standardized coefficients	t	*p* value
	B	Standard error	Beta		
Weight	-0.311	0.267	-0.701	-1.133	0.274
BMI	0.501	0.373	0.412	1.356	0.182
Waist circumference	0.608	0.231	1.022	2.701	0.010*
Hip circumference	0.243	0.519	0.381	0.472	0.647
Waist-to-height ratio	-66.751	705.172	-0.669	-0.111	0.932
Visceral fat	0.393	1.952	0.253	0.203	0.861
Whole-body subcutaneous fat	0.663	1.551	0.537	0.421	0.672
Subcutaneous fat of Trunk (SCFT)	-0.267	4.631	-0.239	-0.061	0.951
Subcutaneous fat of Arm (SCFA)	-0.419	0.828	-0.461	-0.509	0.623
Muscle mass of Trunk (MMT)	-1.701	3.710	-0.459	-0.462	0.649
Muscle mass of Arm (MMA)	2.672	1.149	0.882	2.331	0.277*

contributing to inflammatory conditions.[14]

TNF-α cytokine produced by immune cells suppresses the expression of SHBG (Sex Hormone Binding Globulin) protein, thus raising the level of free androgen in the circulating system.[11,13] This hyperandrogenism might worsen clinical and fertility issues in PCOS women. TNF-α is also associated with insulin resistance among PCOS, one suspected culprit in PCOS pathophysiology. Monitoring the levels of TNF-α could help predict the risk of inflammatory condition in PCOS.[10]

The rise of macrophage TNF-α expression is subject to which factors affect this condition in PCOS women. The linear regression analysis performed in this study indicated that two variables are significantly correlated with the expression of TNF-α: waist circumference (WC) and muscle mass of arm (MMA). In concordance with this result, Pedersen (2019) also stated that higher WC is associated with low-grade inflammation.[17] Training and physical activity are known to reduce body fat and WC.[17,18] This reduction in WC was then found to have a positive correlation with decreasing TNF-α levels.[17] Another previous research also stated that fatty acid levels correlate positively with the level of TNF-α circulating in the blood system.[19]

MMA was negatively correlated with TNF-α level, meaning that the level of MMA percentage counteracts TNF-α. Decreasing the percentage of MMA was usually followed by increasing the fat level.[18] Another past study might explain the association between TNF-α and the percentage of muscle mass of the arm (MMA). This research indicated that macrophages are more likely to exhibit pro-inflammatory behavior and less anti-inflammatory behavior in individuals with excess weight and body fat.[11] Fatty acid that was released in lipolysis interact with inflammation by altered methylation level of certain cytokine genes. This article also stated that macrophages from obesity patients still respond in a pro-inflammatory way even after being taken out from an obesity environment.[11]

Macrophages, the most abundant immune cells, are essential in preventing infections and maintaining tissue homeostasis.[20,21] In healthy women, the number of macrophages is balanced between pro- and anti-inflammatory cells to prevent inflammatory conditions. Meanwhile, the number of pro-inflammatory macrophages increases and releases more inflammatory cytokines in PCOS women, thus inflicting low-grade chronic inflammatory conditions.[22] The secretion of TNF-α by macrophages also influences the insulin signaling pathway, leading to the development of insulin resistance. The reciprocal and causal relationship between PCOS and low-grade inflammation makes determining the exact pathophysiology challenging.[22,23]

This study did not establish a correlation between TNF-α and other significant anthropometric variables;

however, these findings represent progress toward personalized medicine and the creation of a treatment model tailored to individual patients. The results regarding TNF-α expression may indicate additional approaches for managing and monitoring the inflammatory aspects associated with polycystic ovary syndrome. This research is also constrained by the criteria used for sample grouping. Further study should be subjected to BMI grouping, considering fat and obesity might vary the inflammatory condition. Moreover, grouping control and PCOS women based on their BMI categories might yield different results, considering that PCOS is a metabolic disorder.

CONCLUSION

This study revealed that TNF-α expression in monocyte-derived macrophage was statistically associated with waist circumference and muscle mass of arm with regression equation as follows: -96.971 + 0.601*waist circumference + 2.665*MMA.

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Original Article

The Effect of Smoking Habits on Decreased Liver Function in Active Smokers Aged 20–50 Years old

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Abstract

Background : AA study was conducted to determine the effect of smoking habits on decreased liver function in active smokers aged 20–50 years. This study was motivated by Indonesia's high smoking prevalence and its associated liver-related health issues. The liver is an organ that plays a role in the body's metabolism. Smoking triggers the formation of free radicals, causing a decrease in liver function and inflammation.
Methods : In this study, mix method design was used with active smokers as the smoker group and the control group was respondents not active or passive smokers. Groups are categorized again based on age, ie 20–30, 31–40, and 41–50. The study population is the academic community of the Hermina Health Institute Jatinegara Campus and the community living on Kapitan III Road Tapos District, Depok City. Liver function is evaluated through enzymes SGPT, SGOT, ALP, and CRP as parameters for inflammation. To evaluate the influence of smoking habits and lifestyle factors on liver function risk, an analysis of variance (ANOVA) followed by post-hoc testing and linear regression analysis were performed.
Results : Laboratory results showed that mean levels of ALT, AST, ALP, and CRP were higher in the smoker group compared to controls across all age categories, although most values remained within normal limits. ANOVA revealed statistically significant differences in all biomarkers (p<0.05), and post-hoc tests identified specific age-related group differences. Smokers' habit data indicated that most smokers began smoking between the ages of 1520 and had smoked for over five years. Lifestyle analysis showed a higher prevalence of insufficient physical activity, reduced sleep duration, and higher BMI among smokers. CLDQ scores for both groups generally reported minimal emotional disturbance and no signs of advanced liver disease.
Conclusion : Smoking is associated with elevated liver enzymes (ALT, AST, ALP) and CRP levels, indicating subclinical hepatic and inflammatory alterations. These biochemical changes correlate with lifestyle risk factors and diminished quality of life. Early screening and lifestyle modification are recommended to prevent long-term hepatic damage among smokers.

Keywords : Active smokers, inflammatory event, liver enzymes, productive age, smoking

INTRODUCTION

A wide array of health complications has been linked to habitual tobacco use. According to the World Health Organization (WHO), as of 2023, tobacco smoking contributes to approximately 8 million deaths globally each year, with 1.3 million of these attributed to secondhand smoke exposure.[1] In Indonesia, WHO data from 2020 reported an estimated 225,700 deaths annually due to smoking and other tobacco-related illnesses. Furthermore, the prevalence of active smoking among Indonesian adolescents aged 1019 has shown an alarming increase, rising from 7.2% in 2013 to 9.1% in 2018.[2] The unregulated sale of cigarettes, particularly the lack of age restrictions, significantly hampers tobacco control efforts and exacerbates public health risks.

Cigarettes contain a multitude of toxic compounds, including tar, nicotine, and carbon monoxide, which adversely affect both active smokers and those exposed to secondhand smoke.[3] Additionally, the presence of heavy metals in cigarettes––whether traditional or electronic––poses a substantial threat to human health, primarily through the induction of oxidative stress and systemic inflammation.[4] Cigarette smoke exposure triggers cellular inflammation, which, if unmitigated, may progress to chronic conditions resulting in metabolic syndromes and extensive organ damage.[5]

A 2021 study by Premkumar and Anand highlighted that tobacco consumption can accelerate the oxidation of Nicotinamide Adenine Dinucleotide Phosphate (NADPH), disrupting the body's antioxidant defense mechanisms and promoting free radical formation. This oxidative imbalance contributes to hepatocyte inflammation, cellular injury, fibrotic mediator proliferation, and hepatic iron accumulation. Smoking has also been linked to vascular constriction, endothelial dysfunction, tissue hypoxia, and hepatocellular damage.[6]

Moreover, tobacco smoke is a rich source of free radicals and heavy metals, which provoke oxidative stress and persistent inflammation.[7] Chronic exposure to these radicals significantly elevates the risk of organ impairment.[6] The liver, as a central organ in detoxification and immune response, is particularly vulnerable. Prolonged smoking leads to the accumulation of reactive oxygen species (ROS), which stimulates the production of pro-inflammatory cytokines such as IL-6 and TNF-α. These cytokines subsequently induce hepatic synthesis of C-reactive protein (CRP), resulting in elevated systemic CRP levels. Persistent inflammation, if left uncontrolled, can compromise hepatic function, as reflected in the increased serum levels of liver enzymes including ALT, AST, and ALP.[8,9]

The progression from inflammation to hepatic injury is closely associated with the cumulative effect of oxidative stress, which is influenced by both the quantity of cigarettes consumed daily and the duration of smoking. In addition to smoking, several lifestyle-related risk factors contribute to liver dysfunction, such as frequent consumption of high-fat foods, alcohol intake, physical inactivity, and inadequate rest. These behaviors are often linked to elevated body mass index (BMI), which in turn heightens the risk of hepatic steatosis, non-alcoholic fatty liver disease (NAFLD), and cirrhosis.[10,11]

Although previous studies have explored the association between smoking and the elevation of inflammatory biomarkers and liver enzymes, comprehensive analyses examining the interplay between smoking and additional risk factors remain scarce. The present study aims to investigate the cumulative impact of smoking habitsincluding smoking duration, frequency, and the age at smoking initiationon hepatic function. It further considers non-smoking lifestyle factors such as alcohol use, sleep duration, and physical activity. Participants in this study are restricted to adults aged 2050 years to specifically evaluate the effects of smoking within the productive age range. Individuals over 50 were excluded to minimize confounding factors associated with age-related organ function decline.

The working hypothesis of this study posits that predefined risk factors––particularly active smoking––are significantly associated with impaired hepatic function, as evidenced by elevated inflammatory biomarkers and liver enzyme levels in the smoker group compared to non-smokers.

METHODS

Research Design and Variables

This study employed a mixed-methods approach, integrating both qualitative and quantitative methodologies within an experimental research design to investigate the causal relationship between independent and dependent variables using liver and inflammation biomarker, and we conducted an investigation into lifestyle habits using a questionnaire filled out by means of direct interviews with all respondents. The independent variables comprised smoking-related behaviors––including active smoking status, age at smoking initiation, smoking duration, and the number of cigarettes consumed per day––as well as lifestyle factors such as alcohol consumption, physical activity, sleep duration, and body mass index (BMI). The dependent variables were selected biomarkers indicative of hepatic function and systemic inflammation: C-reactive protein (CRP), alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP).

Participants were divided into two groups: the control group, consisting of individuals who neither

smoked nor lived in environments with active smokers, and the experimental group, consisting of active smokers observed for this study. Confidentiality and anonymity of all respondents were strictly maintained; no names, initials, or complete addresses were recorded or disclosed.

The study sample included individuals aged 20 to 50 years, further classified into three age-based subgroups for comparative analysis: Group 1 (20–30 years), Group 2 (31–40 years), and Group 3 (41–50 years). These categories were designed to evaluate potential age-related differences in the impact of smoking and associated lifestyle factors on liver function biomarkers.

Population and Sampling

Sampling for this study was conducted using a purposive sampling technique, based on the following inclusion criteria:

1. Age between 20 and 50 years.
2. Non-obese individuals.
3. Free from comorbid conditions such as hepatitis, cirrhosis, or liver cancer.
4. Control group: Non-smokers who do not live with or are not regularly exposed to active smokers.
5. Experimental group: Active smokers who have been smoking for at least one year.

The sample size was calculated using Slovin's formula

$$n = \frac{N}{1 + N(e)^2}$$

Where n is the required sample size, N is the population size, and e is the margin of error. With a 10% margin of error and a total population of 1,200 across the two study sites, the minimum sample size was determined to be 92. This number was increased to 102 participants to account for potential deviations associated with the selected error tolerance.

A 10% margin of error was chosen due to the large population size and the difficulty in finding control participants who are completely free from passive smoke exposure (i.e., not exposed to cigarette smoke for more than one hour per day). This level of tolerance is considered acceptable, as the potential variance between the sample and the population is expected to be minimal.

To obtain data on respondents' smoking habits and lifestyle factors, both structured interviews and questionnaire administration were conducted. The questionnaire comprised 14 items on lifestyle history and 29 items related to chronic liver disease, based on the *Chronic Liver Disease Questionnaire* (CLDQ). Reliability testing of the questionnaire was performed using Cronbach's Alpha, and validity was assessed via Pearson's correlation test

Research Location and Duration

The study was conducted over a one-month period from May to June 2024, across two research sites: Babakan Village, Tapos District, Depok City, and the Hermina Health Institute campus. Ethical clearance for the study was obtained from the Ethics Review Board of Binawan University, with approval number 120/KEPK-UBN/VI/2024.

Blood sample collection was performed immediately following participant interviews and questionnaire completion. Blood was drawn into plain evacuated tubes and stored in a sample collection box before being transported to the laboratory for serum preparation. Biochemical analysis of serum levels––specifically SGPT (ALT), SGOT (AST), ALP, and CRP––was conducted at the Clinical Pathology Laboratory of the Hermina Health Institute, Jatinegara Campus.

Data Analysis

The data analysis procedure began with reliability and validity testing of the questionnaire. Normality and homogeneity of the data were assessed using the Kolmogorov-Smirnov and Levene's tests, respectively. To evaluate the influence of smoking habits and lifestyle factors on liver function risk, analysis of variance (ANOVA) followed by post-hoc testing and linear regression analysis were conducted using IBM SPSS version 26.0. These tests aimed to determine statistically significant differences between independent variables and liver function biomarkers. To estimate the probability and strength of association between risk factors and hepatic impairment, linear regression analysis was employed.

RESULTS

This research employed a mixed-methods approach, incorporating both qualitative and quantitative data collection techniques. Primary data was obtained through direct interviews, self-administered questionnaires, and blood specimen collection, which were subsequently analyzed to assess hepatic function and inflammatory biomarkers.

Participants were restricted to individuals aged 20 to 50 years, with no gender-based exclusion criteria applied. The total respondents in this study were 102 people consisting of 50 control respondents spread across 16 people in the 20–30 year group, 17 people in the 31–40 year group, and 17 people in the 41–50 year group. While the total respondents in the smoker group were 52 people consisting of 19 people in the 20–30 year group, 17 people in the 30–40 year group, and 16 people in the 41–50 year group.

TABLE 1
Laboratory Results of Liver Function Parameters and Inflammatory Marker by Age Group

Age Category	Control		Smoker	
	Mean	SD	Mean	SD
ALT (normal value : ≤ 34 U/L)*				
20–30 years old	15.94	6.81	25.42	8.88
31–40 years old	20.18	8.38	33.18	6.82
41–50 years old	22.76	8.35	35↑	8.62
AST (normal value : ≤ 31 U/L)*				
20–30 years old	16.19	16.19	16.19	16.19
31–40 years old	7.53	7.53	7.53	7.53
41–50 years old	16.59	16.59	16.59	16.59
ALP (normal value: ≤ 240 U/L)*				
20–30 years old	156.88	156.88	156.88	156.88
31–40 years old	51.55	51.55	51.55	51.55
41–50 years old	140.24	140.24	140.24	140.24
CRP (normal value : ≤ 3 mg/L)*				
20–30 years old	4.86↑	1.58↑	8.71↑	4.78↑
31–40 years old	4.69↑	2.00↑	6.29↑	1.68↑
41–50 years old	11.02↑	7.27↑	9.51↑	4.42↑

Table 1 presents the biochemical results, including liver function parameters––alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)––as well as the inflammatory biomarker C-reactive protein (CRP).

Alanine aminotransferase (ALT or SGPT) and its isoenzyme aspartate aminotransferase (AST or SGOT) are key enzymes used as primary biomarkers of liver cell damage. They catalyze the transfer of amino groups, playing a vital role in metabolism and ATP production. Normally, small amounts circulate in the blood, but elevated levels indicate hepatocyte injury. [12,13]

Alkaline phosphatase (ALP) is a zinc metalloenzyme produced mainly in bile canaliculi and other tissues like bone and placenta. Increased ALP levels signal cholestasis or bile duct damage, though higher levels are also normal during bone growth in children and adolescents. [12,13]

C-reactive protein (CRP) is an acute-phase inflammatory marker synthesized by hepatocytes. Although CRP is a sensitive indicator of systemic inflammation, it is not organ-specific, as its elevation may result from various inflammatory processes, not solely liver injury. Inflammatory stimuli such as tissue damage activate pro-inflammatory cytokinesIL-6, IL-1β, and TNF-α which in turn stimulate hepatic CRP production. CRP levels typically begin to rise within 6 hours of inflammation and may remain elevated for up to 48 hours, making it a reliable and easily detectable biomarker. [14]

As shown in Table 1, levels of ALT, AST, ALP, and CRP were higher in smokers compared to non-smokers, though most values remained within normal clinical ranges. These findings suggest a potential impact of smoking on liver function and inflammation; however, further analysis is needed to determine whether smoking is the primary cause or if other contributing factors are involved.

In addition to biochemical testing, this study conducted interviews and administered the Chronic Liver Disease Questionnaire (CLDQ) to assess smoking habits, lifestyle factors, and health-related quality of life in individuals with potential liver impairment. Results from the interviews are presented in Tables 2 and 3.

Tables 2 and 3 present the results of interviews conducted with respondents. It is important to note that the reliability of these findings is highly dependent on the honesty of the participants. As shown in Table 3, the

TABLE 2
Smoking Habit Data of the Smoker Group

Categories	Age group		
	20–30 years	31–40 years	41–50 years
Smoking habits	19	17	16
There are family members who smoke	19	17	16
Being in a smoker's environment	19	17	16
Cigarettes smoked per day			
1–10 sticks	12	9	1
11–20 sticks	7	6	5
> 20 sticks	0	2	10
Duration of smoking			
< 1 year	3	0	0
1–5 years	14	0	0
> 5 years	2	17	16
Age of first smoking			
< 15 years old	2	7	3
15–0 years old	17	10	13
> 20 years old	0	0	0

majority of respondents in the smoker group reported having smoked for more than five years. Most of them smoke between 1 to 10 cigarettes per day, and the majority began smoking between the ages of 15 and 20.

Table 3 displays the interview data regarding lifestyle habits that may affect liver function. Behaviors such as smoking, consuming high-calorie foods, lack of physical activity, and insufficient rest can lead to an increase in Body Mass Index (BMI) and elevate the risk of liver disorders, including hepatic steatosis, Non-Alcoholic Fatty Liver Disease (NAFLD), and cirrhosis.[10,11]

In addition to the interviews, this study also utilized a questionnaire instrument based on the Chronic Liver Disease Questionnaire (CLDQ), the results of which are presented in Table 3.

The CLDQ (Chronic Liver Disease Questionnaire) results were subjected to a validation test, which demonstrated that the correlation coefficient (r-calculated) ranged from 0.746 to 0.968, exceeding the critical r-value of 0.162 for a sample size of 102 respondents. Additionally, the *p-value* was less than 0.05, indicating that the CLDQ results are valid. Reliability testing, as presented in Table 3, showed a reliability coefficient greater than 0.8, which reflects a high degree of reliability.

The CLDQ is an instrument designed to assess the quality of life in patients with chronic liver disease, aiming to understand how the condition affects physical, emotional, and social aspects of the patient's life. It consists of 29 questions and utilizes a 7-point Likert scale: 1 = all of the time, 2 = most of the time, 3 = a good bit of the time, 4 = some of the time, 5 = a little of the time, 6 = hardly ever, and 7 = never.

Responses to the CLDQ from the two respondent groups indicate that both the control and smoker groups had an average score of 5 in the abdominal symptoms' domain, suggesting that both groups occasionally experienced abdominal discomfort. However, abdominal symptoms do not necessarily indicate liver dysfunction, and therefore, an analysis of lifestyle habits in both groups is warranted.

In the fatigue domain, differences emerged between the groups. The control group had average responses of 4 and 5, indicating they did not frequently experience debilitating fatigue. In contrast, the smoker group predominantly responded with a score of 3, suggesting frequent fatigue and daytime drowsiness. The majority also reported general weakness and low energy. This level of fatigue likely impacts daily physical activity. For both groups, the average response in the activity domain was 5, suggesting occasional difficulty in performing daily physical tasks.

TABLE 3
Lifestyle factor parameters and Chronic Liver Disease Questionnaire (CLDQ) Analysis

Lifestyle Factor parameters	
Alcohol Consumption	• Most participants in both groups never consumed alcohol. • Smoker group had slightly more individuals with rare alcohol consumption (≥3 months).
Exercise Activites	• Control group: Majority exercise 13x/month or never. • Smoker group: More frequent weekly exercise in younger age group; older groups exercised less or never.
Sleep Duration	• Most participants sleep 5–7 hours/day. • Few participants in either group sleep 8–10 hours/day or <5 hours/day
Body Mass Index (BMI)	• Majority in both groups are within **normal** BMI. • More **overweight** individuals in older age groups, especially in the smoker group.
Chronic Liver Disease Questionnaire (CLDQ) Analysis	
Abdominal Symptoms (α = 0.903)	Slightly higher symptom scores in smokers for bloating, pain, and discomfort.
Fatigue (α = 0.972)	• Control group showed higher mean values for strength and energy. • Smokers reported more drowsiness and tiredness.
Systemic Symptoms (α = 0.926)	• Control group had more dry mouth, itching, and shortness of breath. • Smokers had slightly lower scores on average.
Activity (α = 0.789)	• Control group generally had better activity scores. • Smokers had more trouble with lifting or carrying heavy objects.
Emotional Function (α = 0.932)	• Smokers reported higher levels of irritability and depression. • Control group showed slightly better emotional well-being.
Worry (α = 0.963)	Control group reported more intense worry in all items related to disease progression, family, treatment cost, and transplant.

TABLE 4
Results of ANOVA and Post-Hoc ANOVA Tests

Test	**Parameters**	***P-Value***
ANOVA	ALT	0.000
	AST	0.000
	ALP	0.000
	CRP	0.000

Beyond abdominal symptoms, the CLDQ also evaluates systemic symptoms such as body pain, muscle cramps, dry mouth, and itching. The control group generally reported minimal systemic symptoms, with average scores between 5 and 6. However, among smokers, dry mouth was the most commonly reported symptom.

Additional questions addressed emotional well-being and anxiety levels. Both the control and smoker groups showed similar response patterns, with average scores of 5, indicating that levels of depression and anxiety remained within a manageable range. Overall, no respondents from either group reported symptoms consistent with chronic liver disease.

The statistical test used to determine the effect of smoking habits on liver function and the occurrence of inflammation was a parametric ANOVA with post-hoc analysis. This test was selected based on the results of the normality and homogeneity tests, both of which yielded *p-values* greater than 0.05, indicating that the data met the assumptions required for parametric testing. The results of the ANOVA post-hoc analysis are presented in Table 4.

The results of the ANOVA test indicated significant differences among all groups for all

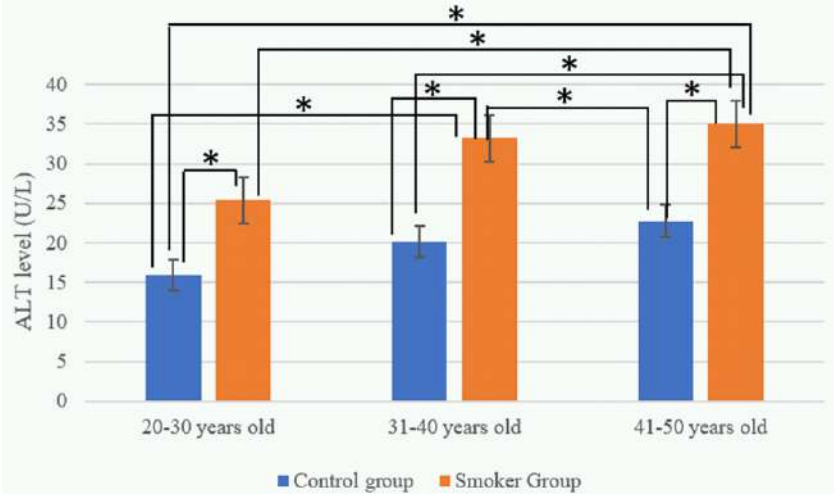

Figure 1. The ANOVA post-hoc analysis for ALT levels (U/L) among the groups (*) indicates a statistically significant difference with $p < 0.05$

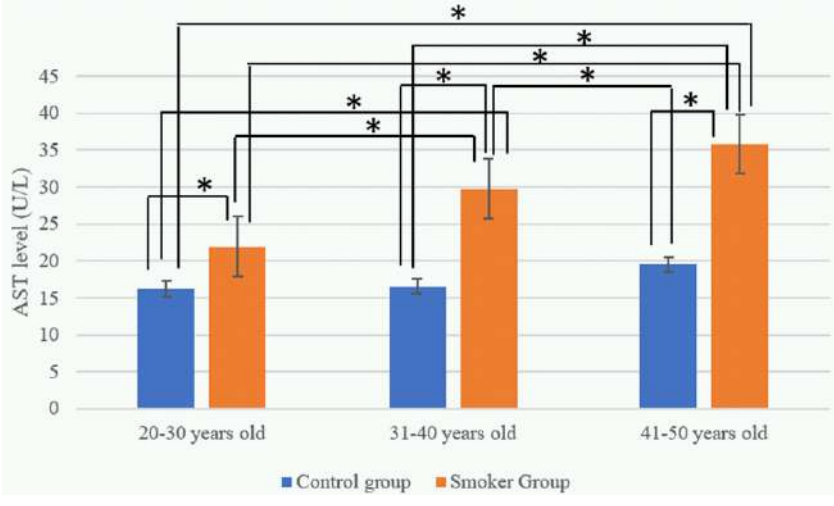

Figure 2. The ANOVA post-hoc analysis for AST levels (U/L) among the groups (*) indicates a statistically significant difference with $p < 0.05$

independent variables, namely ALT, AST, ALP, and CRP. To identify which specific group pairs exhibited the most significant differences, a follow-up post-hoc ANOVA test was conducted, as shown in Table 4. Significant differences in ALT values were observed between all control and smoker groups across all age categories. Similarly, AST values also showed significant differences between control and smoker groups in every age category.

Table 1 presents data indicating that the smoker groups had higher mean AST and ALT levels compared to the control groups. Although these mean values still fall within normal ranges, several smoker respondents exhibited elevated AST and ALT levels. This finding necessitates further testing and analysis of daily lifestyle habits to determine whether these elevated values may serve as early markers of future liver function impairment.

In addition to AST and ALT, ALP levels were also elevated in the smoker groups compared to controls. Post-hoc ANOVA analysis revealed that significant differences in ALP values varied by age. The most pronounced differences were observed between smokers aged 31–40 and 41–50 years and controls aged 31–40 years. The impact of smoking appears statistically significant beginning at age 31, while smokers aged 20–30 did not differ significantly from any other group, likely due to shorter smoking duration.

Smoking habits were also associated with inflammation, as indicated by the CRP parameter across various age groups in both cohorts. Plasma CRP concentrations increased notably in individuals aged 31

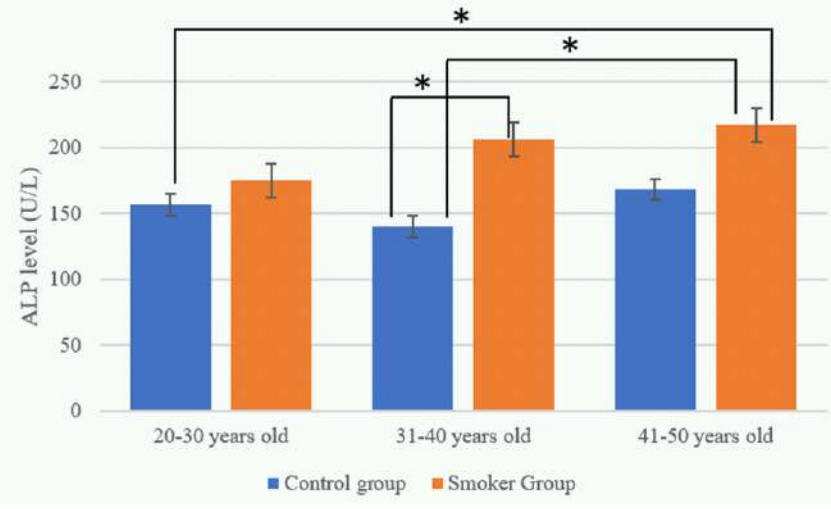

Figure 3. The ANOVA post-hoc analysis for ALP levels (U/L) among the groups (*) indicates a statistically significant difference with $p< 0.05$

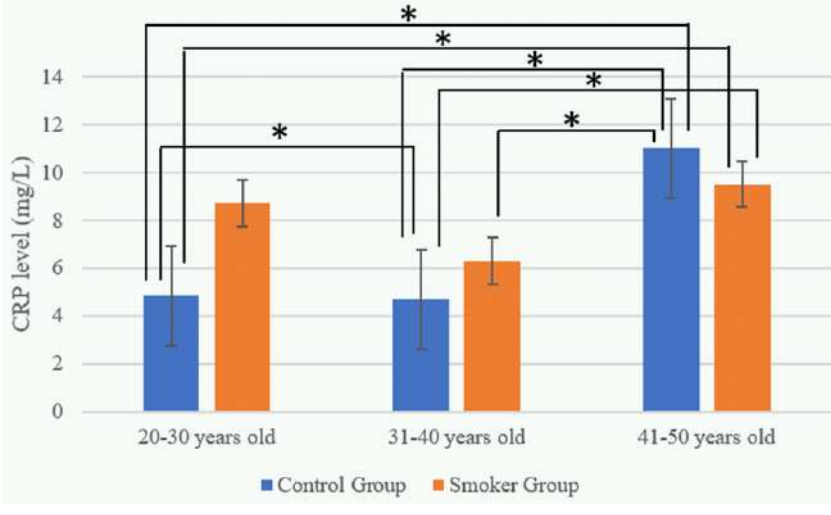

Figure 4. The ANOVA post-hoc analysis for CRP levels (mg/L) among the groups (*) indicates a statistically significant difference with $p< 0.05$

to 50 years and showed significant differences compared to other age categories in both groups. While inflammation can be triggered by multiple factors beyond smoking, smoking reduces immune defenses, making the body more susceptible to antigen or free radical exposure, which induces inflammation. The cumulative effect of long-term smoking and a high number of cigarettes smoked daily contributes to increased inflammation.

The parallel increase in AST, ALT, and ALP levels between ages 31 and 50 corresponds with elevated CRP levels in the same age range, serving as a warning signal for potential future liver function deterioration. The relationship between age ranges across groups and liver function and inflammation parameters was further analyzed using linear regression, the results of which are presented in Table 5.

Table 5 presents a summary of the linear regression results, indicating the strength of the correlation between age range and the concentrations of ALT, AST, ALP, and CRP, which are 0.652, 0.714, 0.397, and 0.259, respectively. These values demonstrate a positive correlation, as all coefficients approach 1, meaning that as age increases, the risk of liver function impairment also rises.

Additionally, Table 5 shows the coefficient of determination (r^2), which quantifies the proportion of variance in ALT, AST, ALP, and CRP values explained by age range. The strongest influence of age range was observed on ALT and AST levels, with coefficients of determination of 42.5% and 51%, respectively. This increase suggests that long-term smoking elevates the

TABLE 5
Correlation Between Age Range and Biomarkers of Liver Function and Inflammation

Parameter	Significance	Correlation value	Coefficient of determination (r square)	F count	T table
ALT	0.000	0.652	42.5%	74	0.6769
AST	0.000	0.714	51%	104	
ALP	0.000	0.397	15.8%	18.73	
CRP	0.009	0.259	6.7%	7.20	

risk of liver function damage, as indicated by rising ALT and AST levels with advancing age, particularly among smokers compared to controls (Table 1).

The relationship between age and ALP and CRP levels was less pronounced, with coefficients of determination of 15.8% and 6.7%, respectively. This implies that each increase in age corresponds to a modest increase in ALP and CRP values by these percentages.

Overall, the duration and frequency of smoking, as represented by the age range of smokers, show a strong correlation with increased ALT and AST values, indicating a gradual hepatocellular injury. ALT and AST are enzymes directly released into the bloodstream when hepatocyte damage or inflammation occurs. Conversely, ALP and CRP levels may remain normal or only slightly elevated because liver damage has not yet progressed to systemic inflammation or biliary obstruction.

Therefore, ALT and AST values can serve as important early biomarkers for monitoring potential liver function impairment in individuals who have smoked for prolonged periods.

DISCUSSION

This research analyzed the relationship between smoking and the decline in liver function as well as the occurrence of inflammation in the productive age group (20–50 years). The liver function parameters included ALT, AST, and ALP, along with the inflammatory marker CRP. The study compared these parameters and the incidence of inflammation between smoker and control groups, each subdivided by age categories of 20–30 years, 31–40 years, and 41–50 years.

Based on the results presented in Table 1, a trend of increasing liver function and inflammation parameters with advancing age was observed in both smokers and controls. The mean levels of ALT, AST, ALP, and CRP in the smoker group were consistently higher than those in the control group. Moreover, these parameters increased with age, as evidenced by the highest values observed in the 41–50 year age group. Thus, the data demonstrate an age-related increase in ALT, AST, ALP, and CRP levels, with significantly higher values in the smoking group. Although the majority of liver function values in the respondents remained within normal limits, the smoker subgroup aged 41–50 exceeded the normal range. Meanwhile, inflammatory parameters in both groups were elevated beyond normal thresholds. Comparative analyses between smoker and control groups using ANOVA and post hoc tests revealed significant differences in ALT and AST levels across all age categories, whereas ALP values did not significantly differ between groups. ALP levels in both groups remained within the normal range but tended to rise with age among smokers. For the inflammatory marker CRP, significant differences between smokers and controls emerged in the 31–40- and 41–50-year age groups.

The upward trend of liver function decline and inflammation in smokers with increasing age is suspected to be influenced by lifestyle habits within this group. As shown in Table 2, smokers frequently inhabit environments with other smokers. As age increases, the number of cigarettes smoked per day also rises (>20 cigarettes), along with longer smoking durations (>5 years). Most smokers began their habit during adolescence (ages 15–20). This indicates that increasing age, smoking environment, daily cigarette consumption, and smoking duration collectively contribute to the elevation of ALT, AST, ALP, and CRP levels. Hackshaw *et al*. demonstrated that even smoking a single cigarette per day carries health risks; light smokers (1–5 cigarettes daily) face up to a 65% increased risk of coronary heart disease and stroke, risks that escalate with greater cigarette consumption, especially among heavy smokers (20 cigarettes per day).[15] These findings support the higher liver function impairment and inflammation markers observed in smokers compared to controls. Smoking can disrupt liver function despite the liver not being in direct contact with smoke, as it serves as the organ responsible for the biotransformation of drugs, alcohol, and other harmful substances.[16]

Lifestyle factors also contribute to liver function decline and inflammation. Research by Nivukoski *et al*. revealed associations between unhealthy lifestyle

factors––such as alcohol consumption, smoking, overweight status, and insufficient physical activity––and abnormalities in laboratory tests for liver function, inflammation, and lipid profiles, including ALT, GGT, CRP, and lipid profiles.[17] Respondents' lifestyle habits were assessed via interviews and the Chronic Liver Disease Questionnaire (CLDQ) to clarify whether smoking or other lifestyle factors contributed to liver function decline and inflammation. The CLDQ was employed to evaluate the impact of chronic liver disease on respondents' quality of life.

Table 3 details lifestyle data in smokers and controls. Alcohol consumption (both frequent and occasional) was more prevalent among smokers, although most respondents in both groups reported no alcohol use. Consumption of small amounts of alcohol (1–4 times per day) is not associated with liver damage risk; however, heavy drinking increases the risk of liver damage such as cirrhosis. This is because the liver can regenerate and maintain function if alcohol intake is low, but excessive intake impairs liver function and leads to damage.[18]

Physical activity frequency of 1–3 times per month was more common in the control group than among smokers. More smokers aged 31–40 and 41–50 years reported no exercise, though routine physical activity (1–3 times per week) was more frequent in the 2030-year smoker group. This pattern likely influences liver function parameters, as lower ALT, AST, and ALP levels were observed in the younger smoker subgroup compared to older smokers. This aligns with findings from Hejazi and Hackett, who showed that exercise improves liver function in patients with non-alcoholic fatty liver disease (NAFLD), demonstrated by reductions in ALT and AST following aerobic and resistance training.[19]

Sleep duration in both groups was predominantly 5–7 hours per day, indicating suboptimal rest. Prior studies have associated short sleep duration (<5 hours) with NAFLD incidence, though 5–7 hours has not been linked to significant liver damage.[20] The study also examined body mass index (BMI) to liver function and inflammation parameters, finding a correlation between obesity and elevated ALT and AST levels.[21] More smokers were overweight compared to controls, although most respondents in both groups had normal BMI.

The findings confirm that smoking adversely impacts the liver, evidenced by elevated ALT, AST, and ALP levels in smokers. This corroborates research by Fathima and Kalyanikutty, who reported that smoking increases liver function parameters.[22] Cigarette smoke contains free radicals such as nicotine, tar, and carbon monoxide, which induce oxidative stress and reduce antioxidant defenses, causing elevated ALT and AST in the blood. Prolonged exposure to free radicals and tobacco constituents progressively increases hepatocyte damage risk.[23,24]

Prekumar *et al*. demonstrated a positive association between smoking and elevated ALP levels; however, ALP elevation may also reflect other conditions such as active growth, osteoporosis, and renal impairment, making it a sensitive but nonspecific marker.[25] Correspondingly, Table 1 shows the greatest increases in ALT, AST, and ALP in the 41–50-year age group. As cumulative cigarette consumption rises with age, risk factors for osteoporosis and kidney disease also increase.[26,27]

Regarding inflammation, Table 1 indicates significant CRP differences between smokers and controls only in the 31–40- and 41–50-year age groups. Both groups' CRP levels exceeded normal thresholds, reflecting CRP's nature as a sensitive but nonspecific marker that can rise due to infection, rheumatoid arthritis (RA), cardiovascular diseases, trauma, and progressive cancer.[28] Elevated CRP in the control group likely results from factors other than smoking or liver dysfunction.

Smoking impairs the liver through three mechanisms: toxic, immunologic, and oncogenic pathways.[29] The direct toxic effect involves oxidative stress induced by smoke components, leading to hepatic fibrosis. Smoking increases proinflammatory cytokines such as interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor α, resulting in liver injury. Indirect toxic effects include secondary polycythemia––elevated carboxyhemoglobin due to reduced oxygen-carrying capacity––stimulating erythropoietin production and red blood cell count. Increased hemolysis releases iron, which accumulates in hepatocytes via macrophage uptake, promoting oxidative stress and liver damage.

Immunologically, nicotine suppresses lymphocyte proliferation and differentiation, decreases antibody production, induces lymphocyte apoptosis, increases cytotoxic CD8+ T cells, decreases CD4+ T cells, and impairs natural killer (NK) cell activity. Oncogenic effects stem from carcinogens in cigarettes, such as hydrocarbons, nitrosamines, tar, and vinyl chloride. The compound 4-aminobiphenyl in tobacco elevates hepatocellular carcinoma risk. Smoking also downregulates tumor suppressor genes like p53, facilitating neoplasm development.[29,30] Active smoking is linked to non-alcoholic fatty liver disease (NAFLD), fibrosis, and hepatocellular carcinoma.[31]

In this study, ALT and AST levels in both groups remained within normal ranges except for smokers aged 41–50, whose values exceeded normal limits. ALP levels in both groups stayed within normal ranges. This suggests that liver damage in smokers aged 41–50 likely affecting hepatocytes but not bile ducts. ALT and AST are markers of hepatocyte damage and cell death, while ALP elevation indicates bile duct injury or obstruction.[32] Elevated CRP levels beyond normal in both groups imply

ongoing inflammation potentially triggered by infections, autoimmune disease, obesity, or lifestyle factors such as insufficient sleep, stress, poor diet, and medication use.[33] CLDQ questionnaire results (Table 3) showed no significant differences between groups, indicating that chronic liver disease symptoms were not clinically apparent in either group.

The combination of aging, smoking habits, and unhealthy lifestyle factors contributes to increased liver function impairment and inflammation parameters. Significant elevations in ALT, AST, ALP, and CRP were first observed in the 31–40 age group compared to controls. Respondents aged 31–40 and 41–50 had longer smoking durations (>5 years) and earlier smoking initiation (under 15 years). These groups also reported reduced physical activity and more overweight prevalence. Therefore, the rise in ALT, AST, ALP, and CRP in smokers relative to controls likely reflects smoking, age, and lifestyle factors. Healthy behaviors such as not drinking alcohol, regular exercise, sufficient sleep, and normal body weight may help maintain liver function and inflammatory parameters within normal ranges. This is further supported by CLDQ data showing no deterioration in quality of life from liver disease among respondents.

CONCLUSION

This study demonstrates a significant association between smoking and impaired liver function, as well as increased systemic inflammation, particularly in individuals aged 31–50 years. Smokers exhibited higher levels of ALT, AST, ALP, and CRP compared to non-smokers, with significant elevations emerging from the 31–40 age group onward. These findings suggest a cumulative effect of smoking over time, further influenced by lifestyle factors such as alcohol use, physical inactivity, and higher BMI.

Despite enzyme levels remaining mostly within normal ranges, elevated values in older smokers indicate potential subclinical hepatic injury. Smoking appears to contribute to liver dysfunction via oxidative stress, immune modulation, and inflammatory pathways. While CLDQ scores showed no significant impact on perceived quality of life, the biochemical changes highlight the importance of early intervention. Promoting healthy lifestyles and smoking cessation may help to prevent progressive liver damage and systemic inflammation

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Original Article

Optimization Axial T2WI Lumbar MRI in Spinal Stenosis: Effects of GRAPPA Acceleration Factor on Image Quality and Anatomy

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Abstract

Background : Patients with lumbar spinal stenosis (LSS) who struggle to lie down for long periods may encounter issues during lumbar MRI exams. GRAPPA, a parallel imaging method to speed up MRI scans, can reduce the Signal to Noise Ratio (SNR), affecting image quality and anatomical information. This study aims to find the best GRAPPA acceleration factor by assessing its effect on image quality and anatomical information.
Methods : This study involved scans on 10 Lumbar MRI patients with LSS cases. The scans were performed using a Siemens Magnetom Aera 1.5 Tesla MRI machine with T2WI TSE axial cut. Each patient underwent 4 treatments with acceleration factors of 1 (without GRAPPA acceleration factor), 2, 3, and 4. Image quality was analysed using ROI to obtain SNR and CNR values. The radiologist assessed the anatomical information on the images. The analysis included a one-way ANOVA and the Kruskal-Wallis test was performed for image quality and anatomical information.
Results : The research found that the GRAPPA acceleration factor significantly affects image quality and anatomical information in axial T2WI TSE Lumbar MRI scans for patients with LSS (*p-value* < 0.01). A factor of 3 reduces examination time by 65.35% without significant differences ($p > 0.05$) in image quality and anatomical information.
Conclusion : The acceleration factor in axial T2WI TSE lumbar MRI significantly affects image quality and anatomical information for lumbar spinal stenosis cases. An acceleration factor of 3 is optimal for maintaining quality and anatomical information.

Keywords : GRAPPA acceleration factor; T2WI TSE; image quality; anatomical information

INTRODUCTION

Lumbar spinal stenosis (LSS) is the narrowing of the vertebral canal and/or intervertebral foramen, which leads to compression of the spinal cord or nerve roots, resulting in low back and leg pain.[1] Additionally, calcification or ossification of the posterior longitudinal ligament or ligamentum flavum, along with the development of intraspinal synovial cysts, may also contribute to spinal stenosis.[2] MRI is widely considered the gold standard for diagnosing LSS.[1] Lumbar MRI for diagnosis of patients with LSS cases using Sagittal T2WI and T1WI, along with Axial T2WI protocols.[3,4] Axial sections are important for determining the severity (grade) associated with clinical symptoms felt by patients with LSS.[5-7]

MRI poses a drawback due to its longer examination duration compared to conventional radiology or CT scans.[8] Prolonged MRI exams may cause patient discomfort and potential movements,[9] leading to artefacts in the images.[10] Parallel imaging offers a method to accelerate MRI data acquisition.[11]

GRAPPA is a widely used parallel imaging method aimed at accelerating acquisition time.[12] However, it does have certain drawbacks that can effect the Signal To Noise Ratio (SNR), Contrast to Noise Ratio (CNR), and scan time. Specifically, employing acceleration factor or R-factor values to speed up acquisition leads to a reduction in SNR due to a decrease in the number of k-space lines, consequently affecting the anatomical information captured by the image.[13]

The author observed that motion artefacts are more common in axial cuts because these cuts are generated at the end. This study aims to determine the best GRAPPA acceleration factor by evaluating its effect on the image quality and anatomical information in axial T2WI TSE lumbar MRI scans of Lumbar Spinal Stenosis cases.

METHODS

This research is a quantitative study employing an experimental approach. Data was gathered at Prof. Dr. R. Soeharso Orthopedic Hospital in Surakarta using a Siemens Magnetom Aera 1.5 T MRI and a standard spine coil. The study involved selecting 10 samples from the entire population of lumbar MRI images of patients with LSS. The sample images were obtained using axial T2WI TSE cuts without varying the GRAPPA acceleration factor and with acceleration factors of 2, 3, and 4.

Image quality is assessed using SNR and CNR which are sought by performing ROI (Region of interest) organ images with anatomy on the nucleus pulposus, ligamentum flavum, cerebrospinal liquid, and spinal cord. The SNR assessed is the SNR of the organ nucleus pulposus, ligamentum flavum, cerebrospinal fluid, and spinal cord. SNR was calculated using the formula:

$$\text{SNR} = \frac{\text{mean organ value}}{\text{standar deviation background noise value}}$$

The mean organ value is the average value of the organ points after performing ROI on the organ. The standard deviation value represents the signal noise in the background of the image, outside the organ.

CNR measurement involves calculating the difference in SNR at adjacent organ points using the following formula:

$$CNR = |SNR_1 - SNR_2|$$

SNR1 represents the SNR at organ point 1. SNR2 represents the SNR at organ point 2. The assessed CNRs are liquid cerebrospinal-ligamentum flavum, liquid cerebrospinal-medulla spinalis, and medulla spinalis-ligamentum flavum.

The anatomical information value of Axial Cut Lumbar MRI images with varying GRAPPA acceleration factors was evaluated by three radiologist. They assessed the clarity of the nucleus pulposus, ligamentum flavum, cerebrospinal fluid, and spinal cord using a scoring system (1=unclear, 2=less clear, 3=moderately clear, and 4=clear).

The data underwent analysis using the statistical software SPSS version 25. In determining the most optimal GRAPPA acceleration factor variation that closely aligns with non-accelerated image quality, one-way ANOVA and the Kruskal-Wallis test were performed for image quality and anatomical information, respectively. A p-value of less than 0.05 indicates statistically significant results. An analysis was performed to assess scan time based on the duration of image acquisition for each GRAPPA acceleration factor variation.

This research was carried out with a strong emphasis on respecting: a) Human dignity, b) The privacy and confidentiality of research subjects, c) Justice and inclusiveness, and d) The benefits and losses incurred. This research has undergone an ethical review and has complied with the ethical clearance letter bearing reference number IR.03.01/D.XXV.2.3/49/2024. The following statement was issued by the ethics team of Prof. Dr. R. Soeharso Surakarta Orthopedic Hospital.

RESULTS

The visible image features were reconstructed through different techniques, including standard (non-accelerated) methods and GRAPPA acceleration factors 2, 3, and 4, can be objectively observed and assessed based on signal intensity. Furthermore, radiologists can subjectively evaluate the contrast, sharpness, and

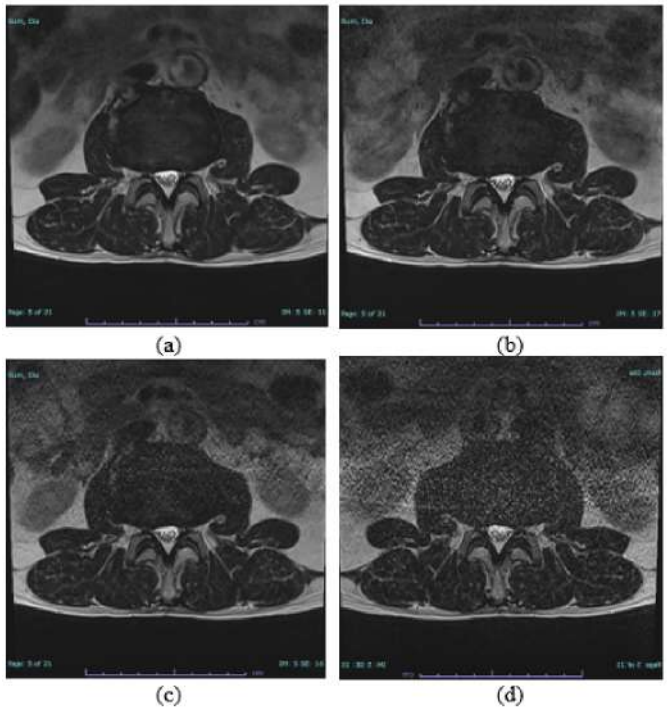

Figure 1. Image result of Lumbar MRI Axial cut T2WI TSE using GRAPPA Acceleration Factor variation (a) without GRAPPA (we designate this as AF 1) (b) GRAPPA AF 2 (c) GRAPPA AF 3 (d) GRAPPA AF 4

TABLE 1
Average SNR of each anatomical organ

GRAPPA variation	*Nucleus pulposus*	*Ligamentum flavum*	*Liquid cerebrospinal*	*Medulla spinalis*
AF 1	55.49098	24.49181	337.4425	172.9885
AF 2	35.85072	20.64401	230.652	130.2049
AF 3	40.87231	18.36493	213.62	116.5652
AF 4	25.52507	16.96186	104.7124	62.97111

intricate details of the resultant images. Figure 1 shows the resulting image in that particular variation.

Image Quality Assessment

Table 1 and 2 show the average SNR and CNR of 10 images in each organ with acceleration factor variation.

Table 2 and 3 show the results of the normality test for the overall SNR and CNR value of each axial T2WI TSE lumbar MRI image with GRAPPA acceleration factors of 1, 2, 3, and 4. *P-value* > 0.05 indicates the SNR and CNR data are normal.

A one-way ANOVA Post hoc test was conducted to determine which GRAPPA acceleration factor had the closest or almost the same quality as the variation without the GRAPPA acceleration factor (AF 1).

From Table 5 we can see that the values of AF (GRAPPA acceleration factor) 2 and 3 have a *p-value* > 0.05 against AF 1 (without GRAPPA acceleration factor) in each organ that we studied. This means that the image quality produced by AF 2 and 3 does not have a significant difference compared to the image quality produced by AF 1.

Anatomical Information Assessment

Anatomical information assessment was carried out with radiologists on the scoring system by comparing the image produced in every variation of the GRAPPA acceleration factor. Score 1 means unclear, 2 means less

TABLE 2
Average CNR in particular organ

GRAPPA Variation	*Liquid cerebrospinal-Ligamentum flavum*	*Liquid cerebrospinal-Medulla spinalis*	*Medulla spinalis-Ligamentum flavum*
AF 1	311.6867	164.454	147.2327
AF 2	209.9069	100.4471	109.4598
AF 3	195.545	97.05482	98.49021
AF 4	88.82571	41.74132	47.08439

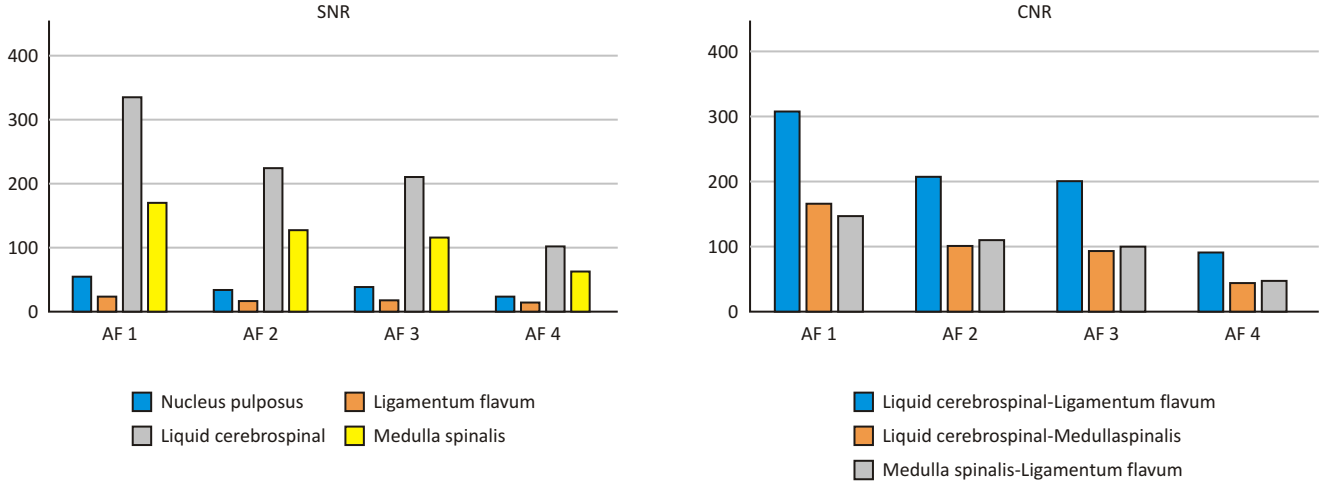

Figure 2. SNR and CNR graph with GRAPPA acceleration factor variation

clear, 3 means moderately clear, and 4 means clear. The total assessment of 10 images for every variation. This assessment can be seen in Table 6.

Table 7 shows the mean rank of anatomical information at every GRAPPA variation level, significantly decreasing with increasing GRAPPA acceleration factor. The correlation test (Table 8) reveals a strong and significant correlation between acceleration factors and anatomical information (*p-value* < 0.01). The data in Table 9 illustrates that the comparison of GRAPPA acceleration factors 2, 3, and 4 with those not employing the GRAPPA acceleration factor did not yield statistically significant differences, except for variation 4.

Scan Time Assessement

According to Table 10, the scan time is significantly slower when GRAPPA acceleration is not utilized than when the acceleration factor is employed; specifically, variation 4 produces the fastest results.

DISCUSSION

Any increase in the GRAPPA acceleration factor will cause a decrease in SNR.[11,13] This is due to the k-space used to make the image was decreased by an increased GRAPPA acceleration factor.[13]

Maulidya & Murniati's (2018) research indicated that the image quality remains optimal for GRAPPA acceleration factors 2 and 3, providing good diagnostic information quality.[14] However, variation with a GRAPPA acceleration factor of 4 results in poor image quality.[15] This is consistent with the findings of our study, where the post hoc test results show that the general SNR organ with a variation of GRAPPA acceleration factor 3 has a significance level >0.05 (*p-value* > 0.05) compared to the variation without GRAPPA acceleration factor. This indicates that the image quality of the variation with GRAPPA acceleration factor 3 is not significantly different from the image quality of the variation without GRAPPA acceleration factor. On the other hand, for the variation with GRAPPA acceleration factor 4, a

TABLE 3
SNR normality test results for each organ

SNR	AF Variation	Shapiro-Wilk	
		N	Sig.
Nucleus pulposus	1	10	0.153
	2	10	0.130
	3	10	0.081
	4	10	0.116
Ligamentum flavum	1	10	0.658
	2	10	0.079
	3	10	0.523
	4	10	0.094
Liquid cerebrospinal	1	10	0.504
	2	10	0.490
	3	10	0.412
	4	10	0.129
Medulla spinalis	1	10	0.329
	2	10	0.428
	3	10	0.083
	4	10	0.064

TABLE 4
CNR Normality Test Results

CNR	AF Variation	Shapiro-Wilk	
		N	Sig.
Liquid cerebrospinal-Ligamentum flavum	1	10	0.743
	2	10	0.846
	3	10	0.431
	4	10	0.161
Liquid cerebrospinal-Medulla spinalis	1	10	0.217
	2	10	0.072
	3	10	0.104
	4	10	0.291
Medulla spinalis-Ligamentum flavum	1	10	0.967
	2	10	0.831
	3	10	0.089
	4	10	0.436

TABLE 5
Post Hoc SNR

Organ	AF	AF	Sig.	Annotation
SNR Nucleus pulposus	1	2	0.151	Not significantly different
		3	0.383	Not significantly different
		4	0.011	Significantly different
	2	1	0.151	Not significantly different
		3	0.945	Not significantly different
		4	0.667	Not significantly different
	3	1	0.383	Not significantly different
		2	0.945	Not significantly different
		4	0.341	Not significantly different
	4	1	0.011	Significantly different
		2	0.667	Not significantly different
		3	0.341	Not significantly different
SNR Ligamentum flavum	1	2	0.557	Not significantly different
		3	0.199	Not significantly different
		4	0.062	Not significantly different
	2	1	0.557	Not significantly different
		3	0.895	Not significantly different
		4	0.582	Not significantly different
	3	1	0.199	Not significantly different
		2	0.895	Not significantly different
		4	0.939	Not significantly different
	4	1	0.062	Not significantly different
		2	0.582	Not significantly different
		3	0.939	Not significantly different
SNR Liquid cerebrospinal	1	2	0.003	Significantly different
		3	0.000	Significantly different
		4	0.000	Significantly different
	2	1	0.003	Significantly different
		3	0.927	Not significantly different
		4	0.000	Significantly different
	3	1	0.000	Significantly different
		2	0.927	Not significantly different
		4	0.002	Significantly different
	4	1	0.000	Significantly different
		2	0.000	Significantly different
		3	0.002	Significantly different

TABLE 5. Continued

Organ	AF	AF	Sig.	Annotation
SNR Medulla spinalis	1	2	0.210	Not significantly different
		3	0.058	Not significantly different
		4	0.000	Significantly different
	2	1	0.210	Not significantly different
		3	0.920	Not significantly different
		4	0.017	Significantly different
	3	1	0.058	Not significantly different
		2	0.920	Not significantly different
		4	0.078	Not significantly different
	4	1	0.000	Significantly different
		2	0.017	Significantly different
		3	0.078	Not significantly different

TABLE 6
Anatomical information assessment of MRI lumbal axial T2WI TSE on GRAPPA acceleration factor variation

AF Variation	Score	Total Assessment				Score Annotation
		Nucleus pulposus	*Ligamentum flavum*	*Liquid cerebrospinal*	*Medulla spinalsis*	
AF 1	1	0	0	0	0	1 = unclear
	2	0	0	1	0	2 = less clear
	3	0	9	6	8	3 = moderately clear
	4	10	1	3	2	4 = clear
AF 2	1	0	0	0	0	
	2	0	0	0	2	
	3	10	10	9	8	
	4	0	0	1	2	
AF 3	1	1	0	0	1	
	2	9	4	2	1	
	3	0	6	7	8	
	4	0	0	1	0	
AF 4	1	10	2	0	1	
	2	0	8	6	8	
	3	0	0	4	1	
	4	0	0	0	0	

TABLE 7
Friedman's rank based on anatomical information assessment

GPAPPA *acceleration factor*	Mean Rank
AF 1	3.45
AF 2	2.89
AF 3	2.36
AF 4	1.30

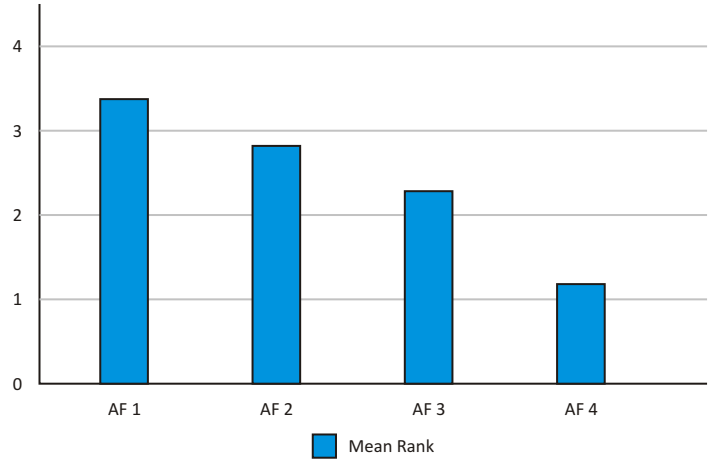

Figure 3. Mean rank graph

TABLE 8
Kendall's Tau correlation test results on variations in GRAPPA acceleration factor on anatomical information

GPAPPA *acceleration factor*	Sig. (2-tailed)	Annotation
AF vs Informasi Anatomi	0.000	Significant, Strong

TABLE 9
Pairwise comparison Kruskal Willis test result

Acceleration Factor	Sig.	Annotation
AF1 vs AF2	0.172	Not significantly different
AF1 vs AF3	0.173	Not significantly different
AF1 vs AF4	0.005	Significantly different

significance level < 0.05 (*p-value* < 0.05) was obtained, indicating a significantly different image quality compared to the image quality without GRAPPA acceleration factor. Based on the visual grading assessment using the Kruskal-Wallis test, it is evident that the GRAPPA acceleration factor 3 produces anatomical information comparable to the results obtained without GRAPPA acceleration factor variation.

In its application parallel imaging (GRAPPA) is used to reduce scan time.[13,16] Based on the findings of this study, employing a GRAPPA acceleration factor of 2 leads to a 44.74% reduction in scan time compared to the absence of GRAPPA acceleration (AF 1). Furthermore, utilizing a GRAPPA acceleration factor of 3 results in a

TABLE 10
Scan time on GRAPPA acceleration factor variation

Variation	*Scan time*	Reduction persentage (%)
AF 1	3.48	- %
AF 2	2.06	44.74%
AF 3	1.19	65.35%
AF 4	0.56	75.44%

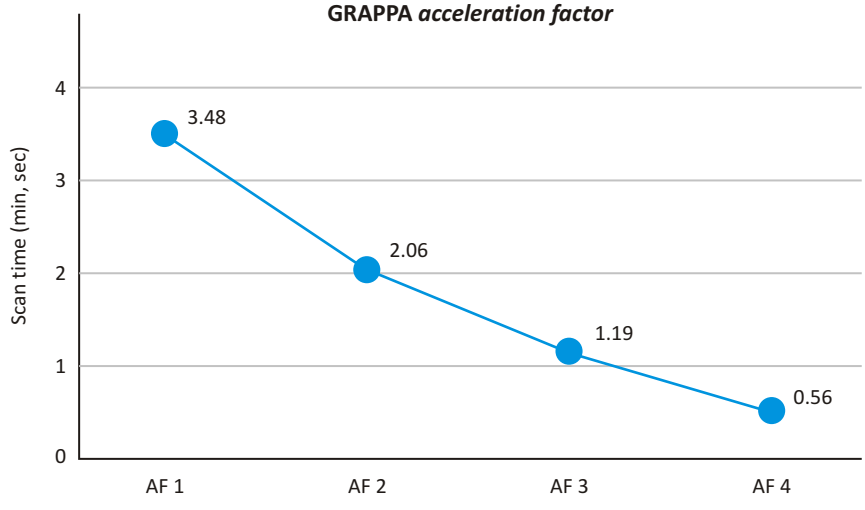

Figure 4. Scan time graph on GRAPPA variation

65.35% decrease in scan time, while a factor of 4 yields a 75.44% reduction, both in comparison to the scenario without GRAPPA acceleration (AF 1). In another study by Nölte *et al*. (2008), the use of GRAPPA acceleration factor 2 will reduce scan time by about 50% of the original sequence (without variation of the GRAPPA acceleration factor).[16]

Numerous studies, mainly conducted using 1.5T scanners, have demonstrated that the implementation of parallel imaging can significantly reduce examination time while maintaining the image quality. The advantages of shorter breath-hold times in parallel MRI have been evidenced in cardiac, thoracic, and liver imaging,[17] as well as in cardiac MR imaging with free breathing.[18]

In non-cooperative patients, or in patients who are in pain when lying down for a long time on examination such as LSS cases,[6,19] parallel imaging is expected to avoid motion artefact,[15] and several other benefits in examinations with patients who have difficulty lying down for a long time.[16] With a scan time reduction of up to 65.35%, acceleration factor 3 is particularly advantageous for examining non-cooperative patients.

CONCLUSION

The optimal acceleration factor value used in Lumbar MRI Axial Cut T2WI TSE with LSS Case is acceleration factor 3 where there is no significant difference in image quality and anatomical information produced by variations without GRAPPA acceleration factor (*p-value* >0.05). With a time reduction of 65.35% in acceleration factor 3, it is very helpful in examining non-cooperative patients or patients with conditions that cannot survive lying down for a long time. The use of GRAPPA acceleration factor 3 in MRI examinations can still maintain the optimal image quality and anatomical information with a fast scan time.

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Original Article

Effects of 4G 2100 MHz Radiation on Lymphocyte Levels: An Experimental Study in Wistar Rats

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Abstract

Background : Electromagnetic radiation (EMR) from mobile phones has become a global concern due to its potential impact on the immune system. However, the effects of EMR on lymphocyte levels remain inconsistent across studies. This study aimed to evaluate the effects of 4G 2100 MHz EMR exposure on lymphocyte levels in Wistar rats.
Methods : This research employed a true experimental design with a Post Test Only Controlled Group Design. Four groups of male Wistar rats were used: a control group (G1) and three exposure groups subjected to EMR for 15 (G2), 30 (G3), and 45 days (G4). The exposed groups were subjected to 2 hours per day of 4G 2100 MHz EMR using a Redmi A2 mobile phone in WhatsApp call mode. Lymphocyte levels were analyzed using ANOVA to determine differences among the groups.
Results : The average lymphocyte levels in groups G1, G2, G3, and G4 were 68.92 ± 4.78%, 63.37 ± 6.29%, 67.00 ± 11.51%, and 64.35 ± 13.58%, respectively. Statistical analysis revealed no significant differences among the groups ($p = 0.703$).
Conclusion : Exposure to 4G 2100 MHz EMR for 1545 days did not significantly change lymphocyte levels in Wistar rats. These findings support the need for further studies to evaluate other immunological parameters and the long-term effects of EMR exposure.

Keywords : Electromagnetic radiation; Lymphocyte levels; Wistar rats; Mobile phones; Immune system

INTRODUCTION

The rapid advancement of technology has led to a surge in the use of wireless communication devices, especially smartphones. As of 2024, there are more than 7.2 billion smartphone users globally––around 90% of the world's population. In Indonesia, smartphone use is also widespread. According to APJII, 196.71 million Indonesians were smartphone users in 2019, placing the country fourth globally. By 2023, smartphone penetration among adolescents aged 13–18 had reached 98.20%, making them the highest-using age group.[1,2]

This widespread smartphone use raises public health concerns, particularly due to exposure to electromagnetic radiation (EMR), which has been linked to oxidative stress and various health issues, including effects on the immune system.[3,4] Lymphocytes, key players in the adaptive immune response, are susceptible to environmental changes. EMR exposure may reduce lymphocyte levels through mechanisms such as oxidative stress, chronic inflammation, and impaired proliferation.[4]

Exposure to low to high levels of EMR (900 MHz and 1.3 GHz for 15 hours/day) from mobile phones in male albino rats over 28 days showed a trend of decreased lymphocyte levels.[5] Other studies have also reported that exposure to 900 MHz EMF for 2 hours/day in albino rats resulted in a significant decrease in lymphocyte count after two weeks of exposure.[6] Given the increasing public exposure to newer-generation mobile network frequencies, such as 4G operating at 2100 MHz, it is crucial to investigate their potential health impacts. Therefore, this study aims to examine the effects of 4G 2100 MHz electromagnetic radiation on lymphocyte levels in Wistar rats.

METHODS

This study was conducted from August to October 2024 at the Experimental Animal Laboratory, Faculty of Medicine, Universitas Jenderal Soedirman, and the Clinical Pathology Laboratory, Faculty of Medicine, Universitas Muhammadiyah Purwokerto.

The research employed a true experimental design with a Post Test Only Controlled Group Design using Wistar strain rats *(Rattus norvegicus)* as test subjects. Inclusion criteria included healthy and active male Wistar rats aged 24 weeks, weighing 150200 grams.[7] Exclusion criteria included sick or dying rats during acclimatization or the research process.

The study consisted of four groups: the control group (G1), a group exposed to electromagnetic radiation (EMR) for 15 days (G2), a group exposed for 30 days (G3), and a group exposed for 45 days (G4).[7] Each group included seven rats. Before the study began, the rats were acclimatized for seven days under standard animal care conditions. The rats were housed in cages of identical size. All protocols involving animal subjects were approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Jenderal Soedirman, under approval number 041/KEPK/PE/VI/2024.

Groups 2, 3, and 4 were exposed to 4G 2100 MHz EMR using WhatsApp call mode on a Redmi A2 mobile phone for 2 hours daily in treatment cages measuring 7x20x5 cm^2. The phone was positioned at a distance of 3 cm from the cage wall.[7] After maintenance and treatment, the rats were fasted for 8–12 hours, and blood samples were collected via the retro-orbital sinus for lymphocyte level analysis. Blood samples were collected via the retro-orbital sinus and analyzed using an automated Hematoanalyzer Sysmex KX-21 at the Clinical Pathology Laboratory, Faculty of Medicine, Universitas Muhammadiyah Purwokerto. The rats were then terminated using the cervical dislocation technique.

The data obtained were tested for normality using the Shapiro-Wilk test and for homogeneity using Levene's test. The results indicated that the data were normally distributed and homogeneous. Subsequently, the data were analyzed using One-Way ANOVA.

RESULTS

The results of the study showed a *p-value* of 0.703 ($p > 0.05$), indicating that there was no statistically significant differences among the treatment groups. Therefore, exposure to EMR at a frequency of 2100 MHz for 2 hours per day over 15, 30, and 45 days did not result in significant changes in lymphocyte levels in rats.

Body weight was also recorded before and after treatment. The analysis showed no significant differences in body weight among groups ($p > 0.05$), indicating that EMR exposure did not significantly affect the animals' weight.

DISCUSSION

The findings of this study indicate that exposure to 4G 2100 MHz (EMR for 15, 30, and 45 days did not result in significant changes in rat lymphocyte levels ($p = 0.703$). These results contradict the initial hypothesis that prolonged EMR exposure would significantly affect lymphocyte levels. Previous studies have mentioned that exposure to radiofrequency electromagnetic fields did not significantly alter T cell populations or functions in developing rats, suggesting that the immune system may tolerate certain levels of EMR without dysregulation.[8] Another study also mentioned that no significant changes in circulating lymphocyte counts following EMR exposure from a 915 MHz RFID system in healthy adult rats.[9]

Minor fluctuations in lymphocyte levels among the exposed groups (G2, G3, and G4) may reflect adaptive responses of the immune system to EMR exposure.

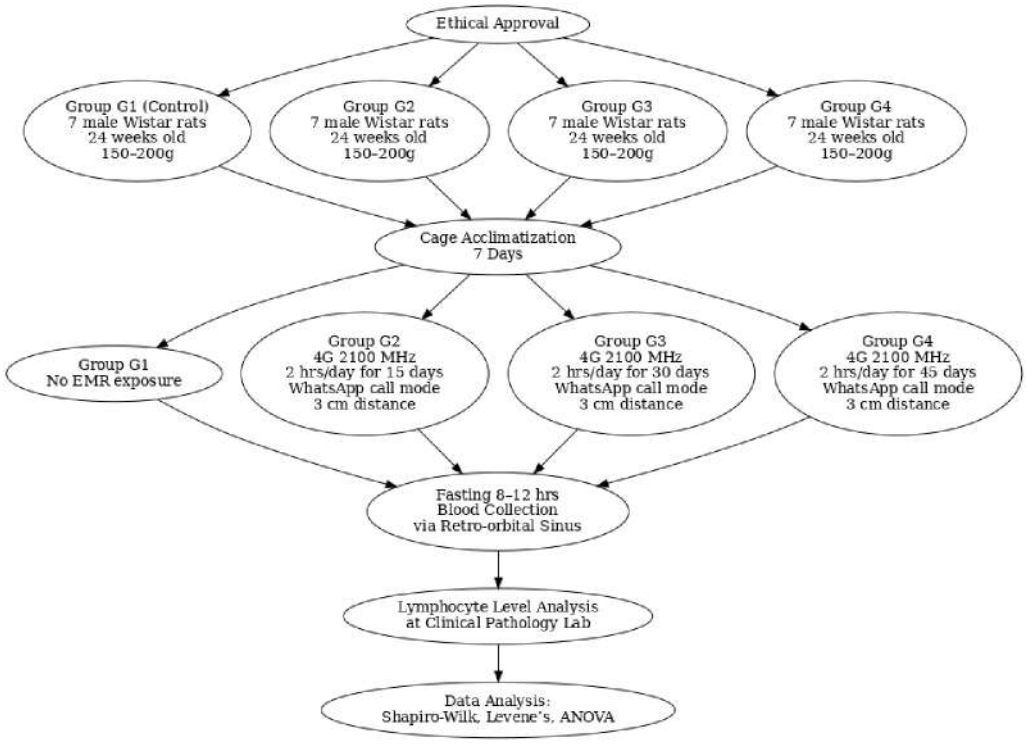

Figure 1. Flowchart of the experimental procedure

TABLE 1
Rat lymphocyte levels

Groups	Mean±SI (%)	*p value*
G1	68.92 ± 4.78	0.703
G2	63.37 ± 6.29	
G3	67.002 ± 11.51	
G4	64.35 ± 13.58	

Lymphocyte homeostasis, a mechanism that regulates cell numbers in response to environmental stimuli, likely contributed to the stable lymphocyte counts observed. Previous studies have mentioned that short-term GSM radiation did not produce measurable physiological effects, suggesting a limited impact of transient EMR exposure on biological systems.[10]

The discrepancy between the results and the initial hypothesis could be attributed to several factors. The cage dimensions may have affected the homogeneity of radiation exposure, potentially leading to variability in EMR absorption among rats. Moreover, biological variation and a relatively small sample size may have limited the statistical power to detect subtle effects. The exposure method––using WhatsApp call mode on a mobile phone––could have resulted in fluctuating signal strengths, which might not have consistently delivered uniform EMR intensity across individuals. Additionally, external factors such as ambient stress or minor environmental inconsistencies could have confounded the results, as also acknowledged in other studies assessing EMR effects.[11]

The frequency used in this study, 2100 MHz for 2 hours daily, may not have reached the threshold necessary to induce significant immunomodulatory effects. Prior research has emphasized that both the dose and duration of EMR exposure are critical in determining biological outcomes. If the exposure is too short or at insufficient intensity, immunological changes may not manifest.[8,9]

Classified as short-term exposure, the 15–45 day EMR treatments in this study might not have elicited cumulative immune effects. No significant alterations in physiological parameters following short-term EMR

exposure, possibly due to the establishment of systemic tolerance mechanisms.[10]

In addition to lymphocyte levels, this study also monitored the animals' body weight before and after treatment. No marked differences were observed across the groups. Body weight stability has also been reported in other EMR-related animal studies as an indicator of minimal systemic stress or metabolic disruption. Other studies, found no significant body weight changes in Wistar and Sprague Dawley rats exposed to 5G or 150 kHz EMR over several weeks.[12,13] These findings reinforce our interpretation that short-term EMR exposure at 2100 MHz does not appear to produce measurable systemic effects, at least as reflected in body weight parameters.

It is important to note that this study exclusively measured lymphocyte levels, without assessing other immunological markers such as lymphocyte activation status or subtype distributions. Future research should consider evaluating broader immune responses––including functional assays and profiling of T-helper and T-cytotoxic subpopulations––to better understand the scope of EMR impact. Increasing sample size, using more controlled EMR exposure setups, and conducting long-term studies will further clarify the potential health effects of mobile-related EMR exposure.

Additionally, this study did not include direct measurement of signal strength or EMR wavelength, which limits the ability to verify the accuracy and uniformity of the radiation exposure. Moreover, no physiological or behavioral stress markers, such as corticosterone levels or observation of stress-related behaviors were assessed. The absence of these parameters may limit the depth of interpretation regarding systemic or stress-mediated responses to EMR exposure.

Furthermore, since this study used an animal model (Wistar rats), the findings may not fully represent immune responses in humans. Physiological and metabolic differences between rats and humans could influence the outcomes. Therefore, the results should be interpreted with caution, and further studies involving human subjects are necessary to validate these findings.

CONCLUSION

This study demonstrated that exposure to 4G 2100 MHz EMR for 15, 30, and 45 days at a duration of 2 hours per day did not result in significant changes in lymphocyte levels in Wistar rats. However, as this study was conducted using an animal model, the findings should be interpreted with caution and not directly generalized to humans.

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Original Article

Antibacterial and Antioxidant Effect from Nanoparticle of *Andrographis paniculata* Extract on Wistar Rat Infected with *Listeria monocytogenes*

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Abstract

Background : Listeria infection triggers the production of ROS (Reactive Oxygen Species) and radical-waste accumulation such as Malondialdehyde (MDA). *Andrographis paniculata*, triggers the production of Superoxide Dismutase (SOD) as antioxidant and suppresses the bacteria multiplication in host's body. However, these active-phytochemical have poor destruction due to their large size and molecular weight. Nanoparticle technology may overcome this problem to increase bioavailability and benefit of herbal pharmacology. This study was aimed to evaluate the effectivity nanoparticle of *A. paniculata* extract to increase bacteria elimination, increase SOD level, and reduce MDA level in rat infected with *L. monocytogenes*.
Methods : Experimental research with post-test only controlled trial design was conducted using white rats (*Rattus novergicus* strain Wistar) into Normal (N), Negative Control (K-), *A. paniculata* extract (EAP-200), and Nanoparticle of *A. paniculata* extract Group (nEAP-100, nEAP-200, and nEAP-400). Colony was measured using total plate count method. SOD & MDA level were measured using colorimetric method
Results : *L. monocytogenes* bacterial count from group with extract *A. paniculata* was significantly lower than those in negative control (p<0.05). SOD level from group with extract *A. paniculata* was very significantly higher than those in negative control (p<0.01), while MDA level from group with extract *A. paniculata* was very significantly lower than those in negative control (p<0.01). Dose of 200 mg/kgBW in nanoparticle form was the most optimum dose.
Conclusion : Extract of *A. paniculata* at various doses and form, increase bacterial clearance, increase SOD level, and reduce MDA level in rat infected with *L. monocytogenes*.

Keywords : Antibacterial, *Andrographis paniculata*, Antioxidant, *Listeria monocytogenes*, Nanoparticle

INTRODUCTION

WHO reports that 600 million people are suffered from foodborne infection with 175.000 people were died in Southeast Asia.[1] Listeria monocytogenes has highest mortality rate among other foodborne pathogen bacteria in United States (16,9%) and Europe (18,8%).[2] Listeria infections cause diseases such as sepsis, meningitis, and die in some individuals such as children and pregnant woman.[3–7]

Free radical such as *Reactive Oxygen Species* (ROS) are side products of aerobic metabolism, which reactive, destructive, and causing oxidative stress. Infection of *L. monocytogenes* increase production of ROS for killing bacteria. However, an excessive of ROS in host body, causes oxidative stress along with the accumulation of radical waste in the host body such as fatty acid radicals or Malondialdehyde (MDA) that resulted from lipid peroxidation in cell membrane or tissues. It presence, provides effects such as DNA damage and protein degradation which causes apoptosis, neuro-inflammation, and cancer.[8–11] The cell requires antioxidant enzyme such as Superoxide Dismutase (SOD), which is able to neutralize ROS into oxygen (O_2) dan hydrogen peroxide (H_2O_2) and also to prevent ROS adverse effect and protect cells from oxidative stress.[12,13]

Various medicine plants which contains natural active compounds have been used and developed in health sector as one of the alternative therapy for patient with antibiotic resistant.[14,15] *A. paniculata* is one of the Indonesia traditional medicines used for antimalarial, antimicrobial,[16] anti-inflammatory,[17] antioxidant, anti-parasitic,[18] antidiabetic, and anticancer agents in Asia.[19] *A. paniculata* contains active phytochemical such as diterpenoid lactone, flavonoid, quinic acid, tannins, and alkaloid.[20,21]

The active pharmacological effects of herbal plants on various disease are still not optimum in host body because poor distribution due to their large size and molecular weight.[22] Nanoparticle are one of the technology-based product utilization which control the optical, physical and chemical properties of the compound to increase drug absorbtion in body (increase bioavailability).[23] Chitosan is a natural polymer biomaterial derived from chitin compounds which low side effects on humans. *A. paniculata* extract with chitosan may also increase antioxidant capacity.[24]

Studies about the benefit of nanoparticle of *A. paniculata* extract still limited as in vitro studies, therefore this research focus on the effectivity of nanoparticle of *A. paniculata* extract to determine the optimal dose for increasing bacterial clearance and SOD level, also reducing MDA level in rat infected with *L. monocytogenes* (in vivo).

METHODS

An experimental study was done with a post-test-only randomized controlled group design. The study was conducted at the Research and Animal Laboratory, Muhammadiyah Semarang University, Semarang for 7 days after obtaining ethical approval from the Health Ethics Committee. The Ethical clearance issued by The Health Research Ethics Committee Faculty of Medicine Universitas Diponegoro. Colony count from the blood and liver samples of rat was measured at Microbiology Laboratory, Faculty of Medicine, Diponegoro University while the analysis of SOD and MDA level was measured at Ecotoxicology Laboratory, Faculty of Biology, Jenderal Soedirman.

Animals and Experimental Groups

The study used 36 male Wistar Rat aged 812 weeks with a body weight of 200300 grams, divided into 6 groups. The research group division was as follows:

Normal group (N) : Healthy Rat (only general feed)

Negative control group (K-) : Rat infected by *L. monocytogenes*

Treatment group I (EAP) : Rat infected by *L. monocytogenes* and treated with 200 mg/kg *A. paniculata* extract

Treatment group II (nEAP-100) : Rat infected by *L. monocytogenes* and treated with 100 mg/kg Nanoparticle of *A. paniculata* extract

Treatment group III (nEAP-200) : Rat infected by *L. monocytogenes* and treated with 200 mg/kg Nanoparticle of *A. paniculata* extract

Treatment group IV (nEAP-400) : Rat infected by *L. monocytogenes* and treated with 400 mg/kg Nanoparticle of *A. paniculata* extract

The experimental animals were inoculated with *Listeria monocytogenes* ATCC 7644 from Gadjah Mada University. 0.5 mL of *L. monocytogenes* in PBS (109 CFU/mL) was intravena injected into each rat on the first day.[25,26] After bacterial infection, nanoparticles of *A. paniculata* dissolved in 1% DMSO were given orally (gastric tube) on treatment groups at the same time of Biorat feeding on each day for a week. After one week, blood (whole blood and serum) and liver tissue was isolated for analysis.[25]

Preparation of A. paniculata extract

The methanol extract of *A. paniculata* was prepared by maceration using 70% methanol as solvent. In this study, the dried *A. paniculata* leaf powder was obtained from *Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional* (B2P2TOOT), Tawangmangu, Central Java, Indonesia. Approximately 20 g r of simplisia was macerated with 200 mL of 70% methanol (w:v = 1:10). After maceration, this mixture was filtered using

Whatman filter paper number No.1. The filtrate was evaporated using a Vacuum rotary evaporator at 50°C until crude extract was obtained.[27]

Preparation of Nanoparticle of *A. paniculata* leaf extract

The nanoparticle of *A. paniculata* leaf extract was produced by using ionic gelation and ultrasonication method followed by addition of chitosan (polimer) and sodium tripolyphosphate (STPP as crosslinker). The 1% chitosan was dissolved in a 1% acetic acid solution and homogenized. About 0.15 gram of *A. paniculata* extract was diluted in 5 drops of 70% methanol. The diluted *A.paniculata* extract was then added to the chitosan solution. The crosslinker in the form of STPP was dissolved in distilled water until reached 1.5% concentration and a mixture of bitter extract + chitosan was added to the solution and then homogenized. The homogeneous solution was then centrifuged at a speed of 10,000 rpm for 10 minutes and then the natant was separated from the supernatant. The natan solution was then sonicated at a frequency of 20 kHz for 60 minutes.28 Morphological analysis including the size and shape of the nanoparticle compound was measured using a Particle Size Analyzer (PSA) and Scanning Electron Microscope (SEM).[29]

Total Bacteria Colony Count

Blood and liver samples approximately 1 mL/1 gr were diluted in 9 mL of NaCl solution (ratio 1:9).[30] 1 mL of the 10–3 to 10–5 dilution tubes, 1mL was taken and transferred into a petri dish. Next, 20 mL of Nutrient Agar (NA) media was poured into a petri dish and then homogenized. The media was incubated at 37°C for 2x24 hours.[31] The colony formed were then counted using the TPC formula.[32]

$$\text{TPC (CFU/mL)} = \text{total colony x volume sample x } \frac{1}{\text{Dilution factor}}$$

SOD and MDA Level

Measurement of superoxide dismutase (SOD) level was analyzed using the Superoxide Dismutase commercial Kit (Elabscience).[33] A 20 µL of plasma was added 1 mL of SOD buffer solution and 100 µL of xanthine oxidase solution. A blank solution was prepared by adding and mixing 1 mL of SOD buffer solution and 100 µL of xanthine oxidase solution. The absorbance value at λ520 nm was measured using a UV-Vis spectrophotometer. The activity of the SOD enzyme was measured using the following formula.[34]

$$\text{Activity of SOD (U/mL)} = \frac{As}{Ao} \times Ks$$

Note: As: absorbance value of sample solution;
Ao: absorbance value of a standard solution;
Ks: standard concentration (30.65).

Measurement of malondialdehyde (MDA) level was analyzed using the Superoxide Dismutase commercial Kit (Elabscience).[33] A 400 µL of plasma was added 400 uL of TCA 20% solution in reaction tube then centrifuge. After centrifuged, 400 uL supernatant of the mixture added 1 mL of TBA 0,67% solution then incubated on water bath for 10 minutes. A blank solution was prepared by adding and mixing 1 mL of TBA 0,67% solution and 400 µL of TCA 20% solution. The mixture was measured by spectrophotometry at λ532 nm.[35]

Statistical Analysis

The data normality was tested using the Shapiro-Wilk test. The average of Listeria colony in blood, SOD, and MDA level were normally distributed, then were analyzed using One Way Anova and followed by Post Hoc LSD. The average of Listeria colony in hepar was not normally distributed, then were analyzed using Kruskall-Wallis test followed by the Mann-Whitney test to determine the differences between groups in this study. The result of *p-value* was considered significant, if $p < 0.05$.

RESULTS

The condition after 7 days experiment, rats in the Negative Control Group showed different behaviors and clinical conditions from the treatment groups. The rat appeared weak and less active as the day went on, the hair was dirty, and the feces became loose, while in the Treatment Group (EAP-200, nEAP-100, nEAP-200, and nEAP-400), the rats were active with clean hair, normal feces, and physically similar to the mice in the Normal Group.

***Listeria monocytogenes* Colony Count**

The total number of bacteria in the blood and liver samples after being treated for 7 days are shown in Figure 1 and Figure 2. Bacterial colony count was carried out with a dilution until 10^{-3} for blood samples and 10^{-6} for liver samples.

***L. monocytogenes* Colony Count in Blood**

The average number of *L. monocytogenes* bacterial colony in the blood (Figure 1), the treated group (EAP-200, nEAP-100, nEAP-200, and nEAP-400) showed a very low graph compared to the Negative Control.

The result of LSD test showed that the average of *L. monocytogenes* colony in blood for treatment group (EAP-200, nEAP-100, nEAP-200, and nEAP-400) was

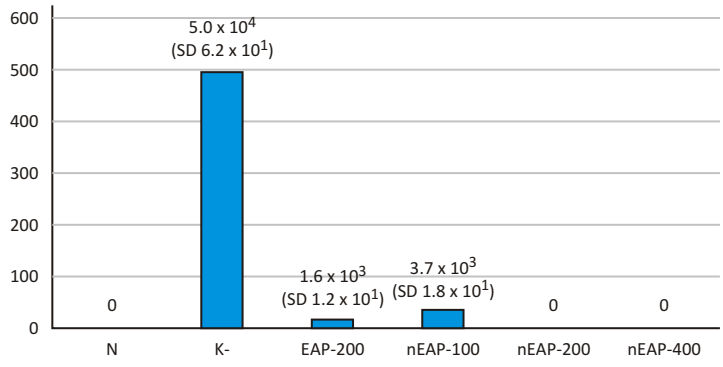

Figure 1. Average Number of *L. monocytogenes* Colony in Blood (CFU/mL)
(N (Normal), K- (Negative Control), EAP-200 (*A. paniculata* Extract 200 mg/kgBW), nEAP-100 (100 mg/kgBW Nanoparticle of *A. paniculata* Extract), nEAP-200 (200 mg/kgBW Nanoparticle of *A. paniculata* Extract), and nEAP-400 (400 mg/kgBW Nanoparticle of *A. paniculata* Extract))

TABLE 1
Statistical Analysis of Average Number of *L. monocytogenes* in Blood

	N	K-	EAP-200	nEAP-100	nEAP-200	nEAP-400
N	–	<0.001*	0.370	0.043	1	1
K-	<0.001	–	<0.001*	<0.001*	<0.001*	<0.001*
EAP-200	0.370	<0.001*	–	0.233	0.370	0.370
nEAP-100	0.043*	<0.001*	0.233	–	0.043*	0.043*
nEAP-200	1	<0.001*	0.370	0.043*	–	1
nEAP-400	1	<0.001*	0.370	0.043*	1	–

(N (Normal), K- (Negative Control), EAP-200 (*A. paniculata* Extract 200 mg/kgBW), nEAP-100 (100 mg/kgBW Nanoparticle of *A. paniculata* Extract), nEAP-200 (200 mg/kgBW Nanoparticle of *A. paniculata* Extract), and nEAP-400 (400 mg/kgBW Nanoparticle of *A. paniculata* Extract); *different results are statistically significant)

significantly lower than of Negative Control group (p<0.01; Table 1). The average of *L. monocytogenes* colony for nEAP-200 and nEAP-400 groups was significantly lower than of EAP200 and nEAP-100 (p<0.05; Table 1), and there was no significant difference in the nEAP-200 group with nEAP-400 (p>0.05; Table 1). The average number of colony in EAP200 was lower than of nEAP-100, but was not statistically significant (p>0.05; Table 1).

L. monocytogenes Colony Count in Liver

The average number of *L. monocytogenes* bacterial colony in the liver (Figure 2), the treated group (EAP-200, nEAP-100, nEAP-200, and nEAP-400) showed a very low graph compared to the Negative Control.

The result of Mann Whitney test showed that the average number of *L. monocytogenes* colony in the blood for EAP-200, nEAP-200, and nEAP-400 groups was very significantly lower than of Negative Control group (p<0.01; Table 2) while the nEAP-100 group was significantly lower than the Negative Control group (p<0.05; Table 2). The average number of *L. monocytogenes* colony in the EAP-200, nEAP-100, and nEAP-200 groups showed there was not statistically significant difference (p>0.05; Table 2).

Superoxide Dismutase (SOD) Plasma Level

Figure 3 shows that the average of SOD plasma level in the treated group (EAP-200, nEAP-100, nEAP-200, and nEAP-400) were higher compared to the Negative Control and Normal Group.

The result of LSD test showed that SOD plasma level in the EAP-200, nEAP-100, nEAP-200, and nEAP-400 groups were very significantly higher than the Normal and Negative Control Groups (p<0.01; Table 3). SOD level in the EAP-200 and nEAP-100 groups were very significantly higher than Negative Control Group,

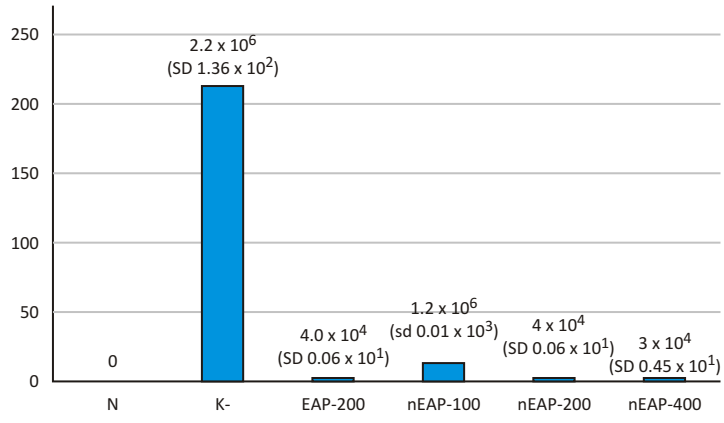

Figure 2. Average Number of *L. monocytogenes* Colony in Liver (CFU/mL)
(N (Normal), K- (Negative Control), EAP-200 (*A. paniculata* Extract 200 mg/kgBW), nEAP-100 (100 mg/kgBW Nanoparticle of *A. paniculata* Extract), nEAP-200 (200 mg/kgBW Nanoparticle of *A. paniculata* Extract), and nEAP-400 (400 mg/kgBW Nanoparticle of *A. paniculata* Extract))

TABLE 2
Statistical Analysis of Average Number of *L. monocytogenes* in Blood

	N	K-	EAP-200	nEAP-100	nEAP-200	nEAP-400
N	–	0.003*	1	0.869	1	1
K-	0.003	–	0.069	0.992	0.069	0.047*
EAP-200	1	0.060	–	1	1	1
nEAP-100	0.869	0.992	1	–	1	1
nEAP-200	1	0.069	1	1	–	1
nEAP-400	1	0.047*	1	1	1	–

(N (Normal), K- (Negative Control), EAP-200 (*A. paniculata* Extract 200 mg/kgBW), nEAP-100 (100 mg/kgBW Nanoparticle of *A. paniculata* Extract), nEAP-200 (200 mg/kgBW Nanoparticle of *A. paniculata* Extract), and nEAP-400 (400 mg/kgBW Nanoparticle of *A. paniculata* Extract); *different results are statistically significant)

but this increase was still significantly lower than of nEAP-200 and nEAP-100 (p<0.05; Table 3). The increase of SOD level between nEAP-200 and nEAP-400 was not statistically significant (p>0.05; Table 3).

Malondialdehyde (SOD) Plasma Level

Figure 4 shows that the average of MDA plasma level in the treated group (EAP-200, nEAP-100, nEAP-200, and nEAP-400) was slightly lower than Negative Control.

The result of LSD test showed that MDA plasma level in all treatment groups (EAP-200, nEAP-100, nEAP-200, and nEAP-400) were significantly lower than of the Negative Control Group (p<0.05; Table 4). The MDA level in nEAP-200 and nEAP-400 groups were significantly lower than of EAP200 group (p<0.05; Table 4). The MDA level between nEAP-200 and nEAP-400 were not statistically significant (p>0.05; Table 4).

DISCUSSION

This study proved that EAP in both crude extracts and nanoparticle form increased the elimination (clearance) of *Listeria monocytogenes* bacteria in wistar rat infected-*L. monocytogenes*. The effect of bacterial clearance were seen on 200 mg/kgBW of crude extract and nanoparticle form. The bacterial clearance effect from active metabolite compounds in EAP might be caused by antibacterial effects and / or the ability to stimulate phagocytosis and intracellular bacterial killing. At this point, we are not sure which mechanism caused effective bacterial clearance in this study.

Previous studies reported that andrographolide (diterpenoid) content in *A. paniculata* has antibacterial activity to inhibit bacterial biofilms form in the host, damage bacterial cell membranes and walls through inhibition of difflux pumps, inhibit the hemolytic

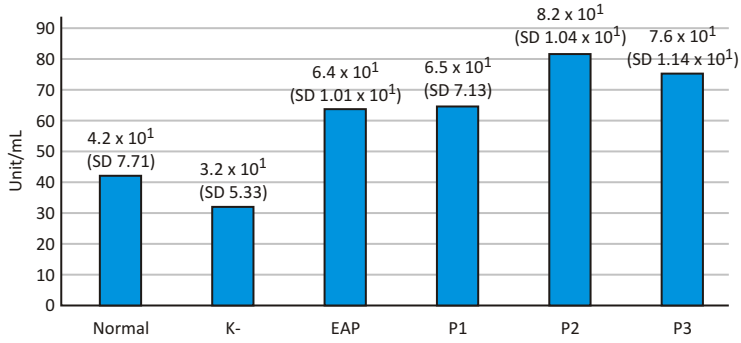

Figure 3. Superoxide Dismutase Serum Level (Unit/mL)
(N (Normal), K- (Negative Control), EAP-200 (*A. paniculata* Extract 200 mg/kgBW), nEAP-100 (100 mg/kgBW Nanoparticle of *A. paniculata* Extract), nEAP-200 (200 mg/kgBW Nanoparticle of *A. paniculata* Extract), and nEAP-400 (400 mg/kgBW Nanoparticle of *A. paniculata* Extract))

TABLE 3
Statistical Analysis of SOD Plasma Level

	N	K-	EAP-200	nEAP-100	nEAP-200	nEAP-400
N	–	0.155	0.001*	<0.001*	<0.001*	<0.001*
K-	0.155	–	<0.001*	<0.001*	<0.001*	<0.001*
EAP-200	0.001*	<0.001*	–	0.676	<0.001*	0.014*
nEAP-100	<0.001*	<0.001*	0.676	–	0.003*	0.035*
nEAP-200	<0.001*	<0.001*	0.001*	0.003*	–	0.299
nEAP-400	<0.001*	<0.001*	0.014*	0.035*	0.299	–

(N (Normal), K- (Negative Control), EAP-200 (*A. paniculata* Extract 200 mg/kgBW), nEAP-100 (100 mg/kgBW Nanoparticle of *A. paniculata* Extract), nEAP-200 (200 mg/kgBW Nanoparticle of *A. paniculata* Extract), and nEAP-400 (400 mg/kgBW Nanoparticle of *A. paniculata* Extract); *different results are statistically significant)

activation of Listeriosin O (LLO), and inhibit communication between bacterial cells.[36–39] The Flavonoid in *A. paniculata* able to damage cell walls bacteria, inhibit synthesis of nucleic acid (through inhibition of the topoisomerase enzyme) and inhibit energy metabolism (through inhibition of NADH-cytochrome C reductase).[40] Previous studies proved that supplementation of silver-nanoparticle *A. paniculata* was effective to inhibit Gram-positive bacteria (*Staphylococcus aureus* with MIC: 25 ug/mL) and Gram-negative bacteria (*E. coli* with MIC: 12.5 ug/mL) in dilution agar method.[41] Paramasivam[42] reported that Aurum(Au)-nanoparticles *A. paniculata* showed a trend of increasing antibacterial effects from a dose of 50 ug/mL to 100 ug/mL using agar diffusion method. On the other hand, andrographolide also triggers lymphocyte proliferation, increases migration of macrophages towards the site of infection, phagocytosis and intracellular killing.[43]

Our findings also showed that *A. paniculata* in various doses and forms increased SOD level very significantly. Dose of 200 mg/kgBW in nanoparticle was significantly more efficaciously increased SOD level compared to dose of 200 mg/kgBW in crude extract form, thus, it can be concluded that the nanoparticle form on dose of 200 mg/kgBW is more effective in increasing SOD level than the crude extract form. There was no significant differences of SOD level between dose of 200 mg/kgBW and 400 mg/kgBW in nanoparticle, implying that the dose of 200 mg/kgBW in nanoparticle form is optimum for increasing SOD level.

SOD is an enzyme which stabilize free radicals of ROS which produced by phagocyte cells in vacuoles to kill bacteria intracellularly and prevent cell/tissue damage due to oxidative stress.[44] According to Bhattacharya's research,[45] ROS is produced after bacteria are phagocytized intracellularly. However, excessive ROS production causes damage to the cells and tissue, therefore SOD production was increased to suppress ROS production and inhibit the apoptosis pathway through the BAX and BCL-2 protein inhibition. In this study, the SOD level was increased on 200 mg/kgBW and 400 mg/kgBW dose in nanoparticle form indicating that

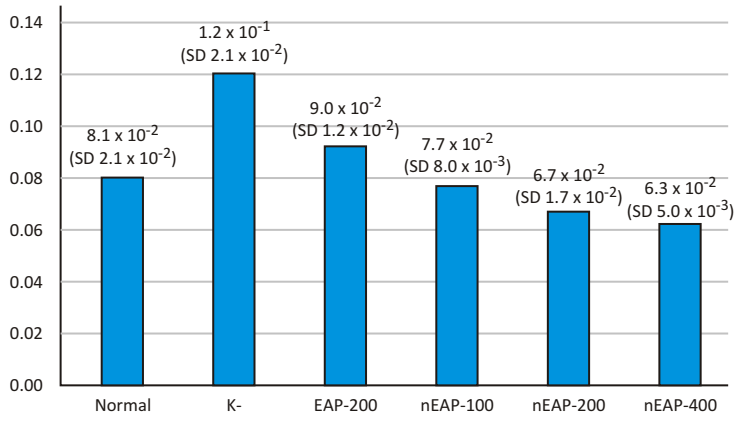

Figure 4. Malondialdehyde Plasma Level (Unit/mL)
(N (Normal), K- (Negative Control), EAP-200 (*A. paniculata* Extract 200 mg/kgBW), nEAP-100 (100 mg/kgBW Nanoparticle of *A. paniculata* Extract), nEAP-200 (200 mg/kgBW Nanoparticle of *A. paniculata* Extract), and nEAP-400 (400 mg/kgBW Nanoparticle of *A. paniculata* Extract))

TABLE 4
Statistical Analysis of MDA Plasma Level

	N	K-	EAP-200	nEAP-100	nEAP-200	nEAP-400
N	–	<0.001*	0.228	0.745	0.187	0.076
K-	<0.001*	–	0.008*	<0.001*	<0.001*	<0.001*
EAP-200	0.228	0.008*	–	0.131	0.016*	0.005*
nEAP-100	0.745	<0.001*	0.131	–	0.313	0.141
nEAP-200	0.187	<0.001*	0.016*	0.313	–	0.626
nEAP-400	0.076	<0.001*	0.005*	0.141	0.626	–

(N (Normal), K- (Negative Control), EAP-200 (*A. paniculata* Extract 200 mg/kgBW), nEAP-100 (100 mg/kgBW Nanoparticle of *A. paniculata* Extract), nEAP-200 (200 mg/kgBW Nanoparticle of *A. paniculata* Extract), and nEAP-400 (400 mg/kgBW Nanoparticle of *A. paniculata* Extract); *different results are statistically significant)

ROS production also increased during bacterial infection. This finding indirectly implied that there has been an increase in phagocytosis and intracellular bacterial killing activity, or other words, the clearance of *L. monocytogenes* bacteria after treatment with *A. paniculata* caused by antibacterial effect and also the immunostimulation effect of *A. paniculata*. Another study is needed to prove that the phagocytosis and intracellular killing are increased by *A. paniculata*. Previous study of Venkata *et al.*,[46] stated that nanoparticle of *A. paniculata* extract at a dose of 80 mg/kgBW increase SOD level in the brains of Rats induced with pentylenetetrazole (PTZ). Research by Kumari *et al.*,[47] showed that Cerium Oxide Nanomaterial of *A. paniculata* extract (cAP-CNP) that contains andrographolid and flavonoid can increase SOD activities until 43.03% by in vitro method through Nitrobule Tetrazolium (NBT) reduction.

Another finding in this study is the *A. paniculata* both in crude extract and nanoparticle also has an antioxidant effect by significantly reduces MDA level. Dose of 200 mg/kgBW and 400 mg/kgBW in nanoparticle form has reducing MDA level better than dose of 200 mg/kgBW in crude extract form and also the effect dose of 200 mg/kg BW in nanoparticle form was equivalent to reduce MDA level in dose of 400 mg/kgBW, implying that, dose of 200 mg/kgBW in nanoparticle form is the most optimal dose for lowering MDA level in the blood. MDA is the end product of peroxidation of unsaturated fatty acids which is used as an indicator of free radicals (ROS) accumulation in the process of intracellular bacterial killing.[48] A high MDA level indicates an increase of ROS content, whereas a low MDA level indicates that the ROS level is also low. MDA, which tends to be low while antioxidant level (SOD) are high, indicates that there is a balance in the cell to minimize cell damage and prevent cell death due to oxidative stress.[8] According to Ibrahimi *et al.*,[49] the flavonoids and alkaloids in EAP trigger SOD production in the body,

suppress the metabolism of toxic substances, and decrease lipid peroxidation (MDA). The study of Venkata *et al*.,[46] also stated that nanoparticle of *A. paniculata* extract at a dose of 80 mg/kgBW decreased MDA level in the brains of pentylenetetrazole-induced rats (PTZ).

This study proved that nanoparticle of *A. paniculata* extract is able to clearance bacterial in liver and blood indirectly through increasing phagocytosis and intracellular killing and also proved antioxidant effects of *A. paniculata* extract by showing the increase of SOD level and decrease of MDA level. The results of this study proved that the nanoparticle form is better than the crude extract form. The weakness of this study is the interval of research doses are wide. Through this study, authors hoped that nanoparticle of *A. paniculata* extract can be develope as supporting therapies for listeria infection.

CONCLUSION

Nanoparticle of *A. paniculata* at various doses and form, increases bacteria clearance and SOD level, and reduce MDA level in rat infected with *L. monocytogenes*. Dose of 200 mg/kgBW in nanoparticle form was the most optimum dose to increases bacteria clearance and SOD level, and reduce MDA level in rat infected with *L. monocytogenes*.

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Case Report

The Role of Mammography in Early Detection of Breast Cancer: A Case Collection Report

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Abstract

Background : Breast cancer is still the main cause of death in women, both globally and nationally. Therefore, mammography screening needs to be carried at to detect breast cancer early.
Cases Presentation : This paper presents the mammography findings of the 83 patients at dr. Ario Wirawan Pulmonary Hospital, Salatiga, Indonesia. Mammography examination used projection cuts, namely craniocaudal (CC) and mediolateral oblique (MLO). The classification used for this case series is BI-RADS.
Discussion : The results showed that of the 83 patients examined with mammography, 2 patients showed BI-RADS 4, indicating breast cancer, and 9 patients showed BI-RADS 2, indicating benign tumors. This case series report is consistent with previous studies explaining that mammography is important for making an accurate diagnosis of breast cancer between benign and malignant calcifications.
Conclusion : Mammography has a role in the early detection of breast cancer. Mammography examination using CC and MLO projections is a standard procedure that aims to obtain high-quality breast images and provide accurate information for early diagnosis of breast cancer.

Keywords : *mammography; BI-RAD; breast cancer*

INTRODUCTION

Breast cancer is the most common tumor that causes death in women. Breast cancer is cancer that originates from the glands, ducts, and tissue of the breast.[1] Based on data from Globocan (International Agency for Research on Cancer – IARC), breast cancer ranks first among all cancers in women.[2] During 2018 – 2022, 7,800,000 cases of breast tumors were reported globally, with 685,000 deaths worldwide.[3] American Cancer Society state that there were around 2,261,419 cases of breast cancer, with 684,996 women dying from it.[2] Meanwhile in the UK, 24,000 women are diagnosed with breast cancer every year, and 15,000 would perish. Until the age of 80 years, a woman's risk of developing breast cancer is 1 in 9.[4] Likewise with Japan, deaths from breast cancer have not decreased in the last 20 years, with the highest incidence in women in their 40s.[5] The Indonesian Ministry of Health released that in 2022 there will be 65,858 breast cancer cases or 16.6% of all diagnosed cancers.[6] Of all breast cancer cases alone, 70 percent of them are already at an advanced stage and treatment becomes more complicated, requires greater costs and the risk of death is higher.[7]

Rapid and accurate diagnosis of breast lesions, including differentiating between cancerous, non-cancerous, and suspicious cancer, plays an important role in breast cancer prognosis.[8] To detect breast cancer, medical imaging is still one of the most reliable tools.[9] Mammography screening with enhanced CT scans contributes to reducing the death rate from breast cancer.[10] The study conducted by Duffy *et al*[11] states that mammography screening significantly reduced the risk of death from breast cancer, and the rate of breast cancer at an advanced stage. Other studies also say that mammography screening has been proven significant in reducing morbidity and mortality from breast cancer, thus having a high survival rate.[12–14] However, scholars reporting the role of mammography in Indonesia is still rare.

Mammography screening as an early detection of breast cancer is also a superior service at dr. Ario Wirawan Pulmonary Hospital. Based on data, since it opened in 2021 to 2023, 83 patients have had mammography examinations. Therefore, this article aimed to reports the role of demographics in the early detection of breast cancer.

CASES PRESENTATIONS

Mammography screening at dr. Ario Wirawan Pulmonary Hospital, Salatiga, began in 2021, with 83 women registered for mammography examinations. The demographic description shows that most people are over 40 years old (see Table 1). Next, the patient comes to the radiology department for a mammography.

Mammography examination uses projection cuts, namely craniocaudal (CC) and mediolateral oblique

TABLE 1
Clinical Data

Fig.	Age	Complaint	Pathology Anatomy Result	Diagnosis	Therapy	Long Care
1	38 years 4 months	Lump in right breast	Medullary carcinoma of the right breast with luminar molecular subtype	BI-RAD 4 Right breast carcinoma	Surgery, Chemotherapy, Radiotherapy	2 days
2	56 years 11 months	Pain in the breast	Medullary Carcinoma of the Breast	BI-RAD 4 Breast carcinoma	Therapy, Surgery, Chemotherapy, Radiotherapy	2 days
3	48 years 4 months	Medical Check Up	Taking medication from an oncology surge	BI-RAD 2 Not operated on	Take medicine	0 days
4	50 years	Medical Check Up	–	BI-RAD 2 -	Refuse operation	–
5	41 years 3 months	Medical Check Up	Usual Ductal Hyperplasia (UDH)	BI-RAD 2 Mammae Sinistra Fibrocystic Cange USD	Operation	1 day
6	35 years 7 months	Feeling of discomfort	Spindle Cell Lipoma	BI-RAD 2 Lipoma	2 day operation	2 days
7	29 years 8 months	Lump in left breast	Cyst of the Left Breast	BI-RAD 2 Cysta Mammae Sinistra	Operation	1 day

TABLE 1. Continued

Fig.	Age	Complaint	Pathology Anatomy Result	Diagnosis	Therapy	Long Care
8	25 years 7 months	Medical Check Up	Fibroadenoma with florid usual ductal hyperplasia UDH, Woman with multiple left mammary tumors	BI-RAD 2 Multiple Fibroadenomas	Operation	2 days
9	33 years 2 months	Medical Check Up	–	BI-RAD 2 –	Refuse surgery, take medication	–
10	67 years 3 months	Medical Check Up	–	BI-RAD 2 –	Refuse operation	–
11	29 years 2 months	Lump in the breast	Single Fibroadenoma	BI-RAD 2 Fibroadenoma (FAM)	1 day operation	1 day

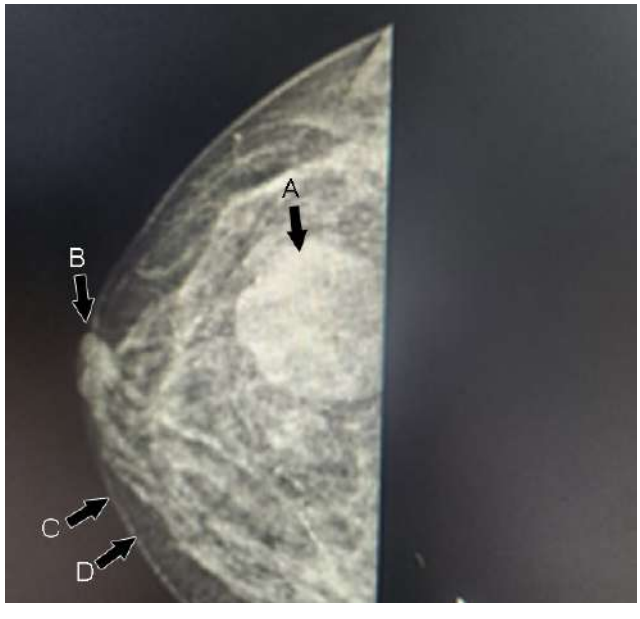

Figure 1. The first patient's mammogaphy with BI-RADS 4 category, found irregular-shaped mass in dextra mammae, partial margins of equal density in the lower outer quadrant (A), papilla mammae (B), cutis (C), and subcutis (D).

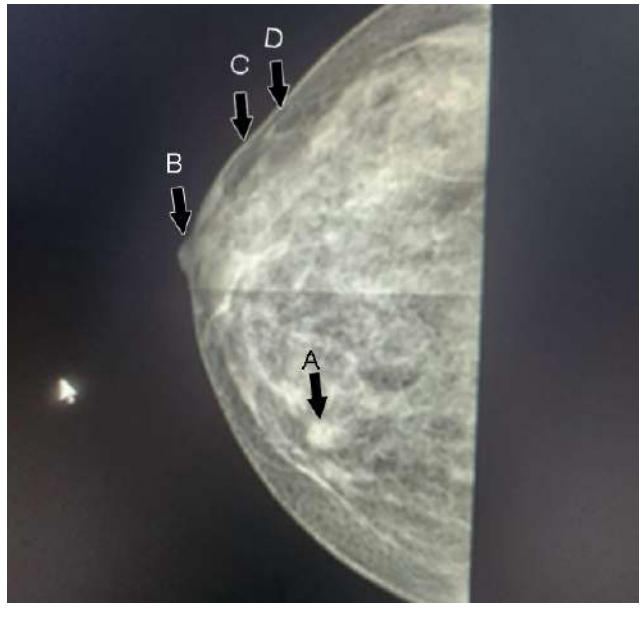

Figure 2. The eleventh patient's mammogaphy with BI-RADS 4 category, found an oval mass, circumscribed margins of equal density in the upper outer and inner quadrants, right mammary measuring 0.5 x 0.3 c. (A), papilla mammae (B), cutis mammae (C), and subcutis mammae (D).

(MLO). The mammography aircraft used is the Siemens brand with digital radiology mammography. The results of the mammography photos are then examined by radiology.

The classification used in the results of this case series mammography examination is Breast Imaging Reporting and Data System (BI-RADS). This system is used to assess findings on mammography and breast ultrasound examinations. BI-RAD consists of6 classifications, namely BI-RADS 1 is no abnormalities found in the breast. BI-RADS 2 is benign changes found that are not cause for concern. BI-RADS 3 is changes found that are most likely benign, but require further examination. BI-RADS 4 is changes suspected of being malignant (cancerous) were found. BI-RADS 5 is changes found that strongly suspect malignancy (cancer). BI-RADS 6 is found cancer which has been confirmed by histopathological examination (examination of tissue under a microscope).

RESULTS

Of the total, 1 patient with breast cancer was less than 40 years old. Two patients with multiple abnormalities

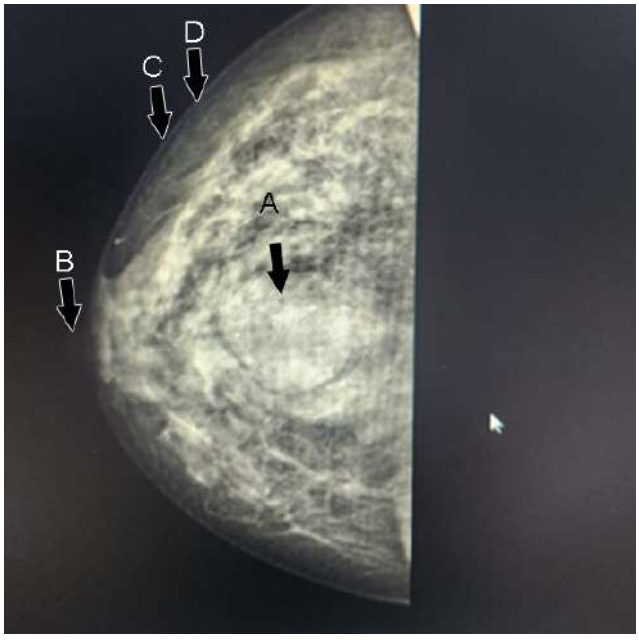

Figure 3. The second patient's mammogaphy with BI-RADS 2 category, found solid mass of relatively oval shape (A), pappila mammae (B), cutis (C), and subcutis (D).

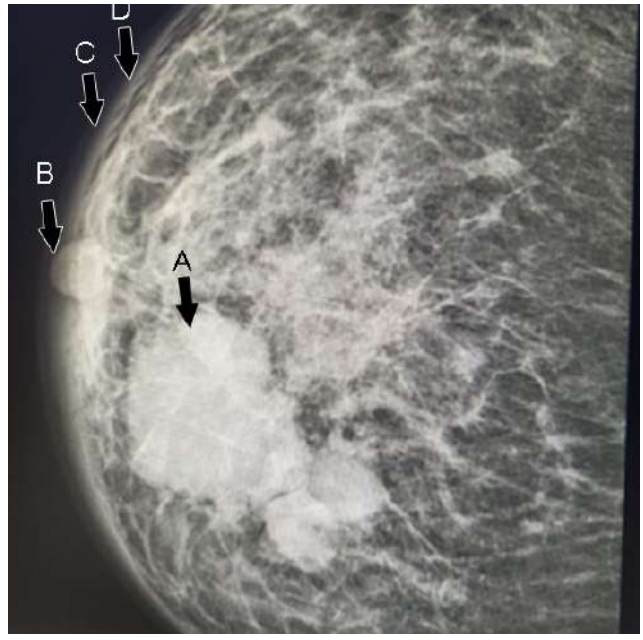

Figure 4. The third patient's mammogaphy with Birads 2 category, found mass with a relatively oval shape and well-defined boundaries (A), pappila mammae (B), cutis (C), and subcutis (D).

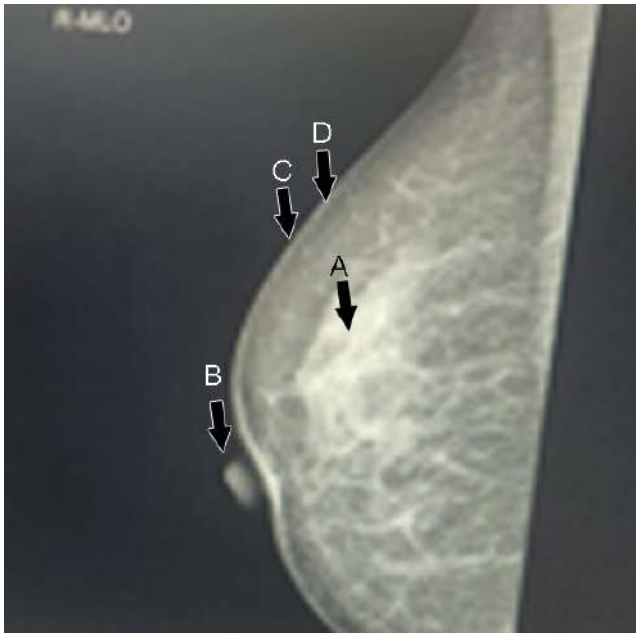

Figure 5. The fourth patient's mammogaphy with BI-RADS 2 category, found mass in the left mammary, firm boundaries, relatively oval shape, and flat edges (A), pappila mammae (B), cutis (C), and subcutis (D).

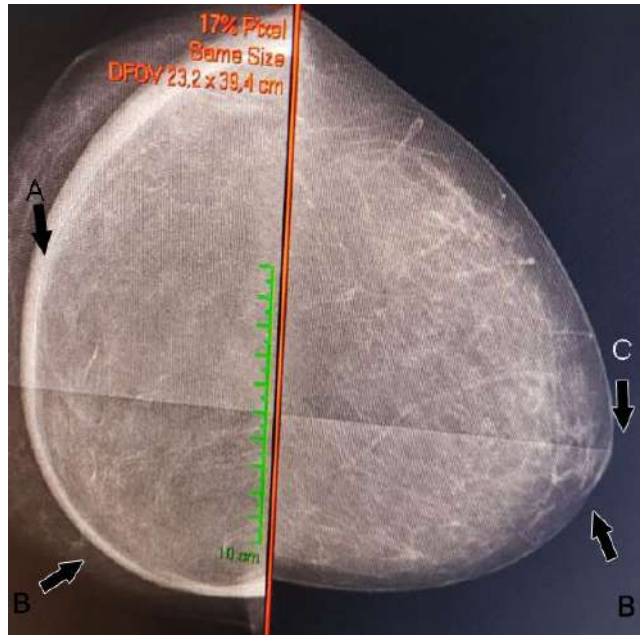

Figure 6. The fifth patient's mammogaphy with BI-RADS 2 category, found the mass has firm borders, round oval, circumscribed edges on the right mammae, no visible architectural distortion, no visible microcalcifications, lymphoma spindle (A), cutis and sub cutis (B), areola mammae (C).

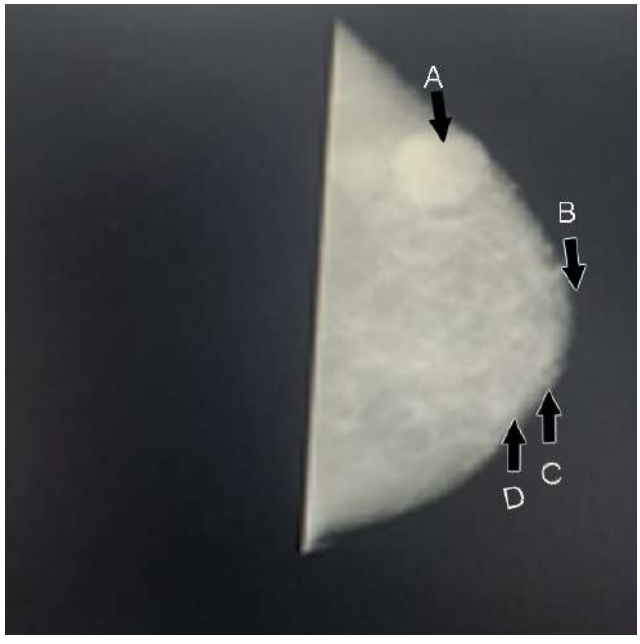

Figure 7. The fifth third patient's mammogaphy with BI-RADS 2 category, found relative mass oval shape, flat edges in the upper-outer left mammary region measuring 3.3 x 3.3 cm (A), areola mammae (B), cutis (C), and subcutis (D).

Figure 8. The seventh patient's mammogaphy with BI-RADS 2 category, found multiple masses in the left mammary, relatively oval shape, flat edges, size 2. 9 X 3.1 and 1.7 X 1.6 cm, no visible microcalcification lesions, no visible macrocalcification lesions (A), papilla mammae (B), cutis mammae (C), and subcutis mammae (D).

Figure 9. The eighth patient's mammogaphy with BI-RADS 2 category, found mass in the left mammary gland, round shape, flat edges, firm boundaries, no visible microcalcification lesions, no visible macrocalcification lesions (A), papilla mammae (B), cutis mammae (C), and subcutis mammae (D).

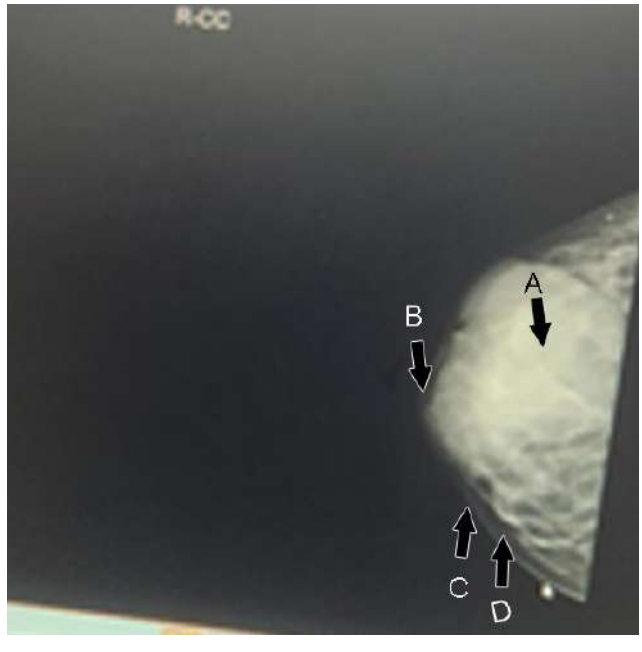

Figure 10. The ninth patient's mammogaphy with BI-RADS 2 category, found solid mass, lump in the right mammae, firm boundaries, relatively oval, in the right mammae, no visible microcalcification lesions, no visible macrocalcifications (A), papilla mammae (B), cutis mammae (C), and subcutis mammae (D)

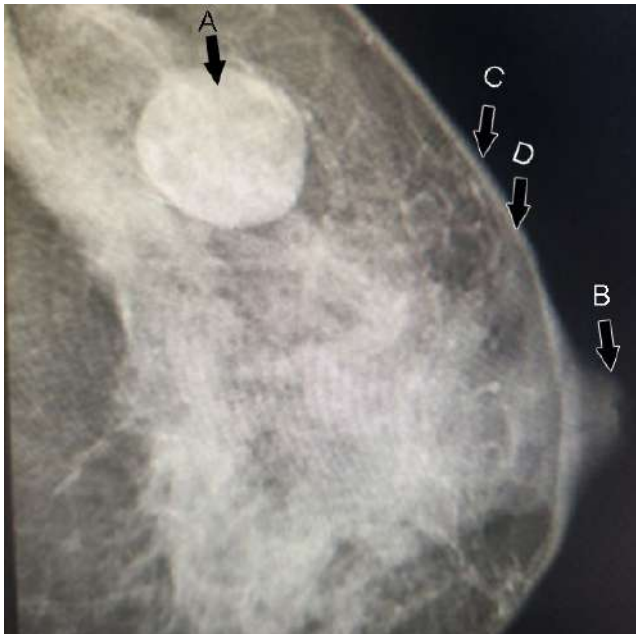

Figure 11. The tenth patient's mammogaphy with BI-RADS 2 category, found The cutis and subcutis are not thickened, the mammary papilla does not appear retracted, there is no visible calcified lesion, there is no visible enlargement of the lymph nodes in the left axilla, the opaq lesion appears round oval with flat edges in the upper-outer region, left mammary measures 1.5 X 1.6 cm, No visible macro or microcalcification lesions, No visible lymphadenopathy in the left axillary region (A), papilla mammae (B), cutis mammae (C), and subcutis mammae (D).

were found in the left and right breasts, aged over 40 years. One patient aged 36 years with malignant cancer, with a history of hereditary. To confirm this, the patient undergoes a biopsy.

Based on mammography examinations, of the 83, it was discovered that two patients was diagnosed with carcinoma malignancy breast cancer (Figure 1 and 2), nine women were diagnosed with benigma tumors (Figure 3 to 11).

DISCUSSION

The role of mammography is very important in women over 40 years. Mammography itself is a breast imaging examination that is often used to detect lumps or changes in breast tissue. In this case report series, mammography examination is carried out by taking several pictures of the breast from various angles to get more complete information. The two most common positions Cranio Caudal (CC) and Mediolateral Oblique (MLO).

The image produced from the CC projection provides information about the thickness of the breast tissue and its internal structure. The MLO projection provides a better view of the breast tissue located at the back and outside of the breast, including the tail of the breast. This projection is intended to view breast tissue from various angles. This helps in detecting lumps, calcifications (calcium buildup), or other tissue changes that may be difficult to see on a single projection. Apart from that, doctors can also obtain more accurate information about the size, shape, and location of the lesion.

Mammography results can be categorized with BI-RAD. For the Birad 2 category it is considered benign so it is sufficient to leave it, while Birad 3 is declared suspended, and Birad 4–5 is categorized as malignant. Therapy given to malignant breast cancer patients includes surgery, chemotherapy, and radiation.

The results of mammography examinations for 9 patients showed that BI-RADS 2 indicated benign tumors. This indicates benign results or no indication of cancer. BI-RADS 1 means negative results or no suspicious findings, while BI-RADS 2 means completely benign findings or no need to worry. The findings of this case are consistent with previous studies which revealed that BI-RADS can be utilized as a quality assessment tool in breast cancer screening.[14–16] Furthermore, this study supports the statement of several studies that mammography is an important criterion for doctors to make an accurate diagnosis of breast cancer into benign and malignant calcifications.[17–21]

Another finding from this case series was the subsequent mammography results explained that 2 (two) patients showed BI-RADS 4 which means breast cancer in women aged 38 years and 56 years. This category indicates the possibility of cancer. BI-RADS 4 shows findings that are suspicious of the possibility of cancer, so it usually requires a biopsy for further diagnosis. BI-RADS 5 shows very suspicious findings and is almost certainly cancer, so further medical attention is needed. This finding is in line with previous studies that breast cancer cases have increased in women under 40 years.[14, 22, 23]

CONCLUSION

Based on the description of the results and discussion, it can be concluded that mammography has a role in the early detection of breast cancer. Mammography examination using CC and MLO projections is a standard procedure that aims to obtain high-quality breast images and provide accurate information for early diagnosis of breast cancer or other medical conditions. Based on the case series, it can be concluded that the role of mammography is very important in finding abnormalities in the mammary glands. The role of mammography is very important in finding abnormalities in the mammary glands. Apart from

detecting tumors or mammary cancer, it can also detect abnormalities in the lymph nodes in the armpit.

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Case Report

Zero Fluoroscopy Transcatheter Device Closure in Subaortic Ventricular Septal Defect

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Abstract

Background : For the last decade, transcatheter closure of ventricular septal defect (VSD) has been the treatment of choice, using fluoroscopy as a guide. However, the risk of radiation and/or contrast agent exposure has been an issue, especially in young patients. We would like to highlight the first case of zero fluoroscopy transcatheter VSD closure in Central Java.
Case Illustration : A 27-year-old female was referred to outpatient department due to worsening shortness of breath in the last 3 months before admission. She had a history of recurrent respiratory tract infections, feeding difficulty, and failure to thrive. Her vital signs were stable, 99% oxygen saturation, and grade 3/6 pansystolic murmur in the lower left sternal border. Transoesophageal echocardiography showed 3 mm subaortic VSD, left to right shunt. Transcatheter VSD closure was successfully done using Konar-MF™ VSD Occluder No. 8/6 mm retrograde approach without fluoroscopy.
Conclusion : The first zero fluoroscopy transcatheter device closure in Central Java has been successfully done in a 27-year-old female with subaortic VSD. Zero fluoroscopy transcatheter VSD closure is a feasible, safe, and effective procedure.

Keywords : Ventricular septal defect closure, zero fluoroscopy, echocardiography-guided

INTRODUCTION

Ventricular septal defect (VSD), as one of acyanotic congenital heart defect, is common in children and transcatheter closure has been the treatment of choice for the last decade.[1] Even though the gold standard for transcatheter placement is a fluoroscopy-guided procedure, it may expose patient to radiation as much as 5 to 6.5 millisieverts.[2]

Therefore, the risk of radiation has always been an issue, especially in younger patients because the effects are cumulative and may cause serious complication such as malignancy.[2,3] Contrast agent used in fluoroscopy has several side effects as well. The iodinated contrast agent may cause acute contrast reactions such as mild allergic reaction (nausea, vomiting, rash) or more serious anaphylactic shock. Another common adverse effect of contrast agent is contrast-induced nephropathy (CIN).[4,5] Thus, reducing or eliminating radiation and/or contrast agent exposure is a crucial matter in congenital defect closure.

The first zero fluoroscopy transcatheter atrial septal defect closure in Indonesia was successfully done in 2018, followed by Medan, Makassar, Bandung, Malang, and Bali. We would like to highlight this case as the first zero fluoroscopy transcatheter VSD closure in Central Java.

CASE ILLUSTRATION

A 27-year-old female was referred to the outpatient department due to worsening shortness of breath in the last 3 months before admission. She complained of recurrent cough and fever since she was a child. She also complained of feeding difficulty and low body weight. About 8 years ago she felt shortness of breath triggered by heavy physical activity. The shortness of breath got worse when she had cough & fever. She denied neither bluish on lips and nails, history of seizure, nor drugs consumption of her mother during pregnancy. Her routine medication was bisoprolol 1.25 mg OD.

In January 2024 she had fever, cough, and shortness of breath. She was hospitalised and chest X-ray showed cardiomegaly, later she was consulted to cardiologist and was diagnosed to have congenital heart disease. She was referred to Dr. Kariadi General Hospital (RSDK) for further assessment and treatment. Echocardiography in RSDK showed ventricular septal defect (VSD) and she was admitted to get VSD device closure.

In the ward, her blood pressure, heart rate, and respiratory rate were 103/61 mmHg, 83 beats per minute, and 18 breaths per minute respectively. Her peripheral oxygen saturations were 99% in all extremities and she had no fever. Her height & weight were 155 cm & 55 kg. Physical examination revealed regular 1st & 2nd heart sound and a grade 3/6 pansystolic murmur in the lower left sternal border. She had normal sinus rhythm of 60 beats per minute in electrocardiogram. Chest X-ray did not show cardiomegaly. Laboratory test results were unremarkable. Transthoracic echocardiography (TTE) showed 4 mm subaortic VSD, left to right shunt, and 87 mmHg trans-VSD pressure gradient (PG).

PROCEDURE

She was scheduled to get transcatheter VSD device closure under general anaesthesia. Transoesophageal echocardiography (TEE) showed 3 mm subaortic VSD left to right shunt and mild aortic insufficiency due to right coronary cusp (RCC) prolapse. We decided to close the VSD using TEE-guided-retrograde technique.

A 5F sheath was inserted into right femoral artery, then JR-3.5 5F guiding catheter was inserted through the sheath guided by 0.035" guidewire. She was given injection of unfractionated heparin 5000 IU and ampicillin 1375 mg intravenous. Guiding catheter was pushed through the descending aorta, aortic arch, ascending aorta, and then to left ventricle. Later, guiding catheter successfully crossed into right ventricle through the VSD.

Konar-MF™ VSD Occluder No. 8/6 mm was inserted into JR-3.5 5F guiding catheter using classical technique and pushed into right ventricle through the VSD. The low-pressure disk was deployed in right ventricle, then the whole system was pulled into left ventricle, then the high-pressure disk was deployed in left ventricle, so the VSD was closed completely. The device was successfully deployed and there was no residual shunt, then the device was detached from the wire. Follow-up TTE was done the next day and showed the device was stowed in place. There was no residual shunt, left ventricle (LV), or right ventricular outflow tract obstruction (RVOT). She got acetylsalicylic acid 80 mg OD and bisoprolol 1.25 mg OD. Her clinical condition was good at follow-up in outpatient department the following week and the shortness of breath had decreased. Follow-up TTE showed the device was stowed in place, no residual shunt, LVOT, nor RVOT obstruction was seen.

DISCUSSION

Table 1 shows the management of VSD based on 2020 guidelines for the management of adult congenital heart disease by European Society of Cardiology.[6]

Percutaneous VSD closure has been performed in the last 20 years. It is more preferred by patients and families because of less length of stay, scarring, pain, and no intensive care needed.[7]

In 2023 Singab *et al*. conducted a comparative study including 72 patients and divided to transcatheter

TABLE 1
Recommendation for Intervention in Ventricular Septal Defect[6]

Recommendations	Class[a]	Level[b]
In patients with evidence of LV volume overload[c] and no PAH (no non-invasive signs of PAP elevation or invasive confirmation of PVR <3 WU in case of such signs), VSD closure is recommended regardless of symptoms.	I	C
In patients with no significant L–R shunt, but a history of repeated episodes of IE, VSD closure should be considered.	IIa	C
In patients with VSD-associated prolapse of an aortic valve cusp causing progressive AR, surgery should be considered.	IIa	C
In patients who have developed PAH with PVR 3–5 WU, VSD closure should be considered when there is still significant L–R shunt (Qp:Qs >1.5).	IIa	C
In patients who have developed PAH with PVR ≥5 WU, VSD closure may be considered when there is still significant L–R shunt (Qp:Qs >1.5), but careful individual decision in expert centres is required.	IIb	C
VSD closure is not recommended in patients with Eisenmenger physiology and patients with severe PAH (PVR ≥5 WU) presenting with desaturation on exercise.[d]	III	C

AR = aortic regurgitation; IE = infective endocarditis; L–R = left-to-right; LV = left ventricle/ventricular; PAH = pulmonary arterial hypertension; PAP = pulmonary artery pressure; PVR = pulmonary vascular resistance; Qp:Qs = pulmonary to systemic flow ratio; VSD = ventricular septal defect; WU = Wood units.
[a]Class of recommendation.
[b]Level of evidence.
[c]LV enlargement with increased stroke volume.
[d]There are limited data available for a precise cut-off, but by clinical experience, this would be given by a fall of arterial oxygen saturation <90%.

and surgical VSD closure. The result of the study was no significant difference in residual VSD, but there were significant differences in the duration of ventilator, intensive care and total hospital length of stay, and blood transfusion in the transcatheter group.[8]

Study by Chen *et al*. evaluated 65 patients with VSD complicated by mild AR due to aortic valve prolapse (AVP), undergoing transcatheter VSD closure between 2008 and 2014. The procedure, guided by fluoroscopy and transthoracic echocardiography, achieved a high immediate success rate of 96.9% without any death or major complications, although two procedures were halted due to device-related AR worsening. After one year, no residual shunts or serious complications were observed, with 61.9% of patients showing improvement in AR and 36.5% demonstrating reduced AVP severity. The study concluded that transcatheter closure is a feasible, safe, and effective alternative to surgery for selected patients with VSD, AVP, and mild AR. However, careful patient selection and further long-term studies were needed to confirm safety and efficacy.[9]

Another study by Zhang *et al*. evaluated long-term outcomes of transcatheter closure in 164 children with perimembranous ventricular septal defect (pVSD) and AVP, categorizing AVP severity as mild, moderate, or severe. The procedure showed high success rates for mild (93.7%) and moderate (89.9%) AVP with minimal complications and favorable five-year event-free

TABLE 2
Indication for VSD Device Closure[11]

Age <18 years old	Mean±SI (%)
PVRi <4 WU.m^2, PVR/SVR ≤0.3 can undergo complete closure	PAP <40 mmHg without PVD or PVR <3 WU, recommended for closure regardless signs & symptoms.
PVRi 4-6 WU.m^2, PVR/SVR ≤0.3 can undergo closure using perforated device/patch	VSD without significant L to R shunt with recurrent history of infective endocarditis, consideration for closure
PVRi 6-8 WU.m2, PVR/SVR 0.3-0.5 is given pulmonary vasodilator treatment for 1 year then re-evaluated	PVR 3-5 WU and Qp/Qs >1.5, consideration for closure.

survival, while severe AVP had a lower success rate (58.3%) and higher incidence of AR, suggesting surgical intervention may be preferable for severe cases. No serious complications occurred during follow-up, and transient arrhythmias and residual shunts were rare and manageable. The study emphasized the importance of accurate pre-procedural evaluation, device selection, and regular follow-up to monitor complications. They concluded that transcatheter closure is a safe, effective alternative to surgery for pVSD with mild to moderate AVP, though further large-scale studies are needed to optimize treatment strategies.[10]

Indications for VSD closure using device are perimembranous, subaortic, or subarterial doubly committed (SADC) VSD which fulfilled the criteria as showed in Table 2.[11]

However, there are absolute and relative contraindication for VSD device closure. Absolute contraindication is VSD with Eisenmenger syndrome, PVR ≥5 WU, or desaturation in cardiac exercise test. Relative contraindication is sepsis or uncorrected coagulation problem.[11]

The indication for VSD device closure in this patient was the RCC prolapse causing aortic insufficiency and to prevent further complication, such as worsening of the RCC prolapse, endocarditis, or pulmonary arterial hypertension.[12] There was no clinical sign of pulmonary vascular disease (PVD) and TTE showed left-to-right shunt VSD with 87 mmHg trans-VSD PG, so we decided to close the VSD without performing right heart catheterization.

Preparation for patient undergoing percutaneous transcatheter VSD closure starts with a thorough evaluation to determine the patient's eligibility for catheter-based closure. This assessment is primarily conducted using a TTE, which helps in measuring and locating the VSD, then followed by cardiac catheterization to detect pulmonary arterial hypertension. Additional preoperative procedures are chest X-rays, electrocardiograms (ECG), and laboratory tests to assess the patient's general condition and renal function.[13]

Prior to the procedure, all patients scheduled for VSD closure should receive antiplatelet therapy, typically involving daily doses of both aspirin and clopidogrel. For patients requiring long-term antithrombotic treatment, warfarin may be used alongside low-molecular-weight heparin for bridging before and after the procedure. An intravenous dose of antibiotics, usually cefazolin or vancomycin (for those allergic to penicillin), should be administered one hour prior to the procedure. To prevent left atrial hypovolemia, patients should also receive intravenous normal saline before and during the intervention. Furthermore, all patients must be assessed by an anaesthesiologist before the procedure; generally, patients under 10 years old are given general anaesthesia, while conscious sedation is preferred for older patients.[13]

According to 2023 clinical pathway for VSD device closure without fluoroscopy in Harapan Kita National Cardiovascular Centre, the patient should be given injection of unfractionated heparin (UFH) 50–100 IU/kgBW IV (maximum dose 5000 IU) for antithrombotic therapy and cefazolin 50 mg/kgBW IV for endocarditis prophylaxis intra-procedural, and followed by acetylsalicylic acid therapy for 6 months.[11] This patient was given UFH 5500 IU intra-procedural for the antithrombotic therapy and Ampicillin 1375 mg for the endocarditis prophylaxis.

The European VSD registry studied complications related to transcatheter VSD closure in 430 patients with various types of VSDs. The procedure had a high success rate of 95.3%, using mainly Amplatzer devices tailored to the VSD type. Complications occurred in 12.7% of patients, including one death (0.2%), haemolysis, device embolization, vascular issues, infections, tachyarrhythmias, and complete atrioventricular (AV) blocks. Complete AV blocks were noted in 2.8% of patients, with half occurring during or immediately after the procedure and others developing within a week or months later, particularly in those with Amplatzer Perimembranous devices.[14]

Study by Holzer *et al*. on 100 subjects over 5 kg

undergoing perimembranous VSD closure with the Amplatzer device showed a 93% procedural success rate. Complications occurred in 29% of patients, with arrhythmia being the most common at 13%. New or worsening aortic valve regurgitation was noted in 9.2% of subjects, with some cases resolving or remaining mild during follow-up. Similarly, 9.2% experienced new or worsening tricuspid valve regurgitation, with partial resolution or mild severity in most cases. Only one patient experienced moderate or worsening valve regurgitation after a median follow-up of 182 days.[15]

Complications of device embolization can be avoided by choosing the correct size. Size of the device should be determined by the size of the defect, with an additional of 0.5 to 1 mm.[14] Another frequent complication that arises is total AV block, which seems to occur more often in younger patients. In contrast to surgical procedures, where total AV block typically manifests shortly after the operation, its onset in patients undergoing percutaneous closure is unpredictable and can occur much later.[16] Potential mechanisms for this include mechanical compression, tissue reactions, or inflammatory responses, which are generally resolved in the early stages. In some instances, patients have returned to sinus rhythm spontaneously or with the aid of temporary pacemakers, corticosteroids, salicylic acid therapy, or through device removal. The use of the Amplatzer Duct Occluder II may lower the risk of total AV block due to its flexibility and the straightforward implantation process.[7]

CONCLUSION

The first zero fluoroscopy transcatheter device closure in Central Java has been successfully done in a 27-year-old female with subaortic VSD. Zero fluoroscopy transcatheter VSD device closure is feasible, safe, and effective procedure.

Other Information

There is no conflict of interest to disclose.
The patient and her family were informed and agreed that her case as the first zero fluoroscopy VSD device closure in Central Java would be published in medical journal.

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Case Report

Bupivacaine-Fentanyl Induced Anaphylactic Reaction in Cesarean Delivery Undergoing Spinal Anesthesia: A Case Report

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Abstract

Background : Incidences of anaphylaxis during pregnancy are unreported but high risks to both infant and mother due to pharmacokinetic profile changes of local anesthesia agents in pregnancy.
Case : A 36-year-old woman (G4P2A1) delivered an infant at 40 weeks of her gestation using a cesarean surgical procedure with spinal anesthesia technique. The spinal anesthetic was administrated bupivacaine 15 mg and adjuvant analgesic (fentanyl 25 mcg). The delivery proceeded seamlessly. The patient was experiencing itching, swelling, and erythema in almost every part of her body, including her face, neck, arm, thigh, and leg in 43 minutes after drugs administration. This anaphylaxis reaction resolved after 22 minutes using 2 ampoules of intravenous dexamethasone 5 mg/ml and 1 ampoule of intravenous methylprednisolone 125 mg/ml. No data on second phase allergy was reported from the patient and infant who are born healthy and have a good response, indicated by an Apgar score 8–9.
Conclusion : The alterations pharmacokinetic profile of local anaesthesia in pregnancy can cause adverse effects such as anaphylaxis reactions. Anaphylaxis reactions require suitable therapeutic intervention (adrenaline and glucocorticoids) to ensure the safety of both the mother and infant during the delivery operation.

Keywords : *anaphylaxis, bupivacaine, fentanyl, pregnancy, spinal anesthesia*

INTRODUCTION

Spinal anaesthesia is a regional anaesthesia procedure or technique by injecting a local anaesthetic into the subarachnoid which is often used in various surgical procedures, especially in the lower body area. In Indonesia, spinal anaesthesia is commonly used for a variety of surgical operations. In one of Bekasi's hospitals, 99.4% of the population receives spinal anaesthetic during caesarean section operations.[1] The most widely used anaesthetic agent in spinal anaesthesia procedures is bupivacaine.[2]

Bupivacaine is a potent local anaesthetic that suppress action potential in the nerve cell. There are unique components of an aromatic ring structure with an amide group linkage.[3] In recent studies on the effects of using spinal anaesthesia was associated with a decrease in intraoperative blood pressure.[3,4] An earlier study, using of another local anaesthetic in pregnancy caused anaphylactic shock during delivery.[5]

Articles of anaphylactic/immunologic reaction during pregnancy are still lacking. Some incidents of anaphylaxis during pregnancy are unreported, so are in Indonesia. Anaphylactic reactions are becoming more common as time passes. In this study, we discuss anaphylaxis reaction as an adverse event of bupivacaine-fentanyl and management therapy of anaphylaxis reaction undergoing bupivacaine-fentanyl as a spinal anesthetic in pregnancy, especially cesarean delivery.

CASE PRESENTATION

A 36-year-old woman who was four times pregnant, with twice cesarean delivery and once aborted (G4P2A1). She was scheduled for a cesarean operation underlying spinal anesthesia at 40 weeks of her gestation. She had an asthma history but no allergy to the drug. She consistently carries an inhaler to alleviate asthma attacks. In the operating room, she had started hydroxyethyl starch intravenous and crystalloid intravenous fluid (CaCl2, KCl, NaCl, sodium acetate) using a transfusion set. At 10.45 a.m., spinal anesthetic was administrated bupivacaine 15 mg and adjuvant analgesic fentanyl 25 mcg in the sitting position, at the L3-L4 interspace. Observation was conducted for 10 minutes to confirm the patient's experience of foot numbness. The caesarean section operation was initiated by the obstetrician and surgical team at 11:03 a.m. The infant was delivered safely after five minutes, accompanied by loud wailing. Apgar score evaluation of the infant's health status and response is shown in Table 1.

At 11.28 a.m., the patient was experiencing itching, swelling, and erythema in almost every part of her body, including her face, neck, arm, thigh, and leg (Figure 1). The patient's blood pressure, heart rate, and respiratory rate were maintained at 126/65 mmHg, 78 pulses per minute, and 22 times per minute, respectively. The anaesthesiologist administrated 2 ampoules of intravenous dexamethasone 5mg/ml and the patient was observed for15 minutes. Section caesarean operation finished at 11.50 a.m. and the patient was moved to the recovery room.

In the recovery room, the patient continues to experience itching and increased burning sensations. Then, the anaesthesiologist administrated 1 ampoule of intravenous methylprednisolone 125mg/ml. It was observed in 20 minutes, the patient no longer experienced itching, and the redness had resolved. Vital signs remained stable, including blood pressure 138/87 mmHg, heart rate 76 pulses per minute, and respiratory rate 22 times per minute. Maternal breathing also remained stable at 99% of saturation oxygen.

DISCUSSION

This case describes an anaphylactic reaction during cesarean delivery induced by a bupivacaine-fentanyl combination. This combination was administered using a spinal anesthesia procedure. The selection of spinal anesthesia procedures is based on safety and health

TABLE 1
The Apgar Score Evaluation

Details	Time measurement		
	1st minute	5th minute	10th minute
Appearance	2	2	2
Pulse	1	2	2
Grimace	2	2	2
Activity	1	1	1
Respiration	2	2	2
Total Apgar Score	8	9	9

Figure 1. Anaphylactic reaction

TABLE 2
The characteristics of previous case report published

Study	Age	Type of anesthesia	Technique of administration	Side effects	Treatment	Prognosis
Takahashi M, *et al* [5]	42 years old	Mepivacaine	Combination of spinal and epidural anesthesia	Anaphylactic shock	Phenylephrine intermittent, Methylprednisolone 500 mg to prevent second phase of allergic	Resolved immediately
Browne I, *et al* [25]	43 years old	Lidocaine	Spinal anesthesia	Anaphylactic reaction	Not mentioned	Resolved
Iwasaki M, *et al* [26]	33 years old	Bupivacaine, Morphine	Spinal anesthesia	Anaphylaxis: hypotension, decreased saturation, hoarseness, breathlessness, skin flushing	Phenylephrine (total dose 0.4 mg), ephedrine (total dose 25 mg), hydrocortisone and famotidine.	Resolved after 23 minutes

advantages for both the mother and the infant.[6] Spinal anesthesia has been the best choice and effective for cesarean delivery with minimal complications such as low blockade respiratory, immediate patient feedback when evaluating the proximity of surgical instruments to neural elements, and reduced respiration of stomach content.[6–8]

In this case, the bupivacaine-fentanyl combination was used during the spinal anaesthesia procedure, these were injected into the spinal space to induce numbness and weakness in the lower extremities.[2] Bupivacaine was most often utilized due to its prolonged local anaesthetic mechanism of action; it also has special characteristics from amide group and is categorized as a strong local anaesthetic.[9] Fentanyl is an opioid that was co-administered to improve the efficacy of local anaesthetic. The Synergistics analgesic effect of fentanyl has given benefits such as reducing visceral pain that enhanced the efficacy of the block, and reducing the necessary dosage of local anaesthetic, thereby ensuring hemodynamic stability.[10,11]

Structure amide group and ester group of local anesthetics can cause another complex phenomenon is the cross-reactivity hypersensitivity, although patterns vary significantly among patients.[12] Amide derivates e.g. lidocaine, mepivacaine, bupivacaine, ropivacaine, prilocaine. Ester derivates e.g. procaine and tetracaine. Recent studies have shown that inconsistent patterns in cross reactions, variable reactivity factors play a major role in cross reactions. Variable reactivity occurs when a patient is allergic to one local anesthetic of the amide group and shows cross-reactions with other amides but is still tolerated with other amides. For example, a patient had allergies to mepivacaine, cross-reactivity to lidocaine

and ropivacaine but tolerated bupivacaine and levobupivacaine.[12,13] This reaction necessitates the utilization of a skin prick test to confirm cross-reactivity. The limitation of this study is its inability to demonstrate any cross-reactions occurring in patients.

The anaphylactic incident in this case has been investigated by the medical team with considering the patient's anamnesis since exposure to bupivacaine-fentanyl. The physical examination showed notable findings, namely vital signs indicative of bradycardia leading to hypotension if not promptly addressed. The assessment of the skin and mucosal membranes demonstrated itching, swelling, and erythema. This is deemed to have fulfilled the criteria for in determining the incidence of anaphylactic reactions. Recent guideline recommends supporting examinations including skin prick tests, Ig E, serum tryptase levels, and plasma histamine levels to determine etiology of anaphylaxis.[14] However, the patient declined to undergo a skin prick test due to concerns that her skin condition would worsen. The examination of serum tryptase was not conducted due to inadequate equipment and unavailability in the hospital laboratory, representing a limitation of this study. The immunological supporting examination used in this case is an increase in eosinophils up to 2 times (5.3%, normal 1–3%), although eosinophils are not primary effectors, Eosinophils contribute to allergic inflammation and immunological modulation by releasing various mediators, such as cytokines, histamine, and granule proteins, which enhance the manifestation of allergic responses.[15]

The study team has hypothesized the presence of additional causal factors. The investigation's findings indicated that intravenous hydroxyethyl starch and crystalloid fluids administered to patients did not elicit allergic responses. It is confirmed that there is no allergic reaction associated with the administration of intravenous fluids during the pre-operative and post-operative procedures. Recent studies have shown rare cases of anaphylactic reactions caused by intravenous fluids e.g. hydroxyethyl starch and crystalloid.[16] Another suspected causative agent is latex, but no evidence indicates that latex allergies have arisen following the patient's hospitalization.

Pharmacokinetic profile of bupivacaine and fentanyl

Bupivacaine had high lipid solubility which impacted the pharmacokinetic profile of bupivacaine. Lipophilic of bupivacaine determines the potency, duration of action, and plasma protein binding of local anaesthetics.[17] A recent study about the pharmacokinetic profile of bupivacaine changes in the pregnancy population showed higher AUC $_{(0-\sim)}$. AUC $_{(0-\sim)}$ describes the amount of active drug present in systemic circulation, if the AUC $_{(0-\sim)}$ value increased the amount of active drug that has an effect will also be higher.[18] That effect can be therapeutic effects or adverse effects. It's possibly can cause an anaphylactic reaction in the patient in this case. It was substantiated in the interview process; the patient reports no history of allergy to bupivacaine and screening of adverse effects monitoring using the Naranjo algorithm result probably scored (above 6 points).

Fentanyl as a strong opioid had selectively bound to gamma receptors in the peripheral and central nervous system which impacted on pharmacokinetic profile especially the metabolism of fentanyl.[19] Recent study on the pregnancy population, the metabolic ratio of fentanyl significantly was higher than non-pregnant (*p-value* = 0.001), that shown the conversion rate to major metabolite in pregnancy was faster than non-pregnant.[20] Secretion of fentanyl inactive metabolites occurs 48–72 hours by urine, probably plasma fentanyl concentrations are expected to remain elevated because an anaphylactic reaction in this case occurred 25 minutes after fentanyl administration.[21]

The combination of bupivacaine and fentanyl has been studied for pharmacokinetic interactions. Synergistic interactions of analgesic effect showed in randomized controlled studies that these combinations given fast onset duration of sensory blockade, an elevated sensory level, and extended postoperative analgesia.[22] Accumulation of the amount of drug combination in blood serum also increased and led to elevating adverse effects.[23] Other conditions have been studied, especially impaired liver and kidney function would change pharmacokinetic profile, leading to slower metabolism and excretion of these drug.[22,24]

Case study of incidence anaphylactic caused by local anaesthesia

Although anaphylactic reaction after local anaesthetic administration is unpredictable, our study found that it occurred in other pregnancies (Table 2). The patients are between 22 and 43 years old, occurring in Asia (70%). Spinal anaesthesia is a technique administration used in all the case study. The local anaesthetics involved are mepivacaine, lidocaine, and bupivacaine in combination with morphine. High lipid solubility such as mepivacaine and bupivacaine had a potential anaphylactic shock reaction in a pregnancy population.[2]

Takahashi M, *et al* showed mepivacaine as local anaesthesia can cause anaphylactic shock. It was clarified by examination of the causative agent of the anaphylaxis reaction after the operation. Mepivacaine has been reported to cause hypersensitivity reaction (redness) in the hand area instead of the others (lidocaine, procaine, bupivacaine).[5] Iwasaki M, *et al* found a shock anaphylactic reaction after bupivacaine and morphine administration in L3–L4 interspace. The manifestations are hypotension, decreased saturation, shortness of

breath, and skin flushing.[26] It's similar to this patient study. The dose of bupivacaine is 12,5 mg and 15 mg, based on theory, these doses are within the therapeutic dose range. However, pharmacokinetic profile changes during pregnancy such as higher active drugs in plasma and elevated active metabolite can cause adverse effects.[18,26]

Management treatment of anaphylactic reactions

International guidelines of anaphylactic management treatment claim adrenaline agents as first-line and glucocorticoids as adjuvants/second line.[27] Recent study, phenylephrine as adrenaline was used to resolve anaphylactic and glucocorticoids (methylprednisolone, hydrocortisone) as the second line to prevent the second phase of allergy. The results achieved good outcomes not more than 30 minutes.[5,26] However, adrenaline was not administered to treat the adverse effects in this patient. It is because the medical team's investigation indicated that the reaction was not severe. Furthermore, this patient had a history of asthma and pre-hypertension, and using adrenaline could elevate risk adverse effect of uncontrol blood pressure. So, the medical team considered intravenous glucocorticoids dexamethasone and methylprednisolone have been given to achieve therapeutic outcomes.

Glucocorticoids are potent immunosuppressant and anti-allergy agent which reduce the inflammatory process. Glucocorticoids mechanism action reduced activation mass cell and decreased maturation mass cell in central role of anaphylaxis reaction. Glucocorticoid receptors bind specific elements such as activating protein-1 nuclear factor to provide anti-inflammatory effects. Action of glucocorticoids in mast cell reduced transcription cytokines of inflammatory, arachidonic acid molecule, so promptly block the release of histamine from mast cell surface and increased inflammatory mediators.[27,28]

Effective management of anaphylaxis in pregnant women is crucial. Maternal anaphylaxis, although rare during pregnancy, presents a serious threat to both maternal and fetal health condition. Without prompt and effective intervention, it can result in critical complications such as fetal distress, neurological impairment, and even death. During pregnancy, an anaphylactic reaction can induce maternal hypoxemia, potentially leading to intrapartum asphyxia. Additionally, maternal hypotension and vasoconstriction may reduce uterine blood flow, significantly increasing the risk of severe fetal brain injury.[29]

Corticosteroids may be utilized in the management of anaphylactic reactions during pregnancy; however, several critical considerations must be considered regarding their effects during the antenatal period. Synthetic corticosteroids, when administered antenatally, readily cross the placenta and can expose the fetus to supraphysiological concentrations. While the primary therapeutic target is fetal lung maturation, other organ systems––particularly the developing neurological and immune systems––may also be affected. Emerging evidence from observational cohort studies indicates a potential association between antenatal corticosteroid exposure and long-term neurodevelopmental impairments in offspring.[30] Additionally, concerns have been raised regarding an increased risk of rare but serious infections in children exposed to antenatal corticosteroids. Recent large-scale studies conducted in the United States and Taiwan have reported a higher incidence of infections such as sepsis and pneumonia following short-term use of oral corticosteroids in the general population. However, in this case, the infant was not exposed to any medications, as the mother received treatment only after delivery.[31] Therefore, the therapeutic interventions did not affect the baby.

All the infants in these cases were delivered safely after anaphylactic problem was resolved, and the mother did not suffer from recurrent anaphylactic episodes, including the infant in present case was born safely. It is shown in the APGAR (Appearance, Pulse, Grimace, Activity, Respiration) score evaluation. The Apgar score is an assessment that indicates the health and response of infant after birth. The Apgar score for the infant in this case is 8 at first minute, 9 at five minutes, and 9 at ten minutes. A score range of 7–10 indicates that the baby is healthy and able to good respond.

A potential limitation of this case report was unreported data of allergy skin tests to detect specific allergies from some local anaesthesia and inability to demonstrate any cross-reactions occurring in patients in this patient.

CONCLUSION

This research highlights the alterations pharmacokinetic profile of local anaesthesia in pregnancy that can cause adverse effects such as anaphylaxis reactions. This s issue requires suitable therapeutic intervention (adrenaline and glucocorticoids) to ensure the safety of both the mother and infant during the delivery operation.

CONFLICT OF INTEREST

All authors declare no conflict of interest and this research did not receive specific funding. This research had ethical approval number: KE/FK/1883/EC/2024 from The Medical and Health Research Ethics Committee "Faculty Medicine, Public Health and Nursing Universitas Gadjah Mada Dr. Sardjito General Hospital".

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Case Report

Generalized Gingival Enlargement in Young Adult with Chronic Periodontitis: A Case Report

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Abstract

Background : Dental plaque is a natural biofilm that, if inadequately controlled, can lead to gingivitis and chronic inflammatory gingival enlargement. Persistent gingival overgrowth may impair both function and aesthetics, often requiring surgical intervention such as gingivectomy, when non-surgical treatments prove ineffective.
Case Report : A 22-year-old male presented with swollen and bleeding gums. Patient reported no history of systemic diseases or the use of any specific medications. Intraoral examination revealed gingival hyperemia, enlargement, and carious lesions on multiple teeth. Despite initial treatment with scaling root planing (SRP) and oral hygiene instruction, gingival enlargement persisted. Panoramic radiography revealed additional findings, including alveolar crest resorption and impacted third molars. A four-stage gingivectomy was performed under local anesthesia, followed by postoperative care, including antibiotics (Clindamycin), analgesics, mouthwash, and strict oral hygiene maintenance. Restorative treatments and splinting of mobile teeth were also carried out.
Discussion : The patient's gingival enlargement was primarily plaque-induced, further exacerbated by local factors such as dental caries. Scaling and root planing (SRP) alone proved insufficient due to the presence of fibrotic tissue, necessitating surgical intervention via gingivectomy. Postoperative healing was successful, with notable improvements in gingival contour, consistency, and oral hygiene indices. Patient education and adherence to postoperative care were crucial in achieving favorable outcomes.
Conclusion : Effective management of chronic inflammatory gingival enlargement requires accurate diagnosis, appropriate initial therapy, and surgical intervention when indicated. In this case, staged gingivectomy, combined with comprehensive oral care, successfully restored gingival health and aesthetics, emphasizing the importance of individualized treatment planning and patient cooperation.

Keywords : calculus, gingival enlargement, gingivectomy, plaque.

INTRODUCTION

Dental plaque is a structured, grayish-yellow biofilm firmly adhering to intraoral surfaces, including restorations. Composed of bacterial cells and microcolonies within a hydrated exopolymer matrix, its formation follows four phases: bacterial adhesion, phenotypic changes, rapid growth with exopolysaccharide production, and a stable balanced phase.[1,2] While plaque plays a physiological role, excessive accumulation and poor hygiene can lead to mineralization into calculus, which, along with plaque, contributes significantly to oral diseases like gingivitis.[1,3] Clinically, gingivitis presents as changes in gingival contour, color, and consistency without attachment or bone loss, managed by mechanical debridement and improved plaque control. Persistent irritants can lead to chronic inflammatory gingival enlargement, characterized by reddish to bluish, soft, and bleeding-prone gingiva, sometimes covering the tooth crown.[4] In severe cases, surgical treatment such as gingivectomy may be needed.[5,6]

Generalized gingival enlargement is a pathological overgrowth of gingival tissue across both arches, impacting aesthetics, comfort, mastication, and hygiene. It has multifactorial causes, including local factors like plaque and calculus and systemic factors such as hormonal changes, medications (e.g., phenytoin, nifedipine, cyclosporine), and systemic diseases like leukemia or immunological disorders.[7] Differential diagnoses include drug-induced hyperplasia, leukemia-related enlargement, scurvy, and hereditary gingival fibromatosis, requiring thorough clinical evaluation, blood tests, and biopsy.[8] Chronic inflammation from plaque and calculus, hormonal fluctuations in pregnancy or puberty, and systemic diseases like diabetes mellitus or hematologic disorders may exacerbate the enlargement.[9] Treatment should be individualized, addressing dominant contributing factors to achieve optimal long-term outcomes. This article aims to present a comprehensive case report of chronic inflammatory gingival enlargement in a young adult, highlighting the diagnostic approach, and surgical management using conventional gingivectomy.

CASE REPORT

A 22-year-old male patient presented to the central laboratorium of RSND Hospital Semarang, complaining of swollen gums and frequent bleeding while brushing his teeth. The patient had no history of systemic diseases, including hypertension, diabetes, or drug/food allergies. He was neither on any regular medication nor under the care of another physician. Extraoral examination

Figure 1. Initial intraoral presentation of gingival enlargement in the anterior region.
Panoramic radiographic examination revealed radio-opaque structures resembling teeth in the area of teeth 38 and 48 in a mesioangular position, alveolar crest resorption in the regions of teeth 11, 21, 22, 31, 32, 41, and 42, and edentulous areas in the regions of teeth 26 and 36.

Figure 2. Panoramic radiograph showing alveolar bone resorption and mesioangular impaction of third molars.

Figure 3. Gingivectomy Procedure

revealed no abnormalities. Intraoral examination revealed hyperemia accompanied by gingival enlargement, with soft consistency and rounded contours on the facial aspects of the upper and lower teeth. The Oral Hygiene Index (OHIS) score was 0.8 (good), and the O'Leary plaque index was 32.1% (fair). Grade 1 mobility was noted in teeth 11, 21, 22, 31, 32, 41, and 42. Cavities extending to the dentin were found on the facial surfaces of teeth 11, 21, and 22; the occlusal surfaces of teeth 14, 16, 44, 45, and 47; and the mesial surfaces of teeth 27, 46, and 47. Initial treatment consisted of scaling and root planing (SRP) and oral hygiene instructions; however, the gingival enlargement persisted.

Panoramic radiographic examination revealed radio-opaque structures resembling teeth in the area of teeth 38 and 48 in a mesioangular position, alveolar crest

Figure 4. The patient's intraoral examination after completing all treatments.

resorption in the regions of teeth 11, 21, 22, 31, 32, 41, and 42, and edentulous areas in the regions of teeth 26 and 36.

The patient consented to undergo gingivectomy to improve his dental and oral condition. The gingivectomy procedure was performed over four appointments, starting from region 1 and progressing sequentially to region 4. On December 7, 2023, the first stage of gingivectomy was carried out on the gingiva of region 11–15. The second stage was performed on December 12, 2023, in the gingiva of region 21–25. The third stage was performed on February 4, 2024, in the gingiva of region 31–35. The fourth stage was completed on March 7, 2024, in the gingiva of region 4144. Postoperative follow-ups were scheduled for 7 days and 30 days after each gingivectomy session.

The gingivectomy procedure began with the patient rinsing with water and 10% povidone iodine, followed by asepsis with 10% povidone iodine at the surgical site. Pocket probing was performed using a periodontal probe to determine the location of the epithelial attachment. Local anesthesia was administered using pehacain (lidocaine HCl with 1:100,000 epinephrine) at the mucobuccal fold and interdental areas around the surgical site. Bleeding points were created using a pocket marker inserted into the gingival sulcus parallel to the long axis of the tooth, with the beak resting on the tooth surface to accurately mark the base of the pocket. The blunt end of the pocket marker was inserted into the pocket until it touched the base, while the sharp end remained outside the pocket and was pressed to create three bleeding points––mesial, mid, and distal gingiva.

The hyperplastic tissue was incised using a Kirkland knife assisted by a No. 15 blade for the external bevel on the facial and lingual surfaces, and an Orban knife assisted by a No. 12 blade for the interdental areas. The tip of the Kirkland knife or No. 15 blade was positioned approximately 1 mm apical to the bleeding point and directed coronally at a 45° angle, as close as possible to the alveolar bone without exposing it. Incision was performed using a discontinuous cutting motion. Interproximal gingiva was excised using the Orban knife or No. 12 blade with a horizontal mesiodistal direction and subsequently elevated. Scaling and root planing (SRP) were carried out with a Gracey curette to remove plaque and calculus previously covered by the overgrown gingiva. Gingivoplasty followed, using a No. 15 blade in a unidirectional scraping motion to contour and smooth the gingival surface, eliminating any sharp or irregular edges resulting from the incision.

Through the procedure of surgical, the surgical field was periodically irrigated with sterile saline (NaCl) solution. Upon completion of the gingivectomy, the area was thoroughly irrigated and compressed with sterile gauze soaked in saline, applying light pressure and massaging from apical to coronal for approximately 3–5 minutes to facilitate tissue adaptation. A periodontal dressing was then applied over the surgical site to protect

the wound.

Postoperative instructions provided to the patient included: (1) refraining from eating or drinking for 1 hour following surgery and avoiding hot foods and beverages for 24 hours; (2) avoiding vigorous rinsing, sucking, touching, or manipulating the surgical area with the tongue or hands, particularly during the first 24 hours post-surgery; (3) maintaining oral hygiene by brushing twice daily (in the morning after breakfast and at night before sleeping) with a soft-bristled toothbrush, using the Charter method (for six months post-surgery), in which the bristles are angled 45° coronally and vibrated gently; (4) attending follow-up visits at 1 week post-surgery (for removal of the periodontal dressing) and 3 weeks post-surgery (for evaluation of wound healing). If the dressing dislodges before the follow-up and no pain is present, no intervention is necessary. However, if pain occurs, the patient is advised to contact the dentist immediately; and (5) adhering to the prescribed medications as directed.

Restorative procedures were also performed on teeth 11, 21, and 22 using composite resin, along with fiber splinting of the anterior maxillary and mandibular teeth to improve aesthetics and support gingival healing.

Following the completion of gingivectomy in all four regions and restoration of teeth 11, 21, and 22, the patient's intraoral examination (Figure 2) revealed some residual hyperemia on the facial aspect of tooth 11. The Oral Hygiene Index Simplified (OHIS) score improved to 0.67, indicating good oral hygiene. The patient continued to receive education on proper oral hygiene practices to prevent recurrence of similar conditions.

DISCUSSION

Gingival enlargement is an overgrowth of gingival tissue that affects mastication, speech, aesthetics, and oral function. It is primarily caused by plaque-induced inflammation but may also result from mechanical irritation, iatrogenic factors, drugs, systemic diseases, hormonal changes, vitamin C deficiency, genetics, or idiopathic causes.[10,11]

Chronic plaque exposure can lead to fibrotic gingival enlargement and pseudo-pocket formation, complicating oral hygiene. Contributing factors include abnormal tooth morphology, overhanging restorations, and orthodontic appliances. Early signs include papillary and marginal swelling, which may progress to partial or complete crown coverage.[10,12] The severity of enlargement is categorized as follows: grade 0 (no enlargement), grade I (confined to interdental papilla), grade II (involves papilla and marginal gingiva), and grade III (extends over three-quarters or more of the clinical crown).[10,12]

Intraoral examination for this patient was revealed caries on the facial surfaces of multiple teeth, poor oral hygiene, gingival hyperemia, swelling with rounded contours, and soft consistency. The gingival enlargement was attributed to local factors, specifically plaque and calculus accumulation, exacerbated by carious lesions on the facial surfaces of the teeth.

The initial treatment for gingival enlargement involves scaling and root planing (SRP). This approach is indicated when the gingiva appears soft, discolored, and edematous with cellular infiltration. SRP serves as an initial phase of periodontal therapy, aimed at removing plaque and calculus, which are primary sources of infection.[12] If gingival enlargement persists due to fibrotic tissue that does not resolve after SRP, or if the overgrowth obstructs the removal of tooth deposits, surgical intervention (gingivectomy) is necessary.[12] Gingivectomy involves the removal of the lateral wall of the periodontal pocket, eliminating the pocket and gingival inflammation, thereby restoring physiological, functional, and aesthetic gingival tissue. Additionally, gingivectomy helps reduce the depth of the gingival sulcus, facilitating easier daily oral hygiene maintenance.[12]

In this case, gingival enlargement was managed in a staged approach. The initial phase consisted of nonsurgical periodontal therapy with scaling and root planing (SRP), performed on September 14, 2023. During this visit, the patient received education on the importance of maintaining proper oral hygiene.

Follow-up the patient were conducted on October 3 and October 11, 2024 (20 and 27 days post-SRP, respectively), to monitor changes in gingival enlargement. Clinical examination revealed persistent gingival overgrowth characterized by erythema and soft tissue consistency, particularly in the buccal areas of teeth 15–25 and 35–44. Based on these findings, surgical intervention was indicated.

Gingivectomy was performed in four stages as follows:

1) First stage – December 7, 2023: gingiva of region 11–15
2) Second stage – December 12, 2023: gingiva of region 21–25
3) Third stage – February 4, 2024: gingiva of region 31–35
4) Fourth stage – March 7, 2024: gingiva of region 41–44

Following each gingivectomy session, the patient was prescribed Clindamycin, administered three times daily for five days to minimize the risk of postoperative infection. Clindamycin was selected due to the patient's prior unresponsiveness to Amoxicillin and Ciprofloxacin for dental pain. It was preferred for its higher efficacy compared to Penicillin and Lincomycin in managing persistent infections.[13] Tooth with poor prognosis were extracted on September 26 and October 11, 2023.

In addition to antibiotics, the patient was prescribed: (1) Mefenamic acid 500 mg (analgesic), three

times daily for three days; and (2) Chlorhexidine gluconate 0.12% mouthwash, twice daily for seven days to support oral hygiene. The patient was also instructed to maintain oral hygiene by brushing twice daily (morning after breakfast and at night before bed) using the Charter method and to return for control after seven days.

During follow-up after each gingivectomy, no pain reported. Clinically, the gingiva showed well-defined contours and firm consistency across all treated regions. The Oral Hygiene Index-Simplified (OHI-S) and the O'Leary Plaque Index both demonstrated improved scores, indicating good plaque control. Gingival healing was satisfactory, and continuous reinforcement of oral hygiene practices was provided.

The disappearance of gingival enlargement and signs of inflammation after SRP indicated that the enlargement was primarily inflammatory, influenced by the patient's level of oral hygiene. The patient demonstrated a good ability to maintain oral hygiene, as reflected in the improvement of the plaque index from high to low levels. However, despite resolving the inflammation, gingival overgrowth persisted after a month, due to the lack of tissue return to its original state, necessitating surgical intervention.

Gingival enlargement can arise from a variety of etiologies, making differential diagnosis essential when evaluating such cases. One condition to consider is gingival fibromatosis, a rare hereditary disorder characterized by slow, progressive gingival overgrowth without marked inflammation or attachment loss.[6,14] It typically presents with dense, fibrotic tissue and may be associated with certain syndromes or familial traits. Another important differential is drug-induced gingival overgrowth (DIGO), commonly linked to medications such as phenytoin, cyclosporine, and calcium channel blockers like nifedipine, which stimulate fibroblast proliferation and extracellular matrix accumulation.[6,14] In this case, the patient reported no history of systemic medication use, and clinical examination did not support the diagnosis of either DIGO or gingival fibromatosis. Therefore, the diagnosis of chronic inflammatory gingival enlargement, primarily caused by plaque and calculus accumulation, was deemed most appropriate.[6,14]

The treatment approach for gingival enlargement depends on the underlying cause and pathological changes. Although conventional scalpel gingivectomy was selected as the main treatment approach, alternative surgical modalities such as electrosurgery and laser therapy are also viable options.[11,12] Electrosurgery provides advantages such as better intraoperative bleeding control and reduced operative time; however, it may result in thermal damage to surrounding tissues, delayed healing, and patient discomfort.[11,12] Laser gingivectomy, particularly using diode or CO□ lasers, offers benefits including minimal bleeding, reduced pain, and faster healing.[12] Despite these advantages, laser techniques are often limited by higher costs, limited accessibility, and the need for specialized training. In this case, conventional gingivectomy was selected based on its clinical efficacy, operator expertise, and the available equipment at the treatment facility.[11,12]

The prognosis for this patient is good, as oral hygiene is well maintained, oral health conditions have improved, there is no systemic disease, and the patient is cooperative. In this case, after the gingiva healed from gingivectomy, the patient was also given fiber splinting on maxillary and mandibular anterior teeth to prevent the periodontitis from worsening. Long-term evaluations have shown that gingivectomy, as part of periodontal treatment, can achieve and maintain clinically healthy periodontal conditions in patients with advanced periodontal disease. This periodontal health was sustained over a 14-year follow-up period in most patients and sites, with comparable effectiveness observed across both younger and older patient populations.[15–17]

CONCLUSION

This case demonstrates that chronic inflammatory gingival enlargement can occur in systemically healthy young adults and may persist despite initial non-surgical therapy. A structured, individualized management approach involving staged gingivectomy, comprehensive oral hygiene education, and adjunctive restorative care proved successful in restoring gingival health, function, and aesthetics. This report underscores the importance of recognizing plaque as a primary etiologic factor even in the absence of systemic or pharmacologic triggers, and the need for timely surgical intervention when conservative approaches fail.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

Ethics approval and consent to participate

The patient provided written informed consent for the publication of this case and accompanying clinical images.

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Title :
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3. Contains no abbreviation unless standard

Abstract :
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2. Consists of maximum 250 words
3. Consists of 3-8 keywords
4. Is presented in English

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1. Should answer research question
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2. Stresses new or rare cases or new therapies or procedures
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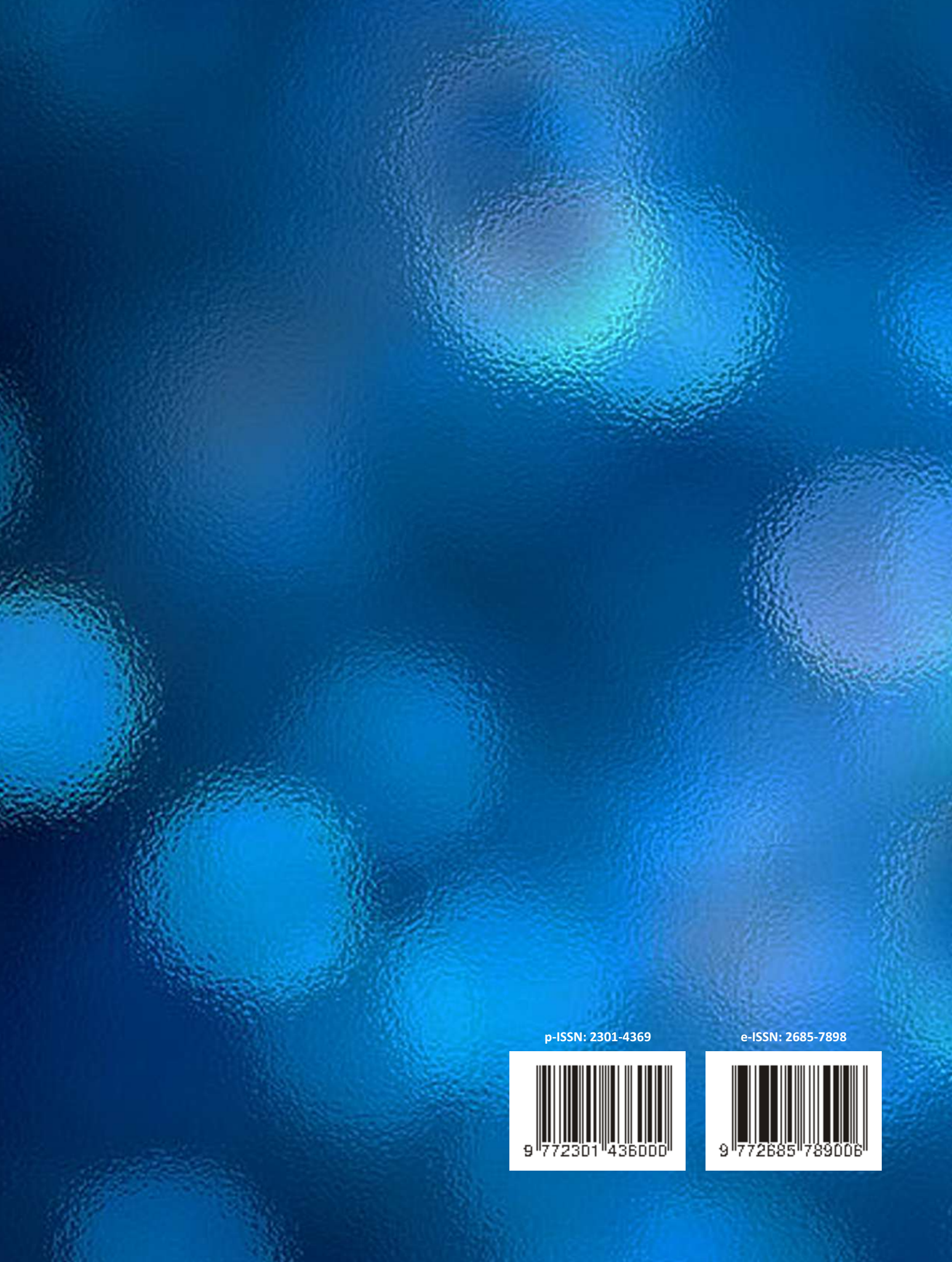

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