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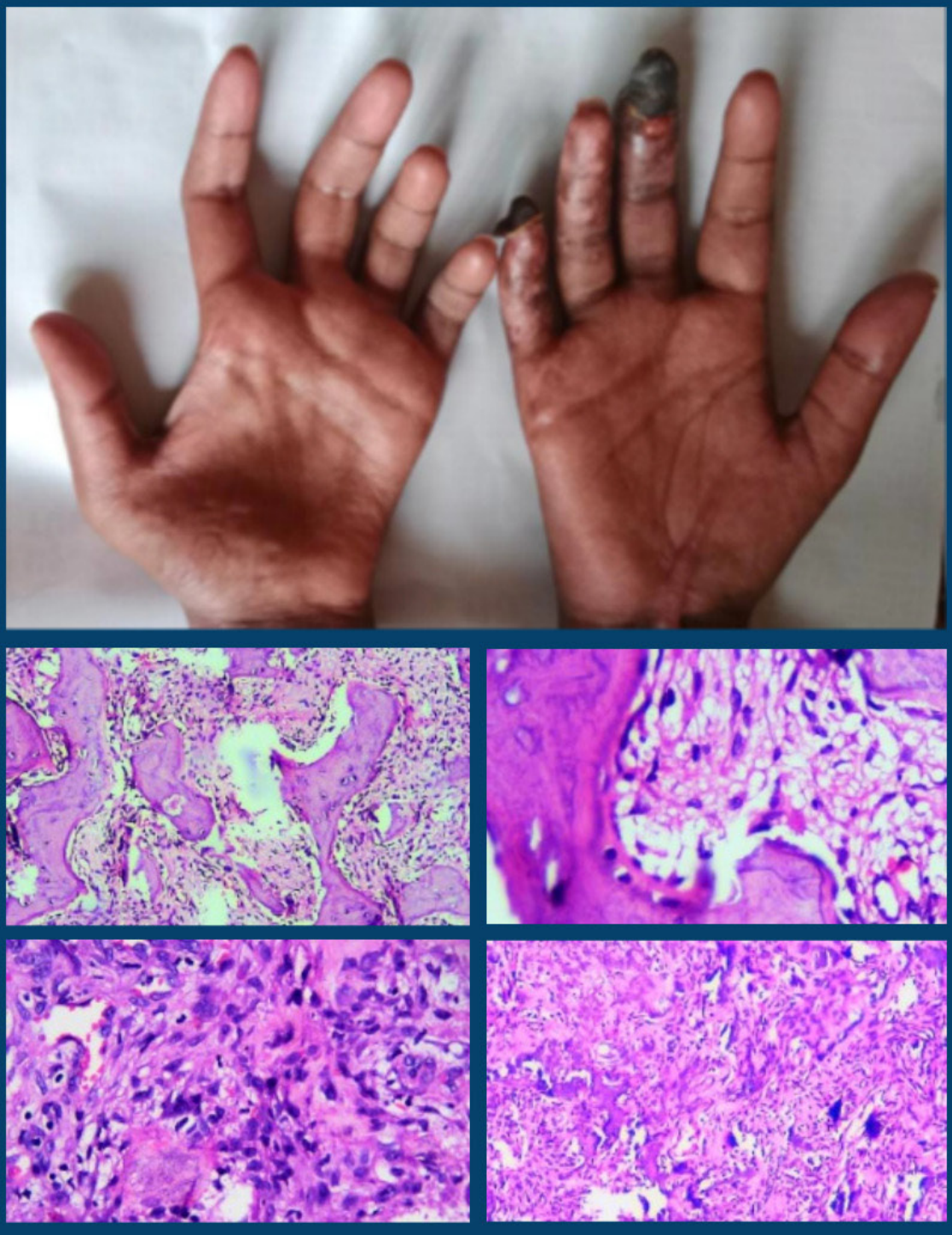

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Chemopreventive effect of dayak onion [*Eleutherine bulbosa,* Mill. (Urb)] against 7,12-dimethylbenz [α] anthracene (DMBA)-induced breast cancer in rats: study on cancer antigen 15-3 (CA 15-3)

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ABSTRACT

Dayak onion [*Eleutherine bulbosa,* Mill. (Urb)] is herbal plant believed to have anticancer effects. It contains triterpenoids, flavonoids, anthraquinones, and naphthoquinones which have antioxidants and anticancer activities. This study aimed to investigate the effect of ethanolic extract of dayak onion bulb (EEDO) on serum cancer antigen 15-3 (CA 15-3) levels in rats induced with 7,12-dimethylbenz [α] anthracene (DMBA). Thirty female Sprague Dawley rats were randomly divided into six groups, namely Normal Group, Positive Control Group (tamoxifen), Negative Control Group (dimethyl sulfoxide/DMSO) 5%), Treatment Group I (EEDO 180 mg/kgBW), Treatment Group II (EEDO 360 mg/kgBW) and Treatment Group III (EEDO 720 mg/kgBW). All groups, except the normal group, were induced with DMBA 20 mg/kg body weight. Serum CA 15-3 levels were determined using enzyme-linked immunosorbent assay (ELISA) method. The results showed significantly lower ($p < 0.05$) CA 15-3 levels in the Treatment Groups compared to the Negative Control Group. The most significant reduction in serum CA 15-3 level was observed in the Treatment Group I receiving EEDO at a dose of 180 mg/kgBW. In conclusion, the EEDO possesses a chemopreventive effect on DMBA-induced breast cancer in rats.

ABSTRAK

Bawang dayak [*Eleutherine bulbosa,* Mill. (Urb)] adalah tanaman herbal yang diyakini memiliki efek antikanker. Tanaman ini mengandung triterpenoid, flavonoid, antrakuinon, dan naftokuinon yang memiliki aktivitas antioksidan dan antikanker. Penelitian ini bertujuan mengkaji efek ekstrak etanol dari umbi bawang dayak (EEDO) terhadap kadar antigen kanker 15-3 (CA 15-3) dalam serum pada tikus yang diinduksi dengan 7,12-dimetilbenzen [α] antrasen (DMBA). Tiga puluh tikus Sprague Dawley betina, dibagi menjadi 6 kelompok secara acak, yaitu Kelompok Normal, Kelompok Kontrol Positif (tamoksifen), Kelompok Kontrol Negatif (dimetil sulfoksida/DMSO 5%), Kelompok Perlakuan I (EEDO 180 mg/kgBB), Kelompok Perlakuan II (EEDO 360 mg/kgBB), dan Kelompok Perlakuan III (EEDO 720 mg/kgBB). Semua kelompok kecuali Kelompok Normal diinduksi dengan DMBA 20 mg/kgBB. Kadar CA 15-3 serum diukur menggunakan metode *enzyme-linked immunosorbent assay* (ELISA). Hasil penelitian menunjukkan bahwa terdapat penurunan signifikan ($p < 0.05$) pada kadar CA 15-3 Kelompok Perlakuan dibandingkan dengan Kelompok Kontrol Negatif. Penurunan kadar CA 15-3 serum yang paling signifikan terlihat pada Kelompok Perlakuan yang menerima EEDO dengan dosis 180 mg/kgBB. Dapat disimpulkan bahwa EEDO memiliki efek kemopreventif terhadap kanker payudara pada tikus yang diinduksi DMBA.

Keywords:
7,12;
dimethylbenz [α]
anthracene;
breast cancer; ca 15-3;
Eleutherine bulbosa, Mill.
(Urb);
rat

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INTRODUCTION

Cancer is the second leading cause of death in the world. Based on data from The International Agency for Research on Cancer (IARC)'s Global Cancer Statistics (GLOBOCAN), in 2018 there were an estimated 18.1 million new cancer cases and 9.6 million cancer deaths. In women, breast cancer is the most prevalent type, accounting 24.2% of the 8.6 million new cases and the highest mortality rate, comprising 15.0% of 4.2 million deaths.[1]

One of the tumor markers in breast cancer is cancer antigen 15-3 (CA 15-3), which is a part of the mucin glycoprotein 1 (MUC1) found in the glandular and luminal epithelial cells of the mammary gland. In malignant conditions, MUC1 in the epithelium will multiply and secrete CA 15-3 in large quantities.[2] This condition causes an increase in serum CA 15-3 levels. In comparison to normal condition, serum CA 15-3 level can be used as a cancer marker. Cancer cells will produce CA 15-3 which can be found in serum or cancer cells tissue.[3] The CA 15-3 levels have been shown to be an independent parameter for breast cancer prognosis and are used to assess chemoprevention and anticancer treatments.[4,5]

It has been reported that DMBA (7,12 dimethylbenz(α)antracene) exposure can cause breast cancer in rats.[6] Metabolic by products from DMBA can damage DNA structure through binding of purines to DNA or depurination, resulting in DNA mutations, inhibition of apoptosis, and causing malignancy in mammary tissue.[6,7] The DMBA induction leads to estrogen receptor positive (ER+) breast cancer.[8]

Tamoxifen was the first FDA-approved chemopreventive agent, which reduces the risk of ER+ breast cancer. A systematic review by The United States Preventive Services Task Force (USPSTF) estimated that compared with placebo, tamoxifen reduced the incidence of invasive breast cancer by 7 events (95% CI) per 1,000 women over 5 years.[9,10]

Dayak onion (*Eleutherine bulbosa*) is a medicinal plant belonging to the Iridaceae family. This plant originates from South America and grows at an altitude of 600 – 2000 meters above sea level. Currently, dayak onion has been cultivated and spread to Indonesia (Kalimantan), Thailand, South China, and South Africa.[11] This plant has been used empirically by people in Southeast Asia as a traditional medicine for hypertension, diabetes, cholesterol, dysentery, anti-inflammatory, laxative, accelerate wound healing, and antifertility.[12] According to previous studies, dayak onion is also believed to have anticancer, antiosteoporosis, antibacterial, antiviral, and cytotoxic effects.[11,13-15] Based on previous studies, it is known that dayak onion has several active compounds such as naphthoquinones, anthraquinones, naphthalene, alkaloids, flavonoids, glycosides, saponins, tannins, triterpenoids, and steroids.[11,13,16,17]

Naphtoquinones and their derivatives such as eleutherine, eleutherol, eleutherinone, and elecanacine, exhibit biological activity as antimicrobial, antiviral, anti-inflammatory, antipyretic, antifungal, and also have cytotoxic effect against colon cancer and breast cancer. *In silico* studies by Lubis *et al.*[13] found that the active compound eleutherinol from dayak onion has the highest affinity among other compound to bind with human estrogen receptor alpha and has anticancer potential.[10] Based on previous *in vitro* studies by Fitri *et al.*[18] the n-hexane, ethyl acetate, and ethanolic extract from dayak onion had a cytotoxic effect on WiDr colon cancer cells and T47D breast cancer cell.[16] However, all previous studies lacked *in vivo* experiments, leading to limitations in evaluating the effects of dayak onion on more complex living organism than single cell line. The *in vivo* experiment provides a better understanding of how the dayak onion affects the

tumorogenesis dan chemoprevention in more complex living organism. The purpose of this present study was to evaluate the *in vivo* chemopreventive effect of ethanolic extract of dayak onion in rats induced with DMBA.

MATERIAL AND METHODS

Animals

Thirty female Sprague dawley rats aged 5 wk at initiation of experiment were used. All animal-use procedures were ethically reviewed and approved by Ethical Clearance Committee of the Faculty of Medicine, University of Tanjungpura prior to experiment in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Animals were housed under a 12 h light/dark cycle with free access to food and water. All efforts were made to minimize suffering and the number of animals used.

Ethanolic extract preparation

Eleutherine *bulbosa* was obtained from Sintang Regency, West Kalimantan Province, Indonesia. It was dried, powdered, and macerated with ethanol 96% then processed using rotary evaporator to get ethanolic extract of *E. bulbosa* bulb (EEDO). The ethanolic extract of dayak onion was dissolved in 5% DMSO according to each group's dosage before being administered to the rats. The DMBA (Tokyo Chemical Industry, Japan) was dissolved in corn oil with a final concentration of 4 mg/mL. Tamoxifen (Kalbe Farma, Indonesia) was dissolved in 5% DMSO with a final concentration of 0.2 mg/mL.

Treatment

After acclimatization for 7 d, 30 rats were randomly divided into 6 groups with each group consist of 5 rats. The groups were as follows: (1) Normal Control Group: only got standard food and water, (2) Negative Control Group: DMBA induction and DMSO 5%, (3) Positive Control Group: DMBA induction and tamoxifen 2 mg/kgBW/d, (4) Treatment Group I: DMBA induction and EEDO 180 mg/kgBW/d, (5) Treatment Group II: DMBA induction and EEDO 360 mg/kgBW/d, and (6) Treatment Group III: DMBA induction and EEDO 720 mg/kgBW/d.

The DMBA was administered intragastrically at a dose 20 mg/kgBW. The induction process was carried out twice weekly for 5 wk. The induction was evaluated by palpation on the chest and abdomen area; the induction was considered successful if a tumor nodule was palpable. Tamoxifen was administered intragastrically every day for 70 d in the Positive Control group (+). The EEDO was administered intragastrically every day for 70 d in Group I, Group II and Group III.

Examination of CA 15-3

After 70 d, all rats were anesthetized using chloroform and euthanized, then blood was drawn through the aorta. The blood was placed in a serum separator tube immediately after being taken from the aorta and stored at room temperature. The blood was allowed to clot for 30 min, then centrifuged for 20 min at 2000 rpm at 8° C. The separated serum sample (supernatant) was aliquoted into 1.5 mL microtube and stored in a freezer at -20° C. After 7 d, the serum was thawed at room temperature and immediately underwent the ELISA procedure to measure the CA 15-3 level. The CA 15-3 level was measured using the appropriate ELISA Kit (Bioenzy, Indonesia, CAT No. BZ-08189311-EB).

Statistical analysis

Data are expressed as mean ± standard deviation (SD) and were

analysed by one-way analysis of variance (one-way Anova). Means between treatment groups were compared using the Least Significant Difference test. All statistical analysis were conducted using SPSS software (version 23, IBM Corp, Armonk, New York). A p value <0.05 was considered statistically significant.

RESULTS

Induction of breast tumors in test animals using DMBA was evaluated by palpating the thoracic and abdominal regions where the breast glands are located. Palpation was performed every week to determine the presence or absence of tumor nodules. Tumor nodules were first palpated in the 8th wk after induction. At the end of the treatment, all rats in all groups had tumor nodules confirmed by palpation.

After measurement with ELISA method, the optical density values of each sample were obtained. Subsequently, we calculated the CA 15-3 level in each sample using the formula from the standard curve. The CA 15-3 serum level are shown in FIGURE 1.

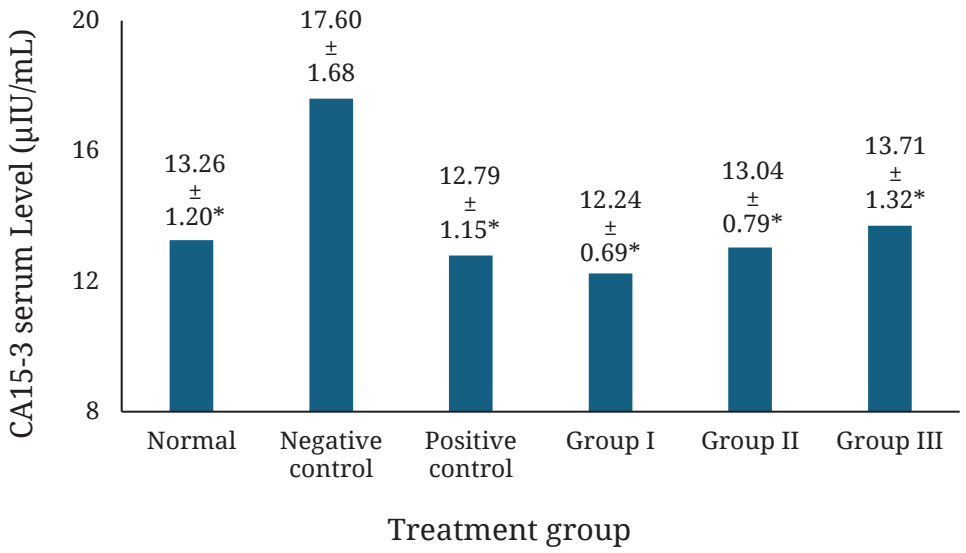

FIGURE 1. Effect of EEDO on CA 15-3 serum level in DMBA-induced breast cancer in rats. The graph shows the mean CA 15-3 serum level (µIU/mL) across different experimental groups. Data are expressed as mean ± SD. *p< 0.05 versus Negative Control group. The groups are as follows: Normal (received standard food and water), Negative Control (DMBA induction and 5% DMSO), Positive Control (DMBA induction and tamoxifen 2 mg/kg body weight/day), Group I (DMBA induction and EEDO 180 mg/kg body weight/day), Group II (DMBA induction and EEDO 360 mg/kg body weight/day), and Group III (DMBA induction and EEDO 720 mg/kg body weight/day). CA 15-3: cancer antigen 15-3; EEDO: ethanolic extract of dayak onion; DMBA: 7,12-dimethylbenz[α]anthracene; SD: standard deviation.

This study showed that the average of serum CA 15-3 level in Normal Group, Negative Control Group, Positive Control Group, Treatment Group I, Treatment Group II, and Treatment Group III were 13.26±1.20 µIU/mL, 17.60±1.68 µIU/mL, 12.79±1.15 µIU /mL, 12.24±0.72 µIU/mL, 14.04±0.69 µIU/mL, and 13.71±1.32 µUI/mL, respectively (FIGURE 1). A significantly different in the serum CA 15-3 level between the Normal Group, Positive Control Group, Treatment Group I, II and III compared to the Negative Control Group was observed ($p<0.05$). However, there was no significance difference between the Normal Group, Positive Control Group, Treatment Group I, II and III (LSD test and HSD test; $p>0.05$). Therefore, the most effective dose of EEDO for chemoprevention on DMBA induced breast cancer in rats cannot be concluded in this study.

DISCUSSION

7,12 Dimethylbenz(α)antracene is a potential carcinogen compound. This compound is an inactive procarcinogen (proximate carcinogen) in the body. It undergoes changes to become a primary carcinogen or an ultimate carcinogen. The ultimate carcinogen is the final metabolite of DMBA, which damages the DNA structure through the formation of radical cations and epoxide dihydrodiol. Epoxide dihydrodiol alters the DNA structure by covalently binding to the exocyclic amino groups of DNA purines and forming a stable DNA adduct, while the radical cation binds to N7 or C8 positions, resulting in unstable DNA adducts due to the loss of purines in DNA or depurination.[19-21]

7,12 Dimethylbenz(α)antracene undergoes activation in mammary gland epithelial cells and produces active metabolites in the form of DNA adducts, which are complexes formed by spesific DNA sections that are covalently bound to DMBA mutagen compounds.[21] Reactive oxygen species (ROS) are also products of DMBA metabolism and are formed during DMBA metabolic activation. Reactive oxygen spesies cause DNA adducts to bind to guanine bases in DNA, causing oxidative damage to the structure and function of DNA, proteins and lipids. This oxidative damage results in DNA to mutation. Cellular DNA can return to normal if the DNA repair mechanism takes place normally, if this mechanism does not function normally then the mutated cells will grow and develop into tumors.[20,21]

There is a difference in serum CA 15-3 levels between the Normal Group and the Negative Control Group, where the serum CA 15-3 level in the Negative Group was higher than the Normal Group. This was one of the pieces of evidence for the success of breast tumor induction in the negative control group.[22] According to Lee *et al.*[22] serum CA 15-3 levels in patients with breast tumors tend to increase compared to normal conditions, this increase tends to be even higher when the tumor becomes invasive and metastasizes to other tissues.

The difference in serum CA 15-3 levels between the Negative Control Group and Treatment Groups I, II, and III demonstrated that there is an effect of the administration of the EEDO on the serum CA 15-3 levels in rats with breast tumors. Spesifically, the EEDO was effective in reducing serum CA 15-3 levels in these rats. The most effective dose in reducing serum CA 15-3 levels in this study was the dose in the Treatment Group I (180 mg/kgBW).

Based on several previous studies, the EEDO has anticancer activities.[13,18,23,24] Dayak onion bulbs contain active compounds such as naphthoquinones, anthraquinones, and naphthalene.[11,13,16,17] Eleutherinol which is one of the derivatives of naphthoquinones, is an active compound known to have the most potent anticancer activity among other active compounds in dayak onion.[13,18]

One of the known effects of dayak onion extract is the suppression of mutant p53 expression. Mutant p53 is a p53 protein that undergoes a missense mutation, resulting in the p53 protein losing its tumor suppression ability and promote tumorigenesis.[25,26]

The results of this study are consistent with *in vitro* studies by Lubis *et al.*[13] which showed a suppressive effect of the EEDO on the expression of mutant p53 protein in T47D cancer cells.[18] According to Fitri *et al.*[18] the anticancer mechanism of dayak onion is achieved by inhibiting the cell cycle at the G0-G1 phase, causing the cell to be trapped in the G0 or G1 phase. This mechanism occurs through the process of inhibiting the expression of cyclin D, so that cyclin D cannot bind to CDK4 and CDK6. As a result, the phosphate group on CDK is unable to phosphorylate Retinoblastoma protein (Rb). Unphosphorylated Rb protein will bind to the E2F transcription factor and inhibit the transcription of cyclin E and cyclin A, which are needed to enter the S phase of cell replication, causing the cell will be stuck in the G1 phase.[18]

The termination of the cell cycle in the G0-G1 phase provides an opportunity for the repair process to occur in the mutated DNA, or if the mutated DNA cannot be repaired, the cell will undergo apoptosis to prevent further spreading. This apoptosis process is trigerred by the inhibition of BCL2, which is an anti-apoptotic protein.[18] The decreased rate of cell proliferation and the cessation of the cell cycle result in the inhibition of the growth and development of mammary tumor cells, preventing the metastasis process from occuring.

Cancer antigen 15-3 protein is the epitope part of Mucin 1 (MUC1). MUC1 is a transmembrane glycoprotein that is found on the surface of epithelial cells of mammary glands, pancreas, prostate, testes, liver, lungs, and kidneys.[27] In normal condition, MUC1 plays a role in cell hydration, defense against microorganisms and degenerative enzymes, as well as inhibiting interactions between cells. When the cells become malignant, MUC1 levels increase above normal, and MUC1 becomes underglycosylated and overexpressed. The location of MUC1 and growth factor receptors also become irregular due to changes in cell shape.[2,28]

Due to its extracellular location, CA 15-3 can be released and go along with the bloodstream. The amount of CA 15-3 is directly proportional to the amount of MUC1. If the levels of MUC1 increases, the levels of CA 15-3 in the blood will also increase.[2,29]

MUCI 1 and CA 15-3 are known to be predictors of prognosis in patients with breast tumors, with elevated MUC1 levels being associated with poor prognosis. MUC1 is likely to play a role in tumorigenesis, progression and metastasis in tumors. The association of MUC1 with EGFRs, β-catenin, and NF-κb can also affect tumor progression and invasiveness.[30] Overexpression of MUC1 has been shown to increase angiogenesis and chemoresistance in breast tumors.[29,31,32] Therefore, a low level of MUC1 is a factor associated with a positive prognosis in breast tumor patients.

Increased tumorigenesis activity and the number of tumor cells in breast tissue lead to increased MUC1, which in turn further worsens the condition of the tumor.[2,28] Conversely, a decrease in the amount of MUC1 increases the possibility of a good prognosis for patients with breast tumors. Inhibition of cyclin D by the EEDO can lead to a decrease in the number of tumor cells, resulting in a reduction in the amount of MUC1 in tumor cells.[2,28] The decrease in the number of MUC1 can be predicted from the decreased CA 15-3 protein levels in the serum, which are lower than in the Negative Control Group that did not receive the EEDO.

This study has some limitations. The

EEDO use in this study is not an isolated pure bioactive compound. In addition, the most effective dose of the EEDO for breast cancer chemoprevention could not be concluded, yet. Therefore, we recommend isolating bioactive compounds from dayak onion and investigating the most effective dose of dayak onion for chemoprevention.

CONCLUSION

In conclusion, the EEDO possesses a chemopreventive effect on DMBA-induced breast cancer in rats. Further study to investigate the most effective dose of the EEDO is recommended.

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EGFR mutation based on lung laterality in adenocarcinoma type of non-small cell lung cancer

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ABSTRACT

Targeted therapies have shown promise in improving survival rates for lung adenocarcinoma, a common and deadly malignancy. *EGFR*-targeting tyrosine kinase inhibitors (TKIs) are particularly effective among these therapies in cases with *EGFR* mutations. Detecting these mutations before TKI treatment is essential. Various radiological features have been linked to *EGFR* mutations. However, the relationship between tumor location and mutation types in Indonesian lung adenocarcinoma patients remains unexplored. This study aimed to identify the frequency of *EGFR* mutation in local lung adenocarcinoma cases based on the tumor location. Clinical data of lung adenocarcinoma patients (n = 272) diagnosed between 2018 and 2022 were retrospectively taken from the Department of Anatomical Pathology, Dr. Sardjito General Hospital, Yogyakarta. The qRT-PCR data of *EGFR* mutation status was obtained from the Department of Anatomical Pathology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta. Descriptive analysis was performed using STATA version 14.0. *EGFR* mutations were found in 60.7% of patients, with 58.2% having exon 19 mutations and 21.2% exhibiting exon 21 L858R mutations. Mutation status was found to be significantly different based on the patient's gender (p = 0.022) and age (p = 0.029) but not with lung laterality (p = 0.093). The proportion of exon 19, exon 21 L858R, and uncommon mutations in the right and left lung adenocarcinoma was similar across all samples. This study found no difference between specific *EGFR* mutation types and tumor location in lung adenocarcinoma.

ABSTRAK

Terapi berbasis target telah menunjukkan hasil yang menjanjikan dalam meningkatkan kesintasan untuk pasien dengan adenokarsinoma paru, suatu penyakit ganas yang sering terjadi dan mematikan. Inhibitor tirosin kinase (TKI) yang menargetkan *EGFR* telah diketahui efektif di antara terapi-terapi yang ada pada kasus dengan mutasi *EGFR*. Mendeteksi mutasi-mutasi ini sebelum pengobatan TKI adalah hal esensial. Berbagai ciri radiologis telah dikaitkan dengan mutasi *EGFR*, namun perbedaan antara lokasi tumor dan tipe mutasi pada pasien adenokarsinoma paru di Indonesia masih belum diketahui. Penelitian ini bertujuan untuk mengidentifikasi frekuensi mutasi *EGFR* pada kasus adenokarsinoma paru lokal berdasarkan lokasi tumor. Data klinis pasien adenokarsinoma paru (n = 272) yang didiagnosis antara tahun 2018 dan 2022 diambil secara retrospektif dari Departemen Patologi Anatomi, Rumah Sakit Umum Pusat Dr. Sardjito, Yogyakarta. Data qRT-PCR dari status mutasi *EGFR* diperoleh dari Departemen Patologi Anatomi, Fakultas Kedokteran, Kesehatan Masyarakat, dan Keperawatan, Universitas Gadjah Mada, Yogyakarta. Analisis deskriptif dilakukan menggunakan STATA versi 14.0. Mutasi *EGFR* ditemukan pada 60,7% pasien, dengan 58,2% memiliki mutasi ekson 19 dan 21,2% menunjukkan mutasi ekson 21 L858R. Status mutasi ditemukan memiliki perbedaan signifikan pada perbedaan jenis kelamin pasien (p = 0,022) dan usia (p = 0,029) tetapi tidak dengan lateralitas paru (p = 0,093). Proporsi mutasi ekson 19, ekson 21 L858R, dan mutasi tidak umum memiliki proporsi yang serupa antara paru kanan dan kiri pada keseluruhan sampel. Penelitian ini tidak menemukan perbedaan signifikan antara tipe mutasi *EGFR* spesifik dan lokasi tumor pada adenokarsinoma paru.

Keywords:
adenocarcinoma;
EGFR;
laterality;
lung;
tumor locationp

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INTRODUCTION

Lung carcinoma, a malignant neoplasm originating from the rapid proliferation of epithelial cells within the pulmonary system,[1] is a considerable health concern. In Southeast Asia, approximately 20% of cancer diagnoses are lung carcinoma.[2] This disease not only stands as a global primary mortality contributor but also has the highest fatality rate (14.1%) including in Indonesia.[3,4] A previously study has found that the five-year survival probability for individuals diagnosed with lung carcinoma between 2010 and 2014 hovered at a mere 10-20%.[5] This worrisome statistic can be attributed to the lack of early-stage lung carcinoma screening methodologies and the suboptimal efficacy of systemic therapeutic approaches, both of which substantially impact survival rates, particularly among cases in advanced stages.[6,7] Notably, an alarming 90% of Indonesian lung carcinoma patients receive their diagnosis only after the disease has progressed significantly.[8]

Recent advancements have given insights into the realm of genetic mutations as an innovative strategy for managing non-small cell lung cancer (NSCLC), particularly the subtype of adenocarcinoma. At present, the identification and treatment stratification for adenocarcinoma hinge upon the molecular characterization of tumors to enhance patient prognoses through more personalized therapies.[6] According to guidelines stipulated by the National Comprehensive Cancer Network (NCCN), among the pivotal molecular assessments is the examination of genetic alterations within the *EGFR* gene.[9] Across Asian populations, *EGFR* mutations manifest in approximately 40-60% of lung adenocarcinoma cases, contrasting with figures of around 12-15% in Caucasian counterparts.[10] The existence of *EGFR* gene mutations gave path for targeted therapy, which are the tyrosine kinase inhibitors (TKIs).

Correct utilization of the first, second or third generation of *EGFR*-TKI therapy in NSCLC significantly improves response rates and enhanced survival outcomes.[11] The effectiveness and resistance of different *EGFR*-TKI generations depend on the subtype of *EGFR* mutation that occurs, which are classified as the common mutations (deletion in exon 19 or L858R mutation in exon 21) and the uncommon mutations (other mutations along exon 18 to exon 21).[10,12,13] This necessitates *EGFR* mutation analysis before initiating therapy. Nonetheless, Indonesia's molecular testing infrastructure could be improved, to reduce treatment delays. As such, investigations are imperative to predict the *EGFR* mutation status.

Research has been undertaken to assess EGFR mutation status in adenocarcinoma based on tumor anatomical location via radiological methodologies. A 2016 study in Taiwan by Tseng *et al.*[14] revealed a higher prevalence of EGFR mutations (71%) in women with upper lobe adenocarcinoma compared to men harboring lower lobe adenocarcinoma (47%). They also reported[14] that the L858R mutation within exon 21 exhibited more significant predominance in upper lobe tumors relative to the exon 19 deletion and wild-type variants. In 2017, Shi *et al.*[15] published a study which assessed both common *EGFR* mutations in comparison to the wild type, and found similar tendency regarding the lobar occurrence, but it was not reported to be statistically different. Nevertheless, similar studies in terms of predicting *EGFR* mutation subtypes based on lung adenocarcinoma tumor localization are still lacking in Indonesia. Thus, additional research in this domain remains essential.

MATERIAL AND METHODS

Study design

This study constitutes a retrospective observational-analytical study employing a cross-sectional

methodology. The primary objective is to assess the difference between tumor location based on radiological discoveries and the *EGFR* mutation status within lung adenocarcinoma cases documented at the Department of Anatomical Pathology, Dr. Sardjito General Hospital, Yogyakarta, between 2018 and 2022. Slides from patients diagnosed with adenocarcinoma type non-small cell lung carcinoma by cytopathology were included. These slides were stored in enclosed drawers at room temperature. The radiological data were accessed via the hospital's electronic medical records system. Initially, 438 lung adenocarcinoma cases were collected. However, cases with inadequate radiological data or specimens unsuitable for PCR examination were excluded. This resulted in a dataset of 272 patients, including 213 who had CT-guided transthoracic needle aspiration (TTNA) and 59 who underwent pleural puncture. PCR examination for *EGFR* mutation was performed at the Department of Anatomical Pathology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada. The study adhered to the principles outlined in the Declaration of Helsinki. Specimen and data collection was approved by the Medical and Health Research Ethics Committee (MHREC), Universitas Gadjah Mada (reference number: KE-FK-0291-EC-2023).

DNA extraction

DNA extraction was performed from cytologic slides or cell blocks using GeneAll® Exgene™ Clinic SV mini 108-101 (Gene All Biotechnology Co., Ltd., Seoul, Korea) in adherence with the manufacturer's protocol.

Quantitative real-time polymerase chain reaction (qRT-PCR)

The DNA assay was done using The Human *EGFR* Gene Mutations Fluorescence Polymerase Chain Reaction (PCR) Diagnostic Kit by Amoy Diagnostics Co., Ltd. (Xiamen, China), which covers G719A, G719S, G719C, T790M, S768I, L858R, L861Q mutations, three insertions in exon 20, and 19 base changes in exon 19. Bioneer Exicycler™ 96 Real-Time Quantitative Thermal Block was utilized for quantitative PCR examination. The application of real-time PCR in this study was preferred as it is more efficient than conventional PCR, while also being in accordance with previous study.[15] The diagnostic kit and the PCR system were per the manufacturer's guidelines.

The DNA region of interest was amplified using PCR with specific primers and probes for mutant gene detection. Amplification continued until double-stranded DNA separation occurred. The mutation detection was based on the cycle threshold (Ct) values and would be determined as positive (present) if there were any signals exceeding the kit background fluorescence. For every cycle we conduct, we consistently utilize both positive and negative controls of the kit to ensure the accuracy of mutation detection. The real-time PCR included an initial denaturation cycle at 95 °C for 5 min, followed by 15 cycles of annealing at 95 °C for 25 sec and 64 °C for 20 sec, and then 31 cycles of extension at 93 °C for 25 sec, 60 °C for 35 sec, and 72 °C for 20 sec.

Data analysis

The PCR results for *EGFR* mutations were classified according to the previous literatures,[12,15] as wild-type, common mutation, and uncommon mutation. Negative *EGFR* mutation PCR result was considered as wild-type. Deletion in exon 19 and L858R mutation in exon 21 were both classified as the common mutations, while the other detected mutations were classified as the uncommon mutations.

Statistical analysis was conducted through STATA software version 14, whereby data consisting of age, gender, tumor location and *EGFR* mutation status was analyzed with chi-square test. In

cases where the data did not align with the prerequisites for the chi-square test, Fisher's exact test was implemented. The proportional difference would be considered statistically different if the p-value is less than 0.05.

RESULTS

Among the study cohort, 153 individuals (56.3%) were female, while 119 (43.7%) were male. The mean age of the participants was 60 yr, predominantly comprising those aged >50 (78.7%), with the oldest participant being 92 y.o. and the youngest being 17 y.o. The prevalence of adenocarcinoma was notably higher in the right lung, accounting for 59.2% of cases, in contrast to the left lung (39.3%), and a minority manifested in both lungs simultaneously (1.5%). Most adenocarcinoma patients (60.7%) exhibited a positive *EGFR* mutation status. The most prevalent EGFR mutation was identified in exon 19, manifesting in 96 individuals (58.2%), followed by the L858R mutation within exon 21, observed in 35 patients (21.2%). A comprehensive overview of the subjects' attributes is provided in TABLE 1.

Within the scope of this investigation, a significant difference was identified between *EGFR* mutations and gender (p=0.022), as well as age (p=0.029). Regarding the tumor's distribution, the *EGFR* mutation status demonstrated no statistically significant link to lung laterality (p=0.226). Nevertheless, an observation emerged indicating a marginally elevated frequency of *EGFR* mutations within the right lung relative to the left lung. The specific difference between *EGFR* mutation status and gender, age, and lung laterality are outlined in TABLE 2.

TABLE 1. Characteristics of study subjects

Characteristics	Frequency
Gender	
Male	119 (43.7)
Female	153 (56.3)
Age [median (min. – max.) yr]	59 (17-92)
≤ 50 [n (%) yr]	58 (21.3)
> 50 yr [n (%) yr]	214 (78.7)
Lung laterality [n (%)]	
Right	161 (59.2)
Left	107 (39.3)
Bilateral	4 (1.5)
EGFR mutation [n (%)]	
Mutation (+)	165 (60.7)
Exon 19	96 (58.2)
Exon 21 (L858R)	35 (21.2)
Uncommon	34 (20.6)
Mutation (-)	107 (39.3)
Total [n (%)]	272 (100)

EGFR: epidermal growth factor receptor

TABLE 2. *EGFR* mutation status based on gender, age and lung laterality

Parameter	Mutation status [n (%)]		p
	Negative	Positive	
Gender			
Male	56 (47.1)	63 (52.9)	0.022*
Female	51 (33.3)	102 (66.7)	
Age			
≤ 50 yr	30 (51.7)	28 (48.3)	0.029*
> 50 yr	77 (36.0)	137 (64.0)	
Laterality			
Right	66 (41.0)	95 (59.0	
Left	38 (35.5)	69 (64.5)	0.226+
Bilateral	3 (75.0)	1 (25.0)	

EGFR: epidermal growth factor receptor; *Analyzed using chi-square test; +Analyzed using Fischer's exact test.

TABLE 3. Frequency of mutation in exon 19, 21 (L858R), and others based on lung laterality

Lung laterality	Exon mutation [n (%)]			p*
	19	21 (L858R)	Uncommon	
Right	55 (57.9)	24 (25.3)	16 (16.8)	
Left	41 (59.4)	10 (14.5)	18 (26.1)	0.093
Bilateral	0 (0.0)	1 (100)	0 (0.0)	

*Analyzed using Fisher's exact test.

The prevailing mutations identified within the right and left lungs during this study predominated on exon 19. Furthermore, it was observed that the proportion of exon 19, exon 21 L858R, and uncommon mutations in the right and left lung adenocarcinoma was similar across all samples. TABLE 3 shows the frequency distribution of common mutations within exon 19, exon 21 (L858R), and the uncommon mutations categorized by lung laterality.

TABLE 4 demonstrates the frequency distribution of exon 19 and exon 21 (L858R) mutations alongside the uncommon mutations, focusing on gender and age aspects within each side of lung. Notably, across all age groups and genders in both lungs, the most prevalent mutations occurred within exon 19. The right lung exhibited the highest prevalence of exon 21 (L858R) mutation among older female patients diagnosed with lung adenocarcinoma. In contrast, it was observed that the uncommon mutations were primarily localized in the left lung of younger female patients. Nevertheless, no differences were identified.

TABLE 4. Frequency of mutation in exon 19, 21 (L858R), and others based on lung laterality, stratified by gender and age

Gender	Age (yr)	Lung laterality	*EGFR* mutation [n (%)]			p*
			19	21(L858R)	Uncommon	
Male	≤ 50	Right	4 (66.7)	1 (16.7)	1 (16.7)	-
		Left	0 (-)	0 (-)	0 (-)	
		Bilateral	0 (-)	0 (-)	0 (-)	
	> 50	Right	19 (65.5)	5 (17.2)	5 (17.2)	1.000
		Left	18 (64.3)	4 (14.3)	6 (21.4)	
		Bilateral	0 (-)	0 (-)	0 (-)	
Female	≤ 50	Right	8 (80)	2 (20)	0 (0)	0.207
		Left	6 (50)	2 (16.7)	4 (33.3)	
		Bilateral	0 (-)	0 (-)	0 (-)	
	> 50	Right	24 (48)	16 (32)	10 (20)	0.156
		Left	17 (58.6)	4 (13.8)	8 (27.6)	
		Bilateral	0 (0)	1 (100)	0 (0)	

EGFR: epidermal growth factor receptor; *Analyzed using Fisher's exact test.

DISCUSSION

Adenocarcinoma of the lung stands as the most documented subtype within non-small cell lung cancer (NSCLC), as indicated by multiple studies[16,17] and is associated with a notably high global mortality rate, as reported by Sung *et al.*[4] in 2021. Notably, *EGFR* mutations exhibit a higher incidence among patients with lung adenocarcinoma in Asia than in other continents. At the same time, Europe showcases the lowest prevalence, as noted in studies by Malapelle *et al.*[10] in 2021 and Melosky *et al.*[18] in 2022. Identifying *EGFR* mutations in adenocarcinoma of the lung opens avenues for targeted therapeutic interventions, potentially yielding improvements in overall prognosis.[11]

In this study, lung adenocarcinoma predominantly affected females (56.3% of all cases), as previously reported by Barta *et al.*,[19] with a more pronounced gender disparity in Asia due to indoor cooking smoke exposure.[20] Individuals over 50 years accounted for 78.7% of diagnoses, consistent with a study by Zhou *et al.*,[21] which is likely to be related to age-associated oncogenic mutation accumulation.[22] Adenocarcinoma was more prevalent in the right lung, attributed to its anatomical features.[23,24] *EGFR* mutations were identified in 60.7% of cases, primarily exon 19 (58.2%) and exon 21 L858R mutations (21.2%), in line with previous research findings.[25,26] The detection of exon 19 deletions and L858R mutations in this study was noteworthy as lung adenocarcinoma with these common mutations are sensitive with the more widely available first-line therapy of EGFR-TKIs, such as gefitinib, erlotinib and afatinib.[13,15] This phenomenon was thought to be related with the specific protein alteration by common mutations that lower the affinity of adenosine triphosphate (ATP) in contrast to the wild-type receptor, and making it more responsive to EGFR-TKI instead.[13]

This study underscores a notable difference in the prevalence of positive *EGFR* mutations between genders, with 66.7% of cases occurring in females. This observation is consistent with earlier research, which reported a heightened

occurrence of *EGFR* mutations in females, with frequencies ranging from 54.9 to 71.5%. This trend is particularly pronounced in Asian populations, including Indonesia, as documented in a Syahruddin *et al*.,[27] study in 2018. One hypothesis was that Asian women may exhibit elevated estrogen levels compared to the Caucasian population.[28] Also, a correlation between estrogen receptors and *EGFR* mutations in lung adenocarcinoma has been suggested.[29]

The high prevalence of lung adenocarcinoma exhibiting positive *EGFR* mutations among the elderly population (64%) in this study aligns with the earlier investigation.[22] *EGFR* mutations, besides being linked to the genetic changes that accumulate with age, are also characterized by their dormant nature, rendering them more challenging to detect at a younger age, as stated by Herceg and Hainaut.[30] In contrast, younger individuals diagnosed with adenocarcinoma of the lung tend to manifest a more aggressive disease profile and a rarer subtype of *EGFR* mutation, culminating in a less favorable prognosis and response to EGFR-TKI therapy, a phenomenon elucidated by Hsu *et al*.[31]

The prevalence of *EGFR*-mutant tumors in the right lung was found to be lower at 59% compared to 64.5% in the left lung, this is in contrast with the non-mutated *EGFR* group. It's important to note that while this observation did not reach statistical significance, it is in contrast with previous study conducted in Asian populations.[24] Furthermore, this study's findings align with those of Rizzo *et al*.,[32] in Wisconsin, United States, involving a sample of 280 patients, which similarly reported no difference between tumor location and *EGFR* mutation status in lung adenocarcinoma.

In this investigation, no difference was observed between the affected lung in adenocarcinoma and the specific subtypes of *EGFR* mutations, a finding consistent with prior research conducted by Rizzo *et al*.[32] However, there were variations in the distribution of *EGFR* mutation frequencies. While the occurrence of exon 19 mutations was comparable in both lungs, the L858R mutation in exon 21 manifested more frequently in the right lung, in contrast to uncommon mutations, which displayed a higher prevalence in the left lung.

A previous study by Shi *et al.*[15] involving 179 EGFR-mutant adenocarcinoma patients, indicated that both exon 19 and L858R mutations tended to occur more often in primary tumors of the right lung, at 59% and 57%, respectively. Similarly, Isaka *et al*.[33] presented a higher frequency of both exon 19 (53.4%) and exon 21 (59.7%) of *EGFR*-mutated tumors in the right lung among 212 Japanese lung adenocarcinoma patients, although statistical significance was not reached. The inconsistencies in these findings compared to our study may stem from differences in the proportional distribution of *EGFR* mutation subtypes. Both previous studies observed a higher frequency of exon 21 mutations relative to exon 19 mutations in their respective populations, hinting at potential racial variations in their prevalence.

The current study provides a comprehensive profile of *EGFR* mutations prevalent in Indonesian patients, revealing that 58.2% have exon 19 mutations and 21.2% exhibit exon 21 L858R mutations. This information is crucial for clinicians to make informed decisions regarding targeted therapies. By analyzing the relationship between tumor location and *EGFR* mutation types, the study concludes that there is no difference between specific *EGFR* mutation types and tumor location. This finding challenges previous assumptions and underscores the need for further investigation in this area. The study focuses on a specific population in Yogyakarta, Indonesia. Future research

should include a more diverse geographic and sample representation to validate these findings and understand regional variations in *EGFR* mutation prevalence. Additionally, the current study did not examine exon 20 mutations of the *EGFR*. Further research is required as new targeted therapies, such as amivantamab, have been developed to target *EGFR* exon 20 mutations previously resistant to tyrosine kinase inhibitors.

CONCLUSION

The prevalence of *EGFR*-mutant lung adenocarcinoma at Dr. Sardjito General Hospital is 60.7%. *EGFR* mutations are more prevalent in females and individuals aged over 50 years. The study did not observe any differences in the proportions of *EGFR* mutation subtypes with respect to lung laterality.

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C-reactive protein (CRP) and lactate dehydrogenase (LDH) as functional outcome predictors in stroke patients

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ABSTRACT

Stroke is a neurologic disorder with high mortality and disability. Its pathophysiology is associated with vascular inflammation. However, studies between vascular inflammatory markers and stroke outcomes are still limited. This study aimed to investigate the association between inflammatory markers and functional outcomes of stroke. This was a retrospective cohort study involving all stroke patients at the Dr. Sardjito General Hospital, Yogyakarta from October 2020 to August 2021 who meet the inclusion and exclusion criteria. Mann-Whitney was used for bivariate analysis, followed by multivariate analysis. A total of 269 subjects, with 213 infarcts (79.2%) and 56 hemorrhagic (20.8%) strokes. There were 83 subjects deceased (30.9%), with 66 infarct (31%) and 17 hemorrhagic (30.4%) strokes. High CRP levels had significant and independent associations with worse GCS, ADL, IADL, NIHSS, BI, SSGM, MRS, and higher mortality rates ($p < 0.05$). High LDH levels had a significant and independent association with worse GCS scores and higher mortality rates ($p < 0.05$). Sub-analysis showed high CRP and LDH had associations with high mortality rates in infarct ($p < 0.001$), but only CRP ($p = 0.029$) had associations with high mortality rates in hemorrhagic. There was no significant association between fibrinogen and procalcitonin with stroke outcomes ($p > 0.05$). Cox-regression analysis showed CRP>24.5 mg/dL and LDH >300U/L associated with hazard ratios of 3.2 ($p < 0.001$) and 1.65 ($p = 0.026$). In conclusion. high CRP and LDH levels are associated with mortality rates in stroke patients.

ABSTRAK

Stroke merupakan kelainan neurologis dengan angka kematian dan kecacatan tinggi. Patofisiologi stroke dikaitkan dengan peradangan pembuluh darah. Namun, penelitian antara penanda inflamasi vaskular dan luaran stroke masih terbatas. Penelitian ini bertujuan untuk mengkaji hubungan antara penanda inflamasi dan luaran fungsional stroke. Penelitian ini merupakan penelitian kohort retrospektif yang melibatkan seluruh pasien stroke di RSUP Dr. Sardjito Yogyakarta pada bulan Oktober 2020 hingga Agustus 2021 yang memenuhi kriteria inklusi dan eksklusi. Mann-Whitney digunakan untuk analisis bivariat, dilanjutkan dengan analisis multivariat. Sebanyak 269 subjek, dengan rincian 213 infark (79,2%) dan 56 stroke hemoragik (20,8%). Subjek meninggal dunia sebanyak 83 orang (30,9%), stroke infark sebanyak 66 orang (31%) dan stroke hemoragik sebanyak 17 orang (30,4%). Tingkat CRP yang tinggi memiliki hubungan yang signifikan dan independen dengan GCS, ADL, IADL, NIHSS, BI, SSGM, MRS yang lebih buruk, dan angka kematian yang lebih tinggi ($p <0,05$). Tingkat LDH yang tinggi memiliki hubungan yang signifikan dan independen dengan skor GCS yang lebih buruk dan angka kematian yang lebih tinggi ($p <0,05$). Sub-analisis menunjukkan CRP dan LDH yang tinggi berhubungan dengan tingkat kematian yang tinggi pada infark ($p <0,001$), namun hanya CRP ($p = 0,029$) yang memiliki hubungan dengan tingginya angka kematian pada hemoragik. Tidak terdapat hubungan bermakna antara fibrinogen dan prokalsitonin dengan luaran stroke ($p > 0,05$). Analisis regresi Cox menunjukkan CRP>24,5 mg/dL dan LDH >300U/L berhubungan dengan rasio bahaya sebesar 3,2 ($p < 0,001$) dan 1,65 ($p = 0,026$). Kesimpulannya, kadar CRP dan LDH yang tinggi berhubungan dengan angka kematian pada pasien stroke.

Keywords:
C-reactive protein; functional outcome; lactate dehydogenes; stroke

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INTRODUCTION

Stroke is a neurological disease with high morbidity and mortality.[1] World Stroke Organization reported 13.7 million new stroke cases and around 5.5 million deaths occur annually.[1] Approximately 87% of all deaths and disabilities due to stroke occur in low and middle-income countries. In Indonesia in 2018, the prevalence of stroke at the age of ≥15 y.o. was 10.9% or an estimated 2.120.362 people. East Kalimantan (14.7%) and Yogyakarta Special Region (14.6%) had the highest stroke prevalence in Indonesia.[2]

The pathophysiology of stroke begins with damage to blood vessel endothelial cells caused by a complex cascade that activates the systemic inflammatory response in both ischemic and hemorrhagic strokes.[3] This inflammatory response activates the immune system, such as macrophages and T cells, then forms a plaque that attracts other inflammatory mediators. Inflammatory mediators of pro-inflammatory cytokines, free radicals, and proteases can induce plaque rupture and thrombosis. Both ischemic and hemorrhagic brain damage can stimulate the movement and migration of immune cells, predominantly neutrophils and macrophages, into the brain and induce a systemic inflammatory response.[4]

C-reactive protein (CRP) is a marker to detect systemic inflammatory status, evaluate therapy, and predict the risk of future atherosclerotic diseases such as stroke and cardiovascular disease.[1,3] It is produced several hr after the onset of tissue injury until it peaks in 48-72 hr.[5] Increasing inflammatory markers such as CRP, IL-1, and IL-6 contribute to the pathogenesis of the ischemic brain and worsen the functional neurological outcome.[6]

Lactate dehydrogenase (LDH) is a cytoplasmic enzyme, the end product of glycolysis, found in many body cells and tissues, including muscle, liver, and brain. The presence of extracellular LDH in serum indicates a process of cell or tissue damage caused by inflammatory processes or pathological conditions.[7,8] It can be found in severe infection and sepsis, malignancy, acute myocardial infarction, hypoxic-ischemic encephalopathy, liver diseases such as hepatic cirrhosis, and hepatic metastases.[7,9] Increased serum LDH is a marker of the process of intravascular hemolysis, thus making LDH a prognostic for patients with stroke. Although LDH is a negative indicator of functional outcomes for various diseases, the relationship between LDH and stroke is still unclear.[9]

Other inflammatory markers, such as procalcitonin and fibrinogen, play a role in the pathogenesis of stroke. Procalcitonin can lead to an inflammatory process that causes endothelial damage, thrombin formation, and microvascular disturbances.[10,11] Conversely, fibrinogen causes endothelial cell damage through an inflammatory process resulting in unstable atherosclerotic plaque progression.[12]

Several scores can be used to assess functional status, cognition, and stroke severity. The degree of severity of stroke can be evaluated by using the NIHSS (National Institutes of Health stroke scale), SSGM (*Skala Stroke Gadjah Mada*), and MRS (modified ranking scale). In contrast, the functional outcomes of stroke patients are measured by BI (Barthel index) scores, ADL (activities of daily Living), and IADL (instrumental activities of daily living). Cognitive outcomes were assessed using MMSE (mini-mental state examination) and Moca-Ina (Indonesian version of Montreal cognitive assessment) scoring. Functional status is essential in determining patient management, as prognostic testing using inflammatory markers is a good option.[1,2,13] However, studies related to LDH, CRP, procalcitonin, and fibrinogen markers on stroke outcomes ae still limited. This study aimed to determine the prognostic relationship between LDH, CRP, procalcitonin, and fibrinogen and stroke patient outcomes.

MATERIALS AND METHODS

Subject

This was a retrospective cohort study involving patients with stroke at Dr. Sardjito General Hospital, Yogyakarta from October 2020 to August 2021 who met the inclusion and exclusion critrai. The inclusion criteria were 1) patients who had been diagnosed with stroke assessed from anamnesis, physical examination, and head CT scan examination, 2) had complete laboratory examination, including CRP, LDH, procalcitonin, and fibrinogen on admission. The exclusion criteria were patients with incomplete variable data.

Variables measurement

C-reactive protein, LDH, fibrinogen, and procalcitonin variables were processed at the Clinical Pathology Laboratory of Dr. Sardjito General Hospital and measured by blood samples from subjects at admission. Measurement of functional status at the end of hospitalization was assessed by BI, ADL, and IADL, while stroke severity was evaluated by NIHSS, SSGM, and MRS. Cognitive status measures were considered by MMSE and Moca-Ina score. The NIHSS had a value range of 0-34, with the higher the value indicating the more severe the degree of stroke. The BI had a value range of 0-100, and SSGM had a value range of 0-38, with low values indicating severe functional impairment. The ADL had a value range of 0-18, and the IADL had a value range of 0-14, with higher values indicating severe functional impairment. The MRS score ranged from 0-6, with higher indicating severe functional impairment. The MMSE and Moca-Ina each have a score range of 0-30, with lower scores indicating worse cognition. Another variable was GCS to measure the level of consciousness. This GCS consists of four scales: Compos Mentis is where a person is fully awake and responsive, somnolence is where a person is drowsy but still responds to the tactile or auditory stimulus, stupor is where a person is difficult to wake and only responds to a vigorous stimulus and coma is where a person does not respond in any stimulus.[14] Follow-up was carried out at the end of hospitalization to determine the duration of hospitalization and the subject's last condition. This study was approved by the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/Dr. Sardjito General Hospital, Yogyakarta (KE/FK/0288/EC).

Statistical analysis

Characteristics of the subject were presented in tabular form. Numerical variables will be analyzed for normality of the data using Shapiro-Wilk, with a $p < 0.05$ indicating that the data was not normally distributed. A multivariate test was performed with adjustments for age, sex, and BMI (body mass index) for each variable with logistic regression (categorical dependent variable) or linear regression (numerical dependent variable). Significant inflammatory variables will be analyzed by the receiver operating characteristic curve (ROC) to determine the cutoff, and then Cox-regression analysis was performed to determine the hazard ratio (HR) of these inflammatory marker variables. SPSS version 20 was used for statistical analysis.

RESULTS

A total of 292 subjects were included in this study, with males 55.5% and females 44.5%. Based on the type of stroke, there were 213 infarction cases (77.7%) and 56 cases of hemorrhage (22.3%). Subjects who died amounted to 83 patients (30.4%). Initial characteristic data of subjects are presented in TABLE 1.

High CRP levels were significantly and independently associated with worsened GCS scores, ADL, IADL,

NIHSS, BI, SSGM, MRS, and higher mortality in stroke patients ($p<0.05$) as presented in TABLE 2. High LDH levels were significantly and independently associated with worsened GCS admissions and higher mortality ($p<0.05$). Conversly, fibrinogen and procalcitonin levels were not associated with stroke functional outcomes or stroke patient mortality ($p>0.05$). Meanwhile, increased levels of procalcitonin correlated with increased levels of CRP ($p <0.007$) and increased levels of fibrinogen associated with increased levels of CRP and LDH ($p<0.05$).

TABLE 1. Characteristics of subjects

Variable	Proportion (%)	Mean ± SD
Age (yr)	-	60.71±13.31
Gender (%)		
Male	162 (55.5)	-
Female	130 (44.0)	-
BMI (kg/m^2)	-	24.11±3.99
GCS (%)		
Coma	27 (9.2)	-
Sopor	27 (9.2)	-
Somnolence	39 (13.4)	-
Compos mentis	199 (68.2)	-
Stroke type (%)		
Infact	227 (77.7)	-
Hemorrhage	65 (22.3)	-
CRP (mg/dL)	-	44.97±54.77
LDH (U/L)	-	331.73±528.41
Procalcitonin (ng/mL)	-	3.79±8.18
Fibrinogen (mg/dL)	-	451.95±157.39
LoS (d)	-	6.76±5.24
ADL	-	12.76±5.68
IADL	-	11.02±4.06
NIHSS	-	10.97±8.99
Barthel Index	-	39.81±30.28
SSGM	-	23.13±11.49
MMSE	-	22.51±6.39
Moca-Ina	-	18.64±7.54
MRS	-	3.71±1.42
Mortality (%)		
Deceased	83 (30.9)	-
Alive	186 (69.1)	-

Note. BMI: body mass index; GCS: Glasgow coma scale; CRP: C-reactive protein; LDH: lactate dehydrogenase; LoS: length of stay; ADL: activities of daily living; IADL: instrumental activities of daily living; NIHSS: National Institutes of Health stroke scale; SSGM: Gadjah Mada stroke scale; MMSE: mini-mental state examination; Mona-Ina: Indonesian version of Montreal cognitive assessment; MRS: modified ranking scale; SD: standard deviasion.

TABLE 2. Relationship between CRP, LDH, fibrinogen, and procalcitonin to various variables in all types of stroke

Variable	CRP	LDH	Procalcitonin	Fibrinogen
Age (yr)[a]				
r-adjusted	0.142	0.043	0.189	0.103
p	0.034	0.475	0.189	0.390
Gender[b]				
Male	43.31±53.45	314.91±182.33	4.15±9.10	486.08±174.87
Female	47.10±56.55	352.82±767.85	3.31±6.86	419.67±133.31
p	0.762	0.084	0.838	0.221
LoS (d)[a]				
r-adjusted	0.034	0.032	0.015	0.175
p	0.611	0.597	0.914	0.141
GCS[a]				
r-adjusted	-0.228	-0.130	0.036	0.002
p	<0.001	0.028	0.797	0.986
ADL[a]				
r-adjusted	0.281	0.119	-0.010	0.022
p	<0.001	0.047	0.941	0.856
IADL[a]				
r-adjusted	0.262	0.063	0.024	-0.048
p	<0.001	0.291	0.864	0.694
NIHSS[a]				
r-adjusted	0.264	0.117	0.631	-0.074
p	<0.001	0.05	0.631	0.544
BI[a]				
r-adjusted	-0.307	-0.071	-0.002	0.065
p	<0.01	0.238	0.991	0.593
SSGM[a]				
r-adjusted	-0.254	-0.039	-	-0.047
p	0.003	0.607	-	0.839
MMSE[a]				
r-adjusted	-0.253	-0.162	-	0.067
p	0.02	0.098	-	0.853
Moca-Ina[a]				
r-adjusted	-0.207	-0.129	-	0.319
p	0.062	0.198	-	0.402
MRS[a]				
r-adjusted	0.283	0.052	0.022	-0.105
p	<0.001	0.419	0.875	0.390
CRP (mg/dL)[a]				
r-adjusted	-	0.319	0.362	0.437
p	-	<0.001	0.007	<0.001

TABLE 2. Cont

Variable	CRP	LDH	Procalcitonin	Fibrinogen
LDH, U/L[a]				
r-adjusted	0.344	-	0.219	0.356
p	<0.001	-	0.115	0.003
Procalcitonin (ng/mL)[a]				
r-adjusted	0.353	0.219	-	-0.035
p	0.011	0.115	-	0.863
Fibrinogen (mg/dL)[a]				
r-adjusted	0.437	0.356	-0.035	-
p	0.001	0.003	0.863	-
Stroke type[b]				
Infarct	49.26±56.27	339.62±587.40	3.97±8.44	473.25±170.89
Hemorrhage	30.03±36.57	304.04±221.78	1.58±3.01	388.05±81.57
p	0.017	0.495	0.165	0.072
Mortality[b]				
Deceased	76.84±58.43	454.12±953.90	5.15±9.90	463.73±170.86
Alive	32.36±47.45	287.97±170.20	1.76±3.93	447.17±146.95
p	<0.001	<0.001	0.061	0.859

Information: [a]Analysis using linear regression adjusted for age, sex, and BMI; [b]Analysis using logistic regression adjusted for age, sex, and BMI; LoS: length of stay; ADL: activities of daily living; CRP: C-reactive protein; GCS: Glasgow coma scale; IADL: instrumental activities of daily living; LDH: lactate dehydrogenase; MMSE: mini-mental state examination; Mona-Ina: Montreal cognitive assessment Indonesian version; MRS: modified ranking scale; NIHSS: National Institutes of Health stroke scale; SSGM: Gadjah Mada stroke scale.

Sub-analysis based on the type of stroke in TABLE 3 showed that high CRP levels significantly correlated with lower GCS, BI, SSGM MMSE, Moca-Ina, and worsened NIHSS, ADL, IADL, MRS scores in stroke infarct patients ($p<0.05$). In cases of stroke infarction who deceased, CRP levels were higher (81.53 mg/dL) than the surviving group (35.03 mg/dL; $p<0.001$). For hemorrhagic stroke, only CRP levels were significantly and independently related to mortality ($p=0.029$). High LDH levels correlated with worse GCS, ADL, and NIHSS scores ($p<0.05$) and were associated with higher mortality in stroke infarction ($p<0.001$). In hemorrhagic stroke, there was no significant relationship between LDH levels and patient outcomes ($p> 0.05$). Fibrinogen and procalcitonin levels were not significantly related to functional outcome and mortality from infarct or hemorrhage stroke ($p> 0.05$).

TABLE 3. Relationship between CRP, LDH, fibrinogen, and procalcitonin to infarction stroke and hemorrhagic stroke

Variable	CRP		LDH		Fibrinogen		Procalcitonin	
	Infarct	Hemor-rhagic	Infarct	Hemorrhagic	Infarct	Hemorrhag-ic	Infarct	Hemorrhagic
LoS[a]								
r-adjusted	0.034	-0.150	0.097	-0.065	-0.049	0.632	0.202	0.505
p	0.611	0.240	0.151	0.614	0.731	0.368	0.144	0.033
GCS[a]								
r-adjusted	-0.265	-0.174	-0.153	-0.097	-0.07	0.833	-0.086	0.140
p	<0.001	0.170	0.023	0.448	0.627	0.167	0.537	0.581
Mortality[b]								
Deceased	81.53±57.28	58.64±61.04	485.69±1069.65	337.11±201.79	5.46±10.31	-	486.91±187.20	387.57±61.48
Alive	35.03±49.23	22.23±38.91	284.18±143.49	302.81±251.01	1.85±4.01	-	463.13±160.14	392.22±68.78
p	<0.001	0.029	<0.001	0.397	0.063	-	0.720	0.918
ADL[a]								
r-adjusted	0.350	0.087	0.136	0.089	0.034	-	0.087	-0.060
p	<0.001	0.506	0.045	0.500	0.814	-	0.531	0.826
IADL[a]								
r-adjusted	0.330	0.107	0.079	0.040	0.068	-	0.011	-0.196
p	<0.001	0.412	0.245	0.764	0.635	-	0.938	0.466
NIHSS[a]								
r-adjusted	0.318	0.098	0.142	0.023	-0.085	0.949	0.004	-0.190
p	<0.001	0.452	0.035	0.863	0.552	0.051	0.975	0.482
BI[a]								
r-adjusted	-0.307	-0.214	-0.073	-0.097	-0.042	0.895	0.018	0.009
p	<0.001	0.098	0.278	0.459	0.772	0.105	0.894	0.974
SSGM[a]								
r-adjusted	-0.255	0.216	-0.047	0.097	-	-	-0.083	0.048
p	0.003	0.164	0.589	0.543	-	-	0.788	0.910
MMSE[a]								
r-adjusted	-0.253	0.081	-0.164	-0.208	-	-	0.120	-
p	0.020	0.727	0.130	0.379	-	-	0.778	-
Moca-Ina[a]								
r-adjusted	-0.270	0.215	-0.138	0.182	-	-	0.301	-
p	0.062	0.392	0.212	0.484	-	-	0.468	-
MRS[a]								
r-adjusted	0.283	0.094	0.068	0.037	0.065	-	-0.004	-0.474
p	<0.001	0.511	0.349	0.795	0.655	-	0.977	0.064

[a]Analysis using linear regression adjusted for age, sex, and BMI; [b]Analysis using logistic regression adjusted for age, sex, and BMI. The - sign indicates that the analysis could not be carried out because the number of subjects in each group did not meet; ADL: activities of daily living; CRP: C-reactive protein; GCS: Glasgow coma scale; IADL: instrumental activities of daily living; LDH: lactate dehydrogenase; MMSE: mini-mental state examination; Mona-Ina: Montreal cognitive assessment Indonesian version; MRS: modified ranking scale; NIHSS: National Institutes of Health stroke scale; SSGM: Gadjah Mada stroke scale.

TABLE 4. Survival analysis CRP dan LDH towards stroke mortality

Variable	Cut off	p	Hazard ratio	95% CI
CRP	>24.5	<0.001	3.225	1.96-5.29
LDH	>300	0.026	1.651	1.06-2.56

CI: confidence interval; CRP: C-reactive protein; LDH: lactate dehydrogenase

Logistic regression analysis included the four inflammatory marker variables above, and the predictor formula was obtained (12 x CRP) + LDH where the total values were 600, 1000, and >2000, indicating a stroke mortality rate of 26.25%, 47.5%, and >75%. TABLE 4 showed analysis using Cox regression, where CRP levels >24.5 mg/dL could increase mortality 3.2 times (p <0.001), and LDH levels >300 U/L could increase mortality 1.6 times in stroke patients (p = 0.026).

DISCUSSION

This study showed that elevated CRP levels (> 24.5) and high LDH (> 300) were associated with increased stroke mortality, especially in stroke infarction. This CRP is a marker of both acute and chronic systemic inflammation, where inflammation plays an essential role in the pathogenesis of cerebrovascular disease through the mechanism of atherosclerosis formation and plaque instability, which results in easy plaque rupture. Several studies had shown that inflammation can affect atherosclerotic plaques' composition, morphology, and stability, the most common cause of stroke infarction.[15,16]

On the other hand, LDH is a marker whose levels will increase if tissue damage occurs. Increased LDH levels are markers of intravascular hemolysis, thus contributing to prognostic factors in stroke.[17] Although the underlying mechanism in the relationship between LDH and stroke outcome and mortality is still unclear, several theories can explain the above conditions. First, LDH is an inflammatory biomarker in which inflammation is related to endothelial cell dysfunction. Second, LDH is an enzyme in many organ systems, and its levels will increase if there is a disturbance in these organs.[8,18]

The mechanism between CRP and hemorrhagic stroke is still unclear. However, several studies showed that CRP levels are associated with the formation of lesions in the white matter, which indicates the involvement of the inflammatory process in the brain's small blood vessels. Bleeding may result from the rupture of small vessels from lipo hyalinosis secondary to hypertension or amyloid angiopathy. Liu *et al.*[15] reported that hs-CRP (high sensitivity CRP) levels were associated with micro brain hemorrhages in the lobar and deeper structures.[14] Other studies showed that there was a relationship between increased CRP levels and the development of stroke, as seen in increased mortality and the incidence of intracerebral hemorrhage after thrombolysis.[18]

In this study, high LDH levels did not significantly correlate with functional outcomes and hemorrhagic stroke mortality. In contrast, previous studies demonstrated that LDH levels had a positive relationship with mortality and poor functional outcome in ischemic stroke patients and in hemorrhagic stroke at three months and one-year

follow-ups.[17] Study with a longer follow-up duration is needed to show a significant relationship between LDH and functional outcome.

In this study, the relationship between fibrinogen and procalcitonin was insignificant to stroke patients' functional outcome and mortality (> 0.05). Another study reported no significant difference between fibrinogen and stroke function outcomes (p = 0.416), and there was no significant difference in fibrinogen levels between stroke patients and the control group.[12,19] This differed from the study by Di Napoli *et al.*,[20] which reported a significant association between high fibrinogen levels and poor stroke function outcomes. For procalcitonin, several studies had shown mixed results. The study by Deng *et al.*[11] reported that procalcitonin levels were associated with stroke functional outcomes, as seen from the NIHSS score (<0.001). In contrast, the study by Miyakis *et al.*,[21] showed no significant association between procalcitonin levels with mortality and stroke function outcomes based on stroke subtype. Further study to investigate the relationship between procalcitonin and fibrinogen on functional outcomes and stroke mortality is still needed.

The limitations of this study were the small number of subjects; we did not differentiate between first stroke or recurrent stroke, and the short follow-up duration. Further research is needed regarding this study. However, based on the researchers' knowledge to the present time, studies have yet to examine the relationship between CRP, LDH, fibrinogen, and procalcitonin on mortality and functional outcome of stroke as assessed by various rating scales comprehensively in Indonesia.

CONCLUSION

This study showed that CRP >24.5 mg/dL and LDH >300 U/L at admission could predict worsened functional outcomes and increased mortality in stroke patients. Mortality and functional outcome of infarction stroke are associated with high CRP and LDH levels at admission, whereas only CRP is associated with mortality and functional development of hemorrhagic stroke.

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Health comorbidities in children with down syndrome (DS) at Dr. Sardjito General Hospital, Yogyakarta

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ABSTRACT

Down syndrome (DS) is a disease caused by trisomy of chromosome 21. The phenotype in DS leads to manifestations in several organ systems. This study aimed to identify the pattern of comorbidities in DS patients. It was a single-center, cross-sectional study at Dr. Sardjito General Hospital, Yogyakarta. Medical records of pediatric patients with DS from a period of January 2022 to May 2023 were included. Descriptive analysis was performed to demonstrate demographic and clinical characteristics. A total of 355 pediatric patients with DS were found at Dr. Sardjito General Hospital and the majority were male (196 children or 55.2%). As much as 339 children (95.49%) had comorbidities. The highest comorbidity was congenital heart disease (230 patients or 67.84%) in specifics were atrial septal defect (41 patients or 12.39%), atrioventricular septal defect (29 patients or 8.17%), and patent ductus arteriosus (28 patients or 7.88%). The second highest comorbidity was endocrine system disorders (102 patients or 30.09%), with 100 patients (28.16%) children suffering hypothyroidism. The number of children who had one comorbidity was 248 patients (69.86%), 74 patients (20.48%) had two comorbidities, and 17 patients (4.79%) had three or more comorbidities. The highest co-prevalence of the two comorbidities was congenital heart disease and endocrine system disorders (36 patients or 10.14%). The highest co-prevalence of 3 or more comorbidities was a combination of congenital heart disease, visual impairment, and hearing impairment (6 patients or 1.69%). In conclusion, 95.49% of children with DS have comorbidities. The most common comorbidity was heart defects. About 25.63% of patients had more than one comorbidity. Children with DS who have comorbidities require more attention to prevent complications and to reduce morbidity.

ABSTRAK

Sindrom Down (SD) merupakan penyakit yang disebabkan oleh trisomi kromosom 21. Fenotipe dari SD menimbulkan manifestasi pada beberapa sistem organ. Penelitian ini bertujuan untuk mengidentifikasi pola penyebaran komorbid pada pasien SD. Penelitian pada satu senter, studi potong lintang di RSUP Dr. Sardjito, Yogyakarta. Rekam medis pasien anak dengan SD periode Januari 2022 sampai Mei 2023 diikutkan dalam penelitian. Analisis deskriptif dilakukan untuk menunjukkan demografis dan karakteristik klinis pasien SD. Total 355 pasien anak dengan SD di RSUP Dr. Sardjito pada kurun waktu penelitian dan mayoritas laki-laki yaitu sebanyak 196 pasien (55,2%). Sebanyak 339 pasien (95,49%) dengan komorbid. Komorbid tertinggi adalah penyakit jantung bawaan (230 pasien atau 67,84%) dengan perincian kasus *atrial septal defect* (41 pasien atau 12.39%), *atrioventricular septal defect* (29 pasien atau 8,17%), dan *patent ductus arteriosus* (28 pasien atau 7.88%). Komorbid tertinggi kedua adalah kelainan sistem endokrin (102 pasien atau 30,09%) dengan 100 pasien (28.16%) yang menderita hipotiroid. Jumlah yang memiliki satu komorbid sebanyak 248 pasien (69,86%), 74 pasien (20,48%) memiliki dua komorbid dan 17 pasien (4,79%) memiliki tiga komorbid atau lebih. Ko-prevalensi dua komorbid tertinggi adalah penyakit jantung bawaan dan kelainan sistem endokrin (36 pasien atau 10,14%). Ko-prevalensi tiga atau lebih komorbid tertinggi adalah kombinasi dari penyakit jantung bawaan, gangguan penglihatan, dan gangguan pendengaran (6 pasien atau 1,69%). Simpulan, anak dengan SD yang memiliki komorbid sebesar 95,49%. Komorbid terbanyak adalah kelainan jantung. Sebanyak 25,63% pasien memiliki komorbid lebih dari satu. Anak SD yang memiliki komorbid memerlukan perhatian lebih untuk mencegah komplikasi dan mengurangi morbiditas.

Keywords:
children;
comorbidities;
down syndrome;
prevalence;
manifestation

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INTRODUCTION

Down syndrome (DS) is one of the chromosomal disorders with the highest prevalence in humans, caused by the trisomy of chromosome 21.[1,2] The phenotype in DS encompasses various clinical manifestations that can affect several organ systems, particularly the nervous, musculoskeletal, and cardiovascular systems.[1] Clinical features include intellectual disabilities, short stature, a flat facial profile, prominent epicanthal folds, upward slanting fissures of the eyelids, and a protruding tongue.[2]

According to WHO's data from 2018, the prevalence of DS is approximately one in every 1,000 births.[2] It is also found that the prevalence of DS has significantly increased as the global population grows. In the United States, the population prevalence of DS increased from around 50,000 in 1950 (3.3 per 10,000 individuals) to about 212,000 in 2013 (6.7 per 10,000 individuals).[1] Currently, in the United States, around 500 live births with DS occur each year, and over 200,000 individuals live with this disorder.[3]

Based on data from the Indonesian Ministry of Health of Republic of Indoensia (*Pusdatin Kemenkes* RI), the incidence of DS cases in Indonesia has risen over the years. In the Basic Health Research (*Riskesdas*) results of 2010, DS cases in children aged 24 to 59 mo were recorded at 0.12%, increasing to 0.13% in the 2013 *Riskesdas*, and further rising to 0.21% in the 2018 *Riskesdas*.[4] According to the Hospital Information System (SIRS), among 2,488 hospitals, 1,657 cases of DS were reported in 2015. In 2016, data from 2,598 hospitals indicated 4,449 cases of DS, while in 2017, 2,776 hospitals reported 4,130 cases of DS.[4]

Down syndrome exhibits diverse clinical phenotypes that affect various organ systems. Over time, a study in England and Wales in 2020 reported that patterns and prevalence of comorbidities in DS may change due to improvements in care and treatment. Therefore, up-to-date information on DS and its comorbidities is essential for clinical practitioners, individuals, families, and caregivers.[5]

Another study in the United Kingdom in 2023 reported multiple morbidities were found in DS patients that show distinct patterns of age-related incidence trajectories and clustering that differ from those found in the general population and in people with other intellectual disabilities. The multiple morbidities have implications for provision and timing of health-care screening, prevention, and treatment for people with DS.[6]

We also found no studies in Indonesia, especially in Yogyakarta Special Region, that show and elaborate different cases of morbidities in DS patients. Therefore, increasing trend of DS cases in Indonesia each year and the need for current information on the pattern of comorbidities in DS cases in Indonesia serve as the fundamentals of this study. This study is expected to contribute valuable information on the patterns of comorbidities in DS cases in Indonesia, particularly in Yogyakarta Special Region.

This study is essential as identifying patterns of common comorbidities, especially in Indonesia, can benefit healthcare services in Indonesia to foresee common comorbidities in DS patients and can benefit DS patients with early intervention and enhance the quality of healthcare.

MATERIAL AND METHODS

Design and subjects

It was a cross-sectional study conducted at Dr. Sardjito General Hospital, Yogyakarta using data of the medical records of pediatric patients who confirmed with a diagnosis of DS in a period of January 2022 to May 2023. The diagnosis of DS was established by a pediatric specialist and consultant endocrinologist based on clinical criteria as documented in the medical records.

Procedure

The data collected for this study include age, gender, and the comorbidity profile of patients with DS. Patients with confirmed diagnoses of DS were included in this study, and patients with incomplete data were excluded from this study. Comorbidities were established by a pediatric specialist based on clinical criteria as documented in the medical records. This study followed the criteria of medical ethics and was accepted by the Institutional Review Board of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada/Dr. Sardjito General Hospital, Yogyakarta (KE/FK/0666/EC/2023).

Data analysis

Data were presented as frequency and percentage. Descriptive analysis was employed to present the demographic and clinical characteristics of the patients.

RESULTS

A total of 355 children with DS were involved in this study. The characteristics of patients are presented in TABLE 1. More male patients were found in this study with the average age was 3.88 ± 3.73 yr.

Among 355 pediatric patients with DS, most the patients (339 children or 95.49%) had comorbidities (FIGURE 1). The highest comorbidity was congenital heart disease (230 children or 67.84%).

The most common comorbidity found was congenital heart disease (230 or 67.84%) in specifics were atrial septal defect (41 patients or 12.39%), atrioventricular septal defect (29 patients or 8.17%), and patent ductus arteriosus (28 patients or 7.88%). The second highest comorbidity was endocrine system disorders (102 patients or 30.09%), with 100 patients (28.16%) children suffering hypothyroidism (TABLE 2).

This study found that some patients has a combination of 2 comorbidities (74 patients or 20.48%). Congenital heart disease and endocrine system disorders are the most common combinations (36 patients or 10.14%). The most minor combination found was visual impairments along with hearing impairments (9 patients or 2.53%) (TABLE 3).

This study also found that some children were diagnosed with 3 combinations of comorbidities (TABLE 4). The highest combination of comorbidities found was congenital heart disease with visual impairments and hearing impairments (6 patients or 1.69%).

TABLE 1. Characteristics of pediatric patients with DS (n=355) at Dr. Sardjito General Hospital, Yogyakarta

Characteristics	Number
Age (mean ±SD yr)	3.88 ± 3.73
Gender [n (%)]	
• Male	196 (55.2)
• Female	159 (44.8)

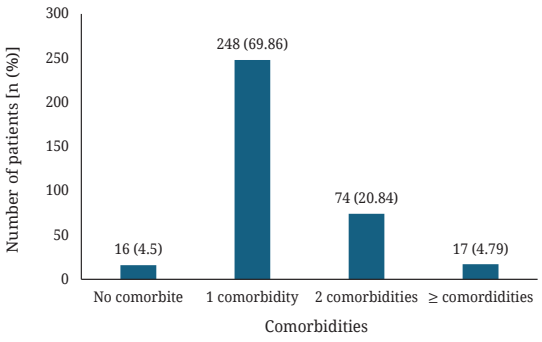

FIGURE 1. Number of comorbidities of pediatric patients with DS (n=339) at Dr. Sardjito General Hospital, Yogyakarta

TABLE 2. Detailed comorbidities profile of pediatric patients with DS at Dr. Sardjito General Hospital

Comorbidities	Number [n (%)]
Central nervous system disorders	21
• Global developmental delay	12 (3.38)
• Epilepsy	7 (1.97)
• Sleep apnea	2 (0.56)
Congenital heart disease	230
• Atrial septal defect	41 (12.39)
• Atrioventricular septal defect	29 (8.17)
• Patent ductus arteriosus	28 (7.88)
• Ventricular septal defect	16 (4.60)
• Tricuspid insufficiency	10 (2.81)
• Tetralogy of fallot	4 (1.12)
• Mitral insufficiency	3 (0.84)
• Atresia of pulmonary artery	3 (0.84)
• Pulmonary valve insufficiency	3 (0.84)
• Pulmonary valve stenosis	1 (0.28)
• Persistent foramen ovale	1 (0.28
• Tricuspid stenosis	1 (0.28)
• Ebstein anomaly	1 (0.28)
• Dextrocardia	1 (0.28)
Endocrine system disorders	102
• Hypothyroidism	100 (28.16)
• Growth and skeletal anomalies	3 (0.84)
• Congenital foot deformities	3 (0.84)
• Thyrotoxicosis	1 (0.28)
• Diabetes mellitus	1 (0.28)
Genitourinary system disorders	10
• Cryptorchidism	10 (2.81)
Gastrointestinal system disorders	28
• Biliary obstruction	9 (2.53)
• Hirschsprung's disease	9 (2.53)
• Duodenal atresia and/or stenosis	6 (1.69)
• Anal atresia and/or stenosis	4 (1.12)
Visual impairments	27
• Refractive errors	13 (3.66)
• Cataracts	6 (1.69)
• Strabismus	4 (1.12)
• Nystagmus	4 (1.12)
Hearing impairments	31
• Sensori-neural hearing loss	29 (8.16)
• Auditory perception disorders	2 (0.56)
Total	339 (95.49)

TABLE 3. Co-prevalence of 2 comorbidities of pediatric patients with DS at Dr. Sardjito General Hospital, Yogyakarta

Co-prevalence comorbidities of patients with DS	Number [n (%)]
Congenital heart disease + endocrine system disorders	36 (10.1)
Congenital heart disease + hearing impairments	14 (3.94)
Congenital heart disease + visual impairments	12 (3.38)
Visual impairments + hearing impairments	9 (2.53)
Total	71 (19.95)

TABLE 4. Co-prevalence of 3 comorbidities of pediatric patients with DS at Dr. Sardjito General Hospital, Yogyakarta

Co-prevalence comorbidities of patients with DS	Number [n (%)]
Congenital heart disease + visual impairments + hearing impairments	6 (1.69)
Congenital heart disease + endocrine system disorders + hearing impairments	2 (0.56)
Congenital heart disease + gastrointestinal system disorders + visual Impairments	1 (0.28)
Congenital heart disease + endocrine system disorders + genitourinary system disorders	1 (0.28)
Visual impairments + growth and skeletal anomalies + hearing impairments	1 (0.28)
Endocrine system disorders + growth and skeletal anomalies + gastrointestinal system disorders	1 (0.28)

DISCUSSION

In this study, it was observed that children with DS were predominantly male, consistent with findings from other studies indicating a higher prevalence of males among individuals with DS.[5,7]

Based on other studies, children with DS are known to have a higher risk of congenital anomalies such as heart and abdominal wall disorders. Additionally, they are more susceptible to vision and hearing impairments, sleep apnea, as well as growth and skeletal disorders. Individuals with DS also exhibit vulnerabilities to immune and endocrine system disorders.[8] Our study indicates that patients with DS may have multiple comorbidities, potentially more than one concurrent condition.

In this study, several children with DS were found to have comorbid epilepsy, aligning with a study by Altuna *et al.*,[9] which revealed a vulnerability to epilepsy in individuals with DS. This susceptibility may be attributed to various mechanisms, including frontal and temporal lobe hypoplasia, dendritic dyskinesia, abnormal neuronal lamination, reduced neuron density, disruptions in inhibitory GABAergic interneuron function, changes in membrane ion channel activities, and other metabolic abnormalities.[9]

Patients with DS in this study were also identified with global developmental delay. This aligns with the findings of Ferreira-Vaques and Lamonica, indicating that individuals with DS perform lower, especially in language and fine motor domains.[10] According to other studies, individuals with DS are more vulnerable to brain function disorders due to several factors, including morphological disorders of pyramidal neurons or excessive changes in inhibitory function.[11] Other studies have proposed hypotheses causing developmental delays, especially in motor skills. These include 1) Changes in the shape and size of neurons and the cerebrum; 2) Maturity disorders of the central nervous system; 3) Pathophysiological processes such as degeneration of the nervous system, disorders in the regulation of neuron apoptosis, excessive gene expression, and decreased neurotransmitter release.[12]

The majority of patients with DS in this study were found to have congenital heart disease. This aligns with several studies that found congenital heart disease to be the most frequently encountered anomaly in individuals with DS.[7,8] In this study, atrial septal defect was the most prevalent congenital heart disease, although other studies have reported atrioventricular septal defect as the most common.[8,13] However, geographical variations may lead to differences in the types of congenital heart diseases in individuals with DS.[13]

According to other studies, individuals with DS are prone to congenital heart disease due to two hypotheses. The first is the gene dosage amplification theory, stating that an increased genetic dosage from chromosome 21 (Hsa 21) in DS can enhance genetic expression leading to congenital heart disease. The second theory, the gene mutation theory, suggests that mutations on the trisomy 21 locus can increase the occurrence of congenital heart disease.[14]

Most patients with DS in this study, with comorbid endocrine system disorders, suffered from hypothyroidism. This aligns with other studies reporting that individuals with DS are significantly more vulnerable to hypothyroidism compared to normal children, up to 25-38 times higher.[8,15-17] Theoretically, this vulnerability can occur due to several hypotheses, including 1) Excessive TRH stimulation, causing delayed maturation of the hypothalamus-pituitary-thyroid axis, leading to high TSH levels with normal fT4 and fT3 and negative anti-thyroid antibodies at three years of age; 2) Peripheral resistance to thyroid hormones, causing reduced TRH secretion; 3) Lack of TSH release due to central disorders or disruption of dopaminergic control; 4) TSH insensitivity; and 5) Reduced TSH bioactivity.[15]

Patients with DS in this study were also found to have growth and skeletal anomalies. This finding aligns with other studies suggesting that individuals with DS may experience arthropathy, joint weakness, hypermobility, foot deformities, scoliosis, and hip instability.[9] This aligns with the study by Foley and Killeen, which found that the majority of patients with DS suffer from Pes Planus.[18]

The study also identified patients with DS who had genitourinary system disorders, all presenting with cryptorchidism. Another study found that individuals with DS may

experience disorders in the genitourinary system, such as posterior urethral valves, pyelectasis, megaureter, kidney malformations (renal hypoplasia, horseshoe kidney, renal ectopia), hypospadias, cryptorchidism, and small penis.[8]

In this study, comorbid gastrointestinal system disorders were also found, consistent with other studies that reported congenital anomalies in the gastrointestinal system in individuals with DS, such as duodenal atresia, anal atresia/stenosis, esophageal atresia, and esophageal fistula. Hirschsprung's disease, constipation, celiac disease, and biliary obstruction were also identified.[8,19]

Patients with DS in this study were also found to have visual impairments. Visual impairment findings in this study align with other studies indicating an increased incidence of refractive disorders due to the failure of emmetropization processes. Additionally, oblique astigmatism, cataracts, blepharitis, and nystagmus were found in patients with DS.[8,20]

The study also identified patients with DS with comorbid hearing impairments, especially sensorineural hearing loss. Another study found that 38%-78% of individuals with DS experience hearing impairments, most commonly caused by otitis media effusion and sensorineural hearing loss.[8] Another study found that the likelihood of sensorineural hearing loss is due to cochlear nerve deficiency, genetic factors, or excessive noise exposure.[21]

In this study, several patients with DS were diagnosed with multiple comorbidities. The most prevalent combination of comorbidities in this study was congenital heart disease and endocrine system disorders. This aligns with the study conducted by Mulu and Fantahun, which found a significant association between hypothyroidism and congenital heart disease in patients with DS.[13] Yaqoob *et al.*[22] also found a high number of individuals with DS having thyroid function disorders and congenital heart diseases. According to Lerner *et al.*,[23] patients with congenital heart disease have a high prevalence of thyroid dysfunction, both genetically and embryonically determined.

This study provides new insights into characteristics and patterns of comorbidities in children with DS in Indonesia, especially in Yogyakarta. These new insights can benefit healthcare services, especially in Indonesia, to foresee common comorbidities in children with DS and benefit patients with early diagnosis, early intervention, and enhanced healthcare quality.

On the downside, this study can only provide a descriptive analysis. However, this study is a cornerstone for future studies, especially in Indonesia, to better understand comorbidities and DS in children, especially in Indonesia, with more complete data and analysis in the future. Future studies to include nutritional data and analysis on comorbid outcomes of DS patients are recommended.

CONCLUSION

This study finds that most children with DS in Dr. Sardjito General Hospital, Yogyakarta suffer at least one comorbid. The most common comorbid found was congenital heart disease. Future studies are needed to enhance health care, especially for DS patients in Indonesia.

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Effects of garlic tablet (GARLET) in combination with telmisartan on high blood pressure

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ABSTRACT

Hypertension (HTN) is a health problem worldwide affecting tens of millions of people. Giving modern antihypertensive alone is not enough to cure this disease. Garlic has been used to treat and cure various diseases, including high blood pressure (BP). This study aims to evaluate the effectiveness of administering telmisartan with garlic in reducing BP. In this study, 96 people with mild to moderate HTN were randomly selected and assigned to three groups consisting of 32 subjects; each participant in the garlic group received 400mg BD, the telmisartan group received 20mg OD, and the mixed group received 400 mg garlic BD and 20mg telmisartan OD for 8 wk of treatment. Blood pressure was measured at the beginning of each week, as well as on the closing day of the 8 wk. Combining telmisartan with garlic is more effective in reducing systolic blood pressure/SBP (113.0±22.9 mmHg) compared to telmisartan (147.7±4.6 mmHg; p=0.015) and diastolic blood pressure/DBP (71.0±21.2 mmHg vs 90.75±11.9 mmHg; p=0.038) during 8 wk of treatment in hypertensive patients. No significant side effects were identified during the treatment period. In conclusion, garlic, like other BP control pills, increases the effectiveness of chemical pills to lower SBP and DBP.

Keywords:
garlic;
telmisartan;
systolic blood pressure;
diastolic blood pressure;
hypertension

ABSTRAK

Hipertensi (HTN) merupakan masalah kesehatan di seluruh dunia yang mempengaruhi puluhan juta orang. Pemberian antihipertensi saja tidak cukup untuk menyembuhkan penyakit ini. Bawang putih telah digunakan untuk mengobati dan menyembuhkan berbagai penyakit, termasuk tekanan darah tinggi. Penelitian ini bertujuan untuk mengevaluasi efektivitas pemberian telmisartan dengan bawang putih dalam menurunkan tekanan darah. Dalam penelitian ini, 96 orang penderita HTN ringan hingga sedang dipilih secara acak dan dibagi menjadi tiga kelompok yang terdiri dari 32 subjek; setiap peserta dalam kelompok bawang putih menerima BD 400mg, kelompok telmisartan menerima OD 20mg, dan kelompok campuran menerima BD bawang putih 400 mg dan OD telmisartan 20mg selama 8 minggu pengobatan. Tekanan darah diukur pada awal setiap minggu, serta pada hari penutupan minggu ke-8. Kombinasi telmisartan dengan bawang putih lebih efektif menurunkan tekanan darah sistolik/SBP (113,0±22,9 mmHg) dibandingkan telmisartan (147,7±4,6 mmHg; p=0,015) dan tekanan darah/DBP diastolik (71,0±21,2 mmHg vs 90,75±11,9 mmHg; p=0,038) selama 8 minggu pengobatan pada pasien hipertensi. Tidak ada efek samping signifikan yang diidentifikasi selama masa pengobatan. Kesimpulannya, bawang putih, seperti pil pengontrol tekanan darah lainnya, meningkatkan efektivitas pil kimia untuk menurunkan SBP dan DBP.

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INTRODUCTION

Hypertension (HTN) is alternatively referred to as high or elevated blood pressure (BP). The vessels deliver blood from the heart to each location of the body. When blood is pumped by the heart, it presses on the walls of blood vessels, or arteries, creating BP. The heart pumps blood throughout the body with a force known as systolic pressure, represented by a greater number. Meanwhile, the resistance to blood flow in the blood arteries between heartbeats as blood circulates in the heart is known as the diastolic pressure (lower number).[1] High BP is one of most prevalent factors globally that brings about considerable risks of death and morbidity. High systolic blood pressure (SBP), poor diet, and cigarette use are the main risk factors for death and morbidity based on the latest findings from the Global Burden of Diseases (GBD).[2] Some of the fatal risk factors of high BP pointed out are stroke, coronary artery disease, failure of the heart, chronic renal illness, peripheral arterial disease, and arterial aneurysms.[3-4]

Morbidity and mortality rates are increasing along with the increase in the global elderly population caused by increasing vascular hypertension and the prevalence of systemic hypertension.[5] According to the World Health Organization, there will be close to 1.6 billion adults worldwide by 2025 who have high BP and its related consequences, including cardiovascular diseases (CVD).[2]

A SBP of >140 mmHg and a diastolic blood pressure (DBP) of > 90 mmHg are signs of the complex condition known as HTN. However, adults who are assessed to be at the pre-HTN stage and who have a DBP of 80 to 89 mmHg and an SBP of 120 to 139 mmHg are at a higher risk of having high BP.[4,6] Secondary HTN can be treated if the motivating causes are adequately identified. On the other hand, 90% of all instances of identified cases are embodied by critical HTN.

Although the underlying pathological processes are partially unclear, it is considered that lifestyle is one of several contributing variables. Luckily, most of these factors can be regulated to control the commonness of morbidity. Diet is thought to have a vital role in the emergence of HTN and associated illness, through a host of lifestyle variables. Age, gender, race, heredity, and genetic susceptibility are other extrinsic variables.[7]

Hydrogen sulfide (H_2S) was identified by Abe and Kimura as an endogenous gas transmitter in 1996. This discovery is regarded as a turning point for the emerging field of study that has had a substantial influence on physiopharmacology.[8] In the previous 25 yr, H_2S has been proven as a fundamental compound for maintaining the equilibrium of various systems. The cardiovascular (CV) system is among the most substantial domains in which the function and importance of H_2S have been studied.[9] Since various mechanisms of action, such as the activation of potassium channels like vascular potassium channel (Kv7) or ATP-sensitive potassium channel (KATP) and the inhibition of 5-phosphodiesterase (5-PDE) enzymes, are connected, the H_2S compound demonstrated that it was able to promote vasodilation.[10,11] This observation opened the foundation for the investigation of exogenous H_2S-donors, or compounds that can release H_2S, to develop cutting-edge HTN treatment methods of garlic.[12]

This directed the researchers to the finding that sure natural molecules acquired from *Alliaceae* (i.e., garlic) and *Brassicaceae* (i.e., rocket or broccoli) botanical households suggest the profile of sluggish H_2S-donors (i.e., suggesting that they will display an H_2S-liberating profile extra analogous to that of the unhurried endogenous

manufacturing of this gasotransmitter). This belongings puts forth that the *Alliaceae* and *Brassicaceae* derivatives, whether or not they are natural extracts or pure molecules (polysulfides or isothiocyanates), might be incredibly successful in treating hypertension or pre-hypertension symptoms.[9] Garlic (*Allium sativum*) has pharmacologically been used for thousands of years; with a thorough study of the benefits of garlic supplements and fresh garlic in preventing atherosclerosis and protecting the heart, however, little was previously known about and supported by research on its therapeutic and pharmacological features.[13]

Drugs that are used as renin-angiotensin converting enzyme inhibitors (ACEIs) are successfully used for the treatment of HTN at any age.[14] The angiotensin-tow (AT-II) receptor antagonist telmisartan, which connects specifically to the angiotensin one (AT1) receptor, restrains the pro-hypertensive effects associated with AT-II, including sodium chloride preservation, vasoconstriction, vascular and cardiac muscle expansion, and pro-fibrotic characteristics in both the renal and cardiovascular systems.[15] Telmisartan is licensed to treat cardiovascular conditions and systemic hypertension in humans.[16]

Many studies were conducted on the combined effect of garlic with BP-lowering drugs such as calcium channel blockers, beta-blockers, and ACEIs. Telmisartan is an angiotensin II receptor blocker (ARB) and it is a common drug that is used to treat high BP. However, until now no study conducted to evaluate the effect of combination telmisartan with garlic.

MATERIAL AND METHODS

Design of the research

The clinical trial was carried out with 96 outpatient men with HTN (aged 40 to 80 yr) who were randomized and double-blinded. Prior to receiving various medications such as diuretics, ACEIs (angiotensin-converting enzyme inhibitors), beta/alpha-blockers, and other locally sourced herbal remedies that proved ineffective, the patients were all diagnosed with HTN. This study was conducted in the Department of Internal Diseases, Alami Curative Hospital. While conducting this study, we followed the 1975 Declaration of Helsinki Principles and the 1983 amendments. This study turned into permitted via the Institutional Ethical Committee, Department of Research of Medical School, Alberoni University.

Inclusion criteria in this study are male patients between ages 40 and 80 yr, who receive treatment of continuous ARB and are not assisted by other anti-hypertensive drugs. Criteria for exclusion in this study are patients with cancer, heart disease, failure Kidney, the inability to handle garlic or telmisartan, in the age range between 40 and 80 yr. In this investigation, the SBP for patients ranged from 160 to 190 mmHg, while DBP ranged from 90 to 115 mmHg. Patients fulfilling eligibility criteria (inclusion and exclusion criteria) were randomized into three treatment groups as follows: Group A: the patients were given low-dose monotherapy of telmisartan 20 mg, OD, for 8 wk (Getz Pharma Karachi, Pakistan). Group B: patients were given low-dose monotherapy of 400 mg of garlic tablet BD for 8 wk (Amin Pharmaceutical Co., Isfahan, Iran). Group C: patients were given a combination of telmisartan 20 mg OD and a 400 mg garlic tablet BD for 8 wk. Follow-up visits were performed after the time of inclusion of the participants, once every week, and at the end of the 8 wk. The randomization was carried out using a random number generator. All groups' treatment periods came to an end after 8 wk. The participants adopted a low-salt, low-fat

diet and adhered to the same dietary and behavioral recommendations.

Demographic information

Age, weight, height, and length of high BP were noted for the participants under study. When the patient was seated, a mercury sphygmomanometer was used to take their BP. The patient was given a five-minute rest before having their SBP and DBP checked and recorded.

Measurements of BP

Arterial BP was checked at the time of inclusion of the participants, once every week, and after the 8 wk. It was consistently measured in the left and right arms in the supine, sitting, and standing postures early in the morning. The results obtained from both the second and third readings were documented, and using the mean value of the 12 readings, an integral estimate of arterial BP was calculated.

Statistical Analysis

The data were analyzed using SPSS version 10.1.7 (SPSS, Chicago, IL, USA). Following a review of the variable distribution, within-group effect calculations were made using Wilcoxon statistics, and between-group comparisons were made using one-way Anova. Data were presented as means, standard error of the mean (SEM), median for non-parametric data, and 95% confidence interval (CI) where applicable. Significance was determined at the 0.05 level of confidence.

RESULTS

A total of 96 participants involved in this study. Eight patients stopped taking study drugs throughout the treatment period: 2 in the garlic group owing to gastrointestinal discomforts, 3 in the telmisartan group, and 3 in the combination group (garlic + telmisartan) group for unidentified reasons. After the 8 wk therapy period, 88 individuals: 30 in the garlic group, 27 in the telmisartan group, and 31 in the combined group (garlic + telmisartan) were assessed.

Demographic indicators between telmisartan and combination groups

TABLE 1 listed the demographic data, which included height, weight, age, and the number of months that the elevated BP had persisted. The age (62.4±10.4 vs. 61.9±11 yr; p=0.9) and weight (76.2±9.9 vs. 76.7±1.7 kg; p=0.7) as well as height (163±26 vs. 166.4±18 cm; p=0.1) and duration of HTN (33.2±15 vs. 33.1±12 mo; p=0.16) are the demographic data that differentiate the telmisartan group from the combination group. The results unequivocally demonstrate that there were no discernible demographic differences between the combination group and the telmisartan group.

TABLE 1. Demographic of subjects (mean ±SD) between telmisartan and combination groups

Demographic of subjects	Telmisartan (n=32)	Combination (n=32)	p
Age (yr)	62.4±10.4	61.9±11	0.9
Weight (kg)	76.2±9.9	76.7±1.7	0.7
Height (cm)	163±26	166.4±18	0.1
Duration (mo)	33.2±15	33.1±12	0.16

Measuring systemic BP

Blood pressure is the pressure exerted by circulating blood on the walls of arterial blood vessels within the systemic circulatory system. Blood pressure refers to the force exerted by blood flow upon the blood vessel' inner walls. This pressure stems from the rhythmic contraction of the heart, which circulates the blood throughout the body's vascular system. Blood pressure has two components a maximum (systolic) and a minimum (diastolic) pressure throughout each cardiac cycle. Generally, a person's BP is usually indicated in terms of either SBP or DBP. It is quantified in units known as millimeters of mercury (mmHg), often illustrated, for instance, as 120/80.

We evaluated the SBP among telmisartan and combination groups. At first, these individuals undergoing treatment telmisartan 20 mg OD or telmisartan 20 mg plus garlic 400mg BD for 8 wk. The measurement of BP was initially taken, subsequently weekly, and finally after the completion of 8 wk. When the BP were compared after 8 wk of treatment, it was discovered that the group receiving combined treatment demonstrated reduced SBP levels (median:102.5 vs 150.0 mmHg; p=0.015) in contrast to the telmisartan group. However, the level of DBP in the telmisartan group was (90.75±11.9 vs 71.0±21.2 mmHg; p=0.038) in the combination group.

The significant differences were also shown in SBP among the garlic group (176.6±2.6 mmHg) vs telmisartan group (148.7 ± 4.6 mmHg) vs the combination group (113±29.9 mmHg) and DBP in telmisartan (90.7±11.9 mmHg) vs combination group (71±21.2 mmHg) as shown (FIGURE 1A and B). As compared with post treatment either with telmisartan 20mg OD or telmisartan 20mg plus garlic 400mg BID in the study participants, there was no notable variance in the SBP when comparing the telmisartan group with the combination group up to the 5 wk. However, a substantial decline in BP measurements started to be evident from the 6 wk and continued steadily until the 8 wk. On the other hand, the DBP levels experienced a significantly steep decrease, a trend which was noticeable much earlier, commencing from the 3rd wk and maintaining until the 8th wk. In contrast, the degree of DBP weas reduced drastically, which was observed much earlier and maintained continuity from week 3 until week 8. Thus, the results specify a statistically significant decrease in SBP and DBP in the combination group as compared to the telmisartan group (FIGURE 1A and B). Also, the individuals who participated in this study showed good compliance, and none of them developed side effects.

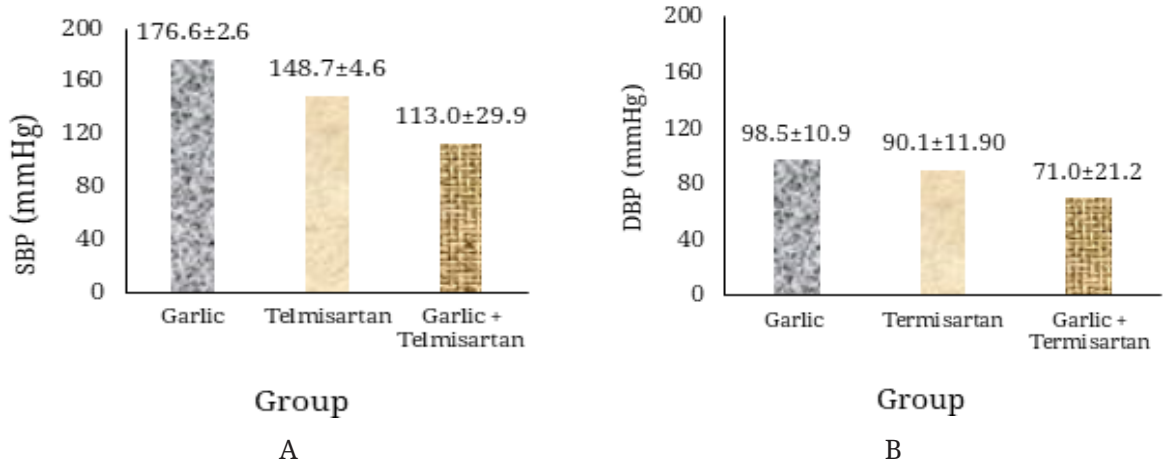

FIGURE 1. The mean of SBP (A) and DBP (B) in the garlic group, telmisartan group and combination group after the 8 wk treatment.

DISCUSSION

Hypertension, characterized by a SBP of 140 mm Hg or higher and a DBP of 90 mm Hg or above, is a pressing global issue affecting nearly a billion people. Current guidelines underscore the significance of treating hypertension and outline various methodologies, including preventive measures for regulating and managing BP levels. These guidelines also address individuals with pre-hypertension (SBP 120-139/DBP 80-89 mm Hg).[17]

The primary approach to managing hypertension hinges on lifestyle alterations like increased physical activity, weight reduction, and dietary modifications, potentially including dietary supplementation. Nevertheless, in cases where lifestyle adjustments fail to yield results or are insufficient, medication becomes a necessary course of action. These alternative therapies are necessitated.

Humans have relied on herbal medicines for decades, for curative purposes. In numerous nations, certain substances are more easily accepted by the human body, resulting in fewer adverse reactions. Garlic, scientifically known as *A. sativum*, is a prime example of such substance, renowned for its anti-hypertensive properties. This common dietary component also plays a significant medicinal role.[18] Multiple scientific investigations have underscored the beneficial impact of garlic on human health. These researches reveal that garlic can lower cholesterol levels, deter platelet clumping, decrease BP, and bolster antioxidant status. It functions as an effective anti-hypertensive agent and also exhibits cardio-protective benefits, these protective effects include reduction of unstable chest pain, improvement of blood vessel elasticity, and reduction of disorders triggered by blockage of peripheral arteries.

Nevertheless, there is a scarcity of clinical trials investigating the combined effects of garlic with contemporary medicine on humans. In carrying out this study, the study found that the garlic tablet combined with telmisartan significantly decreases BP, both (SBO and DBP). This result is the same as previous studies. Telmisartan is a well-known drug for the treatment of high BP, and its effects in reducing BP have been proven in numerous studies, was generally similar in efficacy to enalapril, more effective than submaximal dosages of losartan, and better tolerated than linsoprile.[19] Researchers and clinicians are interested in combining garlic with antihypertensive drugs like amlodipine, diuretics, and they reached satisfying results. This result is analogous to those of the other studies that have shown the hypotensive properties of the products containing garlic.[8,20-22] Thus, this study is carried out to screen the effectiveness of garlic extract in plus telmisartan in patients with high BP.

Blood pressure was measured at baseline, weekly, and at the end of eight weeks. The results revealed that the telmisartan group exhibited low levels of SBP (median:102.5mmHg vs median: 150.0mmHg; p=0.015) as compared to the combination group; whereas, the level of DBP in the telmisartan group was (90.7±11.9 mmHg vs 71.0±21.2 mmHg; p=0.038) in the combination group. Thus, the results specify a statistically significant decrease in SBP and DBP in the combination group as compared to the telmisartan group (FIGURE 1A and B). The result obtained is similar to the study conducted by Satyanand *et al.* in 2013 on the effect of the combination of amlodipine (5 mg) once a day and garlic (8 mg) once a day.[23] Furthermore, the participants involved in the research exhibited robust health, with no instances of side effects. The data we obtained demonstrates that the combination of garlic supplements and telmisartan more effectively

lowers systemic BP compared to when telmisartan is utilized independently in patients with hypertension.

Past scientific research has posited that the BP lowering effect of garlic may be attributed to the production of hydrogen sulfide and the inherent active component, allicin, which is released from alliin with the assistance of the enzyme alliinase, known for its inhibiting effects on angiotensin II and its capacity for vasodilation.[24,25] In a prior study, the daily dosage of garlic powder, ranging from 600-900 mg, delivered 3.6-5.4 mg of allicin; conversely, fresh garlic cloves (approximately 2 g) resulted in an allicin yield of 5-9 mg. Hence, different garlic preparations lead to varying degrees of BP reduction, for instance, aged garlic extract or heat-treated garlic contains a lesser amount of the allicin compound, potentially restricting its BP lowering abilities.[26]

The conclusions drawn from this research bear resemblance to the collective analysis of numerous randomized, placebo-controlled investigations, these studies treated hypertensive individuals (with a SBP of at least 140 mmHg and a DBP of at least 90 mmHg) with a daily dosage of 400 mg of garlic powder showed a mean decrease of 9.5 mmHg in SBP and 8.5 mmHg in DBP. Interestingly, there was no significant reduction in SBP with garlic preparations in studies including normotensives. Similarly, Auer *et al.* showed a reduction in supine DBP in the group receiving garlic treatment from 102 to 91 mm Hg after 8 wk ($p<0.01$) as compared to the placebo group.[21] In another study, Morgan *et al.* research has indicated that the impact of garlic supplements on overall BP is akin to the decrease in SBP by 5 mm Hg, as seen with beta-blocker usage. Similarly, it also matches the 8 mm Hg SBP reduction observed with ACEIs.[27] Additionally, it is comparable to a 10.3 mm Hg decrease in DBP associated with angiotensin II type 1 receptor antagonists.[28]

CONCLUSION

This study indicates that telmisartan with garlic worked better than telmisartan alone to manage SBP and DBP. We suggest adding garlic in addition to other BP-lowering medications. However, more research is needed to confirm the effectiveness of garlic added to traditional anti-hypertension medicines.

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The profile of bacteria isolated from urine culture of adults with urinary tract infection in Yogyakarta 2007-2022

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ABSTRACT

Local data regarding antimicrobial susceptibility patterns of bacteria from urine culture is limited in Indonesia, particularly in Yogyakarta. This study was conducted to provide epidemiology data of bacteria and their resistance profile, including the profile of bacteria that producing extended-spectrum beta-lactamase (ESBL) and carbapenemase in the urine of patients with urinary tract infection (UTI) in Yogyakarta. A descriptive retrospective study was conducted by assessing laboratory records of urine culture from adult patients at the Microbiology Laboratory, Department of Microbiology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta between 2007 and 2022. Of the 842 urine cultures, 464 (55.11%) isolates were recovered. Among these isolates, 50 (10.78%) were fungi, 67 (14.44%) were Gram-positive bacteria, and 347 (74.78%) were Gram-negative bacteria. *Enterococcus* sp. (41 (61.19%)) was the most bacteria found in the Gram-positive bacteria group, while *Escherichia coli* (38.90%) were the most bacteria found in the Gram-negative bacteria group. This study also identified Gram-negative bacteria producing ESBL enzymes (58.70%) and carbapenemases (27.94%). Gram-negative bacteria are the most common bacteria found in urine cultures of adult UTI patients in Yogyakarta, and the resistance profile of these bacteria is concerning.

ABSTRAK

Data lokal terkait pola kepekaan kuman terhadap antimikroba pada urin relatif jarang dijumpai di Indonesia, khususnya di Yogyakarta. Penelitian ini bertujuan untuk menyediakan data epidemiologi terkait bakteri dan profil kepekaannya, termasuk profil bakteri penghasil *extended-spectrum beta-lactamase* (ESBL) dan *carbapenemase* pada urin pasien dewasa dengan infeksi saluran kemih (ISK) di Yogyakarta. Penelitian retrospektif deskriptif dilakukan dengan menilai catatan laboratorium kultur urin dari pasien dewasa di Laboratorium Mikrobiologi, Fakultas Kedokteran, Kesehatan Masyarakat, dan Keperawatan UGM, Yogyakarta antara tahun 2007 hingga 2022. Dari 842 kultur urin, 464 (55,11%) isolat ditemukan. Diantara isolat tersebut, 50 (10.78%) adalah jamur, 67 (14,44%) bakteri Gram positif, dan 347 (74,78%) bakteri Gram negatif. *Enterococcus* sp. (41 (61,19%) merupakan bakteri terbanyak yang ditemukan pada kelompok bakteri Gram positif, sedangkan, sedangkan *Escherichia coli* (38,90%) merupakan bakteri terbanyak yang ditemukan pada kelompok bakteri Gram negatif. Penelitian ini juga mengidentifikasi bakteri Gram negatif penghasil enzim ESBL (58,70%) dan carbapenemase (27,94%). Bakteri Gram negatif adalah bakteri yang paling umum ditemukan pada kultur urin pasien ISK dewasa di Yogyakarta, dan profil resistensi bakteri ini mengkhawatirkan.

Keywords:
bacteria;
infection;
urine;
urinary tract infection;
Yogyakarta

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INTRODUCTION

Urinary tract infections (UTIs) can cause significant public health problems, particularly severe infections which can be a major economic burden on the healthcare system and deter patients from an optimal quality of life.[1] It affects 150 million people each year worldwide with high recurrence rates and rehospitalization.[1,2] UTIs are commonly treated with β-lactam antibiotics that raised a global concern for the emergence of antimicrobial resistance including extended-spectrum beta-lactamase (ESBL) and carbapenemase-producing bacteria.[3,4] These resistance, in addition to the existing patient's comorbidities or risk factors, and the limited choice of effective antibiotics, increase the number of attributable deaths, disability-adjusted life years, and the economic burden of the infections.[1,4,5]

Careful assessment of signs, symptoms, and history of disease are important components to diagnose clinical UTI. However urine culture is crucial to identify the cause of infection and establish the diagnosis.[6] The urine culture is a part of diagnostic stewardship that can supply information on the causative agents and their susceptibility patterns.[6] Therefore, it will subsequently facilitate the most suitable antibiotic treatment, reduce cost, and improve patient outcomes.[6,7] However, urine culture is not always available in Indonesia and many limited resources countries.[8] It also required several days to produce a result.[8]

Local surveillance for UTI-causing pathogens and antimicrobial susceptibility is necessary in the absence of urine culture.[9] It will also share benefits in predicting the cause of infection as well as the empiric antibiotic treatment of UTI patients in the same area or similar characteristics of environment.[9] However, the data was scarcely available or published in limited-resources areas, particularly in Yogyakarta. This study was conducted to supply epidemiology data of bacteria and their resistance profile, including the profile of ESBL- and meropenemase-producing bacteria from the UTI patients in Yogyakarta.

MATERIAL AND METHODS

Design of study

A descriptive retrospective study was conducted by collecting data of urine culture from a register of laboratory records (secondary data) at the Microbiology Laboratory, Department of Microbiology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta during the period of 2007-2022. API (BioMerieux) was used for microorganism identification, then the susceptibility test was conducted with a disk diffusion method to evaluate their sensitivity against antibiotics on Mueller-Hinton agar. Result interpretation was performed following the Performance Standards for Antimicrobial Susceptibility Testing of Clinical and Laboratory Standards Institute (CLSI) M100.

The CLSI definition was used in this study to screen ESBL producer which can be identified from their phenotypic resistance against extended penicillin, monobactam (aztreonam), 3rd generation cephalosporins with or without resistance against 4th generation cephalosporins.[10] Carbapenemase producer was identified from their resistant trait against one or more carbapenems (i.e. meropenem, imipenem, ertapenem, or doripenem) phenotypically.[10] Meropenem is used to screen carbapenemase producers as it offers the best sensitivity and specificity features compared to other carbapenems.[11]

Data analysis

Data were collected using an anonymous data sheet to keep patient confidentiality and analyzed using STATA 17 ME. The species or genus of microbes that were recovered from the urine culture were tabulated, also their susceptibility profile against the antibiotics tested. Summary statistics was conducted for descriptive study using the command "tab" to obtain frequency distribution tables, cross-tabulation, or two-way tables. The positive results were presented in the frequency distribution table [n (%)] with the detail of calculation in the footnote of TABLE 2 and 3. A total of 842 urine cultures from adult (18 y.o. or older) patients were documented during the study period. Before the study initiation, ethical approval was obtained from the Institutional Review Board (The Medical and Health Research Ethics Committee (MHREC)) of Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta (Reference number: KE/FK/0052/EC/2023).

RESULTS

Of the 842 urine cultures, 464 (55.11%) isolates were recovered during examination. Among those isolates, we identified 50 (10.78%) fungal isolates, 67 (14.44%) Gram-positive isolates, and 347 (74.78%) Gram-negative isolates.

Gram-positive isolates recovered from the urine culture

Among Gram-positive bacteria isolates, the most frequent bacteria identified in UTIs were *Enterococcus* sp. [41 (61.19%)], followed by *Staphylococcus aureus* [12 (17.91%)], *Enterococcus faecalis* [11 (16.42%)], *Streptococcus agalactiae* [2 (2.99%)], and *S. pneumoniae* [1 (1.49%)] (TABLE 1).

ESBL-producing and Gram-negative isolates recovered from the urine culture

This research elucidated that the most common Gram-negative bacteria identified from urine patients with UTIs were *E. coli*, accounted for 38.90% of total Gram-negative isolates (TABLE 2). The other Gram-negative bacteria recovered from those cultures were *Pseudomonas* sp. [60 (17.29%)], *P. aeruginosa* [42 (12.10%)], *Klebsiella pneumoniae* [38 (10.95%)], *Enterobacter* sp. [12 (3.46%)], *Proteus mirabilis* [10 (2.88%)], *Klebsiella* sp. [8 (2.31%)], *K. aerogenes* (also known as *E. aerogenes*) [4 (1.15%)], *P. fluorescens* [4 (1.15%)], *Acinetobacter baumannii* [3 (0.86%)], *Citrobacter* sp. [3 (0.86%)], *Providencia rettgeri* [3 (0.86%)], *Proteus penneri* (2 (0.58%)), *Proteus* sp. [2 (0.58%)], *P. putida* [2 (0.58%)], *Serratia* sp. [2 (0.58%)], and less common Gram-negative bacteria [17 (4.90%)].

TABLE 1. Gram-positive bacteria isolated from urine samples of UTI patients in Yogyakarta 2007-2022

Name of bacteria/species	n (%)
Enterococcus sp.	41 (61.19)
S. aureus	12 (17.91)
E. faecalis	11 (16.42)
S. agalactiae	2 (2.99)
S. pneumoniae	1 (1.49)
Total	67 (100.00)

Bacteria with "sp." means that the isolate identification was only up to genus level

TABLE 2. Gram-negative bacteria which isolated from urine samples of UTI patients and ESBL-producing pathogens in Yogyakarta 2007-2022

Name of bacteria/ spesies	Isolates recovered from urine samples [n (%)]*	Isolates tested for ESBL [n (%)]**	Isolates with ESBL phenotypes [n(%)]***
E. coli	135 (38.9)	135(100)	57 (42.22)
Pseudomonas sp.	60 (17.29)	55 (91.67)	49 (89.09)
P. aeruginosa	42 (12.1)	41 (97.62)	35 (85.37)
K. pneumoniae	38 (10.95)	38 (100)	20 (52.63)
Enterobacter sp.	12 (3.45)	12 (100)	7 (58.33)
P. mirabilis	10 (2.88)	9 (90)	1 (11.11)
Klebsiella sp.	8 (2.30)	8 (100)	5 (62.5)
K. aerogenes	4 (1.15)	4 (100)	4 (100)
P. fluorescens	4 (1.15)	4 (100)	3 (75)
A. baumannii	3 (0.86)	3 (100)	1 (33.33)
Citrobacter sp.	3 (0.86)	3 (100)	2 (66.67)
P. rettgeri	3 (0.86)	3 (100)	1 (33.33)
P. penneri	2 (0.58)	2 (100)	1 (50)
Proteus sp.	2 (0.58)	2 (100)	1 (50)
P. putida	2 (0.58)	2 (100)	1 (50)
Serratia sp.	2 (0.58)	2 (100)	1 (50)
S. maltophilia	1 (0.29)	1 (100)	0 (0)
Escherichia sp.	1 (0.29)	1 (100)	0 (0)
E. fergusonii	1 (0.29)	1 (100)	1 (100)
E. cloacae	1 (0.29)	1 (100)	1 (100)
A. caviae	1 (0.29)	1 (100)	1 (100)
B. pseudomallei	1 (0.29)	1 (100)	1 (100)
C. youngae	1 (0.29)	1 (100)	0 (0)
Kluyvera sp.	1 (0.29)	1 (100)	0 (0)
Leclercia sp.	1 (0.29)	1 (100)	0 (0)
P. alcaligenes	1 (0.29)	1 (100)	1 (100)
P. alcalifaciens	1 (0.29)	1 (100)	0 (0)
S. marcescens	1 (0.29)	1 (100)	1 (100)
S. odorifera	1 (0.29)	N/D	N/D
S. liquefaciens	1 (0.29)	1 (100)	1 (100)
Yersinia rohdei	1 (0.29)	1 (100)	1 (100)
Edwardsiella tarda	1 (0.29)	1 (100)	1 (100)
Edwardsiella sp.	1 (0.29)	1 (100)	1 (100)
Total	347 (100)	339 (97.69)	199 (58.70)

N/D: no data; (%)* is the number of Gram-negative isolates divided by total of Gram-negative isolates recovered from the urine culture; (%)** is the number of Gram-negative isolates which were tested for ESBL phenotypes divided by total of respective Gram-negative species or isolates recovered from the urine culture; (%)***) is the number of Gram-negative isolates with ESBL phenotypes divided by total of respective Gram-negative isolates or species which were tested for ESBL phenotypes; Bacteria with "sp." means that the isolate identification was only up to genus level

TABLE 3. Gram-negative carbapenem-resistant bacteria from urine samples of UTI patients in Yogyakarta 2007-2022

Name of bacteria/ Species	Isolates tested for carbapenem-resistant phenotype [n (%)]$^{\phi}$	Isolates with carbapenem-resistant phenotype [n (%)]$^{\phi\phi}$
Pseudomonas sp.	60 (100)	27 (45)
E. coli	135 (100)	23 (17.04)
K. pneumoniae	38 (100)	10 (26.32)
P. aeruginosa	40 (95.24)	10 (25)
Enterobacter sp.	12 (100)	5 (41.67)
Klebsiella sp.	8 (100)	4 (50)
P. fluorescens	4 (100)	3 (75)
Serratia sp.	2 (100)	2 (100)
Proteus sp.	2 (100)	1 (50)
Citrobacter sp.	2 (66.67)	1 (50)
P. rettgeri	3 (100)	1 (33.33)
A. caviae	1 (100)	1 (100)
B. pseudomallei	1 (100)	1 (100)
Edwardsiella sp.	1 (100)	1 (100)
E. tarda	1 (100)	1 (100)
Leclercia sp.	1 (100)	1 (100)
S. liquefaciens	1 (100)	1 (100)
Y. rohdei	1 (100)	1 (100)
P. mirabilis	10 (100)	1 (10)
A. baumannii	3 (100)	0 (0)
C. youngae	1 (100)	0 (0)
E. cloacae	1 (100)	0 (0)
K. aerogenes	4 (100)	0 (0)
E. fergusonii	1 (100)	0 (0)
Escherichia sp.	1 (100)	0 (0)
Kluyvera sp.	1 (100)	0 (0)
P. penneri	1 (50)	0 (0)
P. alcalifaciens	1 (100)	0 (0)
P. alcaligenes	1 (100)	0 (0)
P. putida	2 (100)	0 (0)
S. marcescens	1 (100)	0 (0)
S. odorifera	1 (100)	0 (0)
Total	342	95 (27.94)

(%)$^{\phi}$ is the number of gram-negative isolates or species which were tested for carbapenem-resistant phenotypes divided by total of respective gram-negative isolates which recovered from the urine culture; (%)$^{\phi\phi}$ is the number of gram-negative isolates with carbapene- resistant phenotypes divided by total of respective gram-negative isolates which were tested for carbapenem-resistant phenotypes; Bacteria with “sp.” means that the isolate identification was only up to genus level

During the study period, a total of 199 (58.70%) ESBL-producing gram-negative bacteria were identified from those isolates. We identified that 57 (42.22%) *E. coli* phenotypically exhibited the characteristic of ESBL-producing bacteria. Other gram-negative bacteria were also often phenotypically presented as ESBL-producing bacteria; those including *Pseudomonas* sp. (49 (89.09%)), *P. aeruginosa* (35 (85.37%)), and *K. pneumoniae* (20 (52.63%)). *Enterobacter* sp., *Klebsiella* sp., *K. aerogenes*, and *P. fluorescens* were less identified as ESBL-producing bacteria accounted for 7 (58.33%), 5 (62.5%), 4 (100%), and 3 (75%) isolates respectively. Other bacteria were also shown as ESBL producers, but they are limited in number (TABLE 2).

Carbapenem-resistant Gram-negative bacteria identified from the urine culture

This study recorded that among Gram-negative bacteria which isolated and tested against carbapenem (meropenem), resistance was identified in 27.94% of them (TABLE 3). Although some bacteria isolates revealed a relatively high percentage of carbapenem resistance, this research sample size is limited. Among Gram-negative bacteria isolates tested against carbapenem, *Pseudomonas sp.*, *E. coli, K. pneumoniae, P. aeruginosa, Enterobacter* sp., *Klebsiella* sp., *P. fluorescens,* and *Serratia* sp. were relatively common as carbapenemase producers which accounted for 27 (45%), 23 (17.04%), 10 (26.32%), 10 (24.39%), 5 (41.67%), 4 (50%), 3 (75%), 2 (100%) isolates, respectively.

DISCUSSION

This study described the UTI's etiology and their resistance against antibiotics by observing their phenotypic characteristics as ESBL-producing and or carbapenemase-producing bacteria. This data can serve as local surveillance in Yogyakarta that enables benefits in diagnostic and antimicrobial stewardship programs.[12] This study showed of all recovered isolates from urine culture, Gram-negative bacteria were identified as the predominant (74.78%) uropathogens, whereas Gram-positive bacteria and fungi were represented only in 14.44% and 10.78%, respectively. This finding was similar to a study from East China (2021) that conducted urine culture from 1760 UTIs patients, and reported uropathogens which consisted of 90.5% Gram-negative bacteria, 9.3% Gram-positive bacteria, and 0.2% fungi.[13] In Indonesia, a study of asymptomatic UTI in pregnant women revealed that Gram-negative bacteria (72%) were more frequently isolated from their urine culture, as compared to Gram-positive bacteria (28%).[14] A study in Surabaya, Indonesia also reported similar result, Gram-negative (59.67%) and Gram-positive (14.51%) bacteria, as well as fungi (*Candida* sp.) (25.81%) were identified in the urine culture of diabetic patients with UTI.[15]

Gram - positive bacteria as uropathogens

The East China study reported *E. faecalis* as the most prevalent (31.7%) gram-positive bacteria, then followed by *S. agalactiae* (24.4%), *S. saprophyticus* (18.3%), *E. faecium* (9.1%) and others (16.5%).[13] Our study has slightly different pattern, we reported *Enterococcus* sp., *S. aureus*, and *E. faecalis* as the majority (95.52% in total) of Gram-positive bacteria identified from urine culture, whereas *S. agalactiae* was only accounted for 2.99% of Gram-positive bacteria involved in UTIs. A study in Jakarta revealed that *S. agalactiae* (33.33%), *E. faecalis* (19.04%), and *S. saprophyticus* (14.28%) were the frequently identified gram-positive bacteria of asymptomatic UTI in pregnant women.[14] In Surabaya, *E.*

faecalis (66.66%) was the gram-positive bacteria which frequently isolated from urine culture of diabetic patients with UTI.[15]

This study found that *S. pneumoniae,* which is commonly associated with respiratory or central nervous infection, was detected in a urine sample from a patient with UTI.[16] This extraordinary finding came from a patient who also suffered from *S. pneumoniae* bacteraemia, as suggested by the positive results of two blood cultures (also yielded *S. pneumoniae*) which accompanied the urine culture. Although uncommon, in Munich, Germany, *S. pneumoniae* as urinary tract pathogen was reported in a 82-year-old man with pyelonephritis and urosepsis.[16] Pneumococcosuria where *S. pneumoniae* identified as an agent of infection in urinary tract was scarce.[17] A study at the Department for Infectious Diseases, University Hospital of Heidelberg, reported that 26 urine samples from 18 different patients (age of 3-72 years) contained *S. pneumoniae* between January 2010 and December 2014.[17] The literature suggested that in children, *S. pneumoniae* is rarely identified from urine samples (less than 1%).[18]

Gram - negative bacteria as uropathogens

Escherichia coli was accounted for 75-95% of uropathogens in UTIs all over the world.[19] A study in Japan which included a total of 2049 UTI patients reported that 1682 (82.1%) of UTIs were caused by gram-negative bacteria. It comprised *E. coli* (93.3%), *Klebsiella* sp. (6.2%), and *P. mirabilis* (0.5%).[20] Interestingly, a meta-analysis study in Iran found comparable finding among pregnant women, that *E. coli* and *Klebsiella* were the common gram-negative bacteria causing UTIs which accounted for 61.6% and 13.9% respectively.[21] Similarly, the study in US Veterans Affairs medical centers (in Minnesota and Texas) highlighted that *E. coli* was a predominant uropathogen which accounted for 40.7%.[22]

A study of asymptomatic UTI in pregnant women in Indonesia reported that the gram-negative bacteria which frequently isolated in the urine culture were *E. coli* (37.04%) and *K. pneumoniae* (27.78%).[14] In Surabaya, a study of UTI in diabetic patients reported that the gram-negative bacteria which frequently isolated in the urine culture were *E. coli* (54.05%), *A. baumannii* (10.81%), and *Enterobacter* spp. (8.10%).[15] Similarly, our study reported that *E. coli* was the most prevalent (38.9%) gram-negative bacteria recovered from UTIs, however the second and third most frequent gram-negative uropathogens were *Pseudomonas* sp. (17.29%) and *P. aeruginosa* (12.10%) respectively. We also found a various species of other gram-negative bacteria which involved in UTIs comprising *K. pneumoniae* (10.95%), *Enterobacter* sp. (3.45%), *P. mirabilis* (2.88%), and others (14.43%).

ESBL producing Gram - negative bacteria

Our study highlighted that gram-negative bacteria were the major pathogen (74.78%) causing UTIs, and around 59% of those isolates shared similar phenotypic trait as ESBL producer. ESBLs are defined as a rapidly evolving group of enzymes produced by certain bacteria that can hydrolize extended spectrum cephalosporin.[23,24] These enzymes found to be effective against one or more of third and fourth generation of cephems (such as ceftazidime, ceftriaxone, cefotaxime, cefepime), extended spectrum penicillin (i.e. piperacillin), and monobactam (i.e. aztreonam) but are inhibited by clavulanic acid or tazobactam.[23-25] Therefore the presence of ESBLs in gram-negative bacteria warrant special attention due to the associated risks of

antibiotic therapy failure.[26]

ESBL-producing *Enterobacteriaceae* (i.e. *E. coli, K. pneumoniae*, *Enterobacter* sp., *Proteus* sp.) are the most prevalent causative agents of UTIs.[25] They can be a major threat to the global public as their resistance against β-lactam antibiotics leads to treatment failure in many infections.[25] While our study reported 42.22% ESBL-producing *E. coli* in the urine of UTI patients, a study in Jordan (2019) reported a higher proportion (62%).[27] A systematic review in Ethiopia reported a high rate of ESBL-producing gam-negative bacteria among clinical samples with a pooled rate of 50.1% among different species and varied in the groups of *Klebsiella* spp. (65.7%), *Enterobacter* spp. (62.2%), *Salmonella* spp. (48.4%), *E. coli (*47.0%), *Citrobacter* spp. 46.8%, *Providencia* spp. (43.8%), *Proteus* spp. (28.3%), *P. aeruginosa* (17.4%), *Acinetobacter* spp. (9.4%), and other Gram-negative bacteria (20.8%).[28]

A five-year global surveillance by "SMART program" (2015-2019) reported that the prevalence of non-carbapenem-resistant ESBL-producing *Enterobacteriaceae* was 30% globally, and exceeded 50% in India, Thailand, Vietnam, China, Russia, Mexico, Kenya, and Kuwait.[25] The SMART program estimated that the prevalence of non-carbapenem-resistant ESBL-producing *K. pneumoniae* was 25.4% globally, and more than 40% in Portugal, Chile, Ecuador, Guatemala, Mexico, Israel, Kenya, Morocco, Lithuania, and Kuwait.[25] They also reported that the prevalence of non-carbapenem-resistant ESBL-producing *E. coli* increased significantly ($p < 0.05$) in Asia (excluding China), Australia, New Zealand, and Latin America.[25] Furthermore, the non-carbapenem-resistant ESBL-producing *K. pneumoniae* prevalence increased significantly ($p< 0.05$) in Latin America, USA, and Canada.[25] Unfortunately, no data from Indonesia was included in the SMART surveillance.[25]

A retrospective study in Bali, Indonesia (2019-2020) revealed that ESBL-producing *E. coli* (56.32%) and *K. pneumoniae* (54%) were identified in the urine culture of patients with UTI and chronic kidney disease.[29] Another study in Medan reported that ESBL-producing *E. coli* was contributed to 8.4% of urine associated catheter infections in adult patients who admitted into the intensive care unit from July to August 2018.[30] Differences in clinical settings, study period, study population, and methods might modify the result, thus explaining the heterogeneity of the ESBL-producing bacteria prevalence worldwide.[28]

ESBLs are enzymes encoded in plasmids and can be easily transferred to other bacteria.[31] Apart from ESBL-producing *Klebsiella* and *E. coli*, our study also reported a relatively high prevalence of ESBL-producing Gram-negative bacteria, including non-lactose-fermenting bacteria such as *P. aeruginosa*, *Pseudomonas* spp., and a wide variety of other Gram-negative bacteria causing UTIs.[32] These bacteria have also been reported as the causative pathogen in UTIs, especially in health-care associated UTIs.[32]

Carbapenem - resistant in Gram - negative bacteria

Carbapenems have a β-lactam ring that differs from penicillins by replacing the sulphur atom at C-1 with a carbon atom and adding a double bond between C-2 and C-3.[33] In addition to this characteristic, the side chain of carbapenemis in the trans position instead of the cis- position, making this drug insensitive to the effect of β-lactamases.[33] Carbapenems were previously effective in treatening-drug resistant (MDR) bacteria, including ESBL-producing bacteria which resistant to penicillin and cephalosporins.[34] Unfortunately, the acquisition of carbapenemase genes causes these bacteria to be able to

hydrolize carbapenem, leaving a limited choice of antibiotic treatment for MDR bacteria.[35]

Carbapenemase emergence and spread have increased dramatically over the last decade following its discovery in *K. pneumoniae* in the US in 1996.[34,36] The increasing prevalence of carbapenem-resistant gram-negative bacteria as the cause of infections is a global threat, hence the WHO highlighted those pathogens, particularly carbapenem-resistant *Enterobacterales* (CRE), carbapenem-resistant *P. aeruginosa* (CRPA) and carbapenem-resistant *A. baumanii* (CRAB) in the global priority list of pathogens in 2017.[37] Carbapenem-producing bacteria surveillance is an important strategy to control its spread and enable a positive impact for public health.[38]

Carbapenem-resistant bacteria in this study were reported in 95 (27.94%) of gram-negative bacteria isolated from urine cultures of UTI samples. Our study identified various percentage of carbapenem resistance among different bacterial species isolated from UTI patients, including *Klebsiella* sp. (50%), *K. pneumoniae* (26.32%), *E. coli* (17.04%), *Pseudomonas* sp. (45%), *Enterobacter* sp. (41.67%), *P. fluorescens* (75%), and many more as described in TABLE 3. A review study comprising data mostly from Asia, North America, and Europe (2017) reported that proportion of carbapenem-resistant *Enterobacteriaceae* in community setting was ranged from 0 to 29.5% with the highest proportion of carbapenem- resistant *Enterobacteriaceae* was identified in Asia.[39] In Taiwan, the prevalence of carbapenem-resistant *P. aeruginosa* was increasing from 12% in 2012–2015 to 19-23% in 2018–2021.[40] Surveillance of carbapenem resistance, especially in patients with UTI is remain scattered and fragmented, moreover various surveillance models were adapted to fit with the requirements and capacities of each setting or country.[41] Furthermore, developing countries struggle with political and social dilemmas due to weak laboratory capacity, poor health systems governance, lack of health information systems, and limited resources.[41,42] Despite the challenges, a local antibiogram or data regarding uropathogen and its susceptibility pattern is important to inform local treatment guidelines and promote antimicrobial stewardship program.[43]

Study limitation

This study has limitations, including the small sample size of each bacteria species, limited clinical information, and the passive nature of a secondary laboratory records.

CONCLUSION

This study highlighted the profile of bacteria associated with UTIs in Yogyakarta during 2007-2022 that could be useful for antimicrobial stewardship. The recovery proportion of pathogen of urine culture from UTIs patients was relatively high (55.11%), and like other studies in the world, gram-negative bacteria was the most prevalent pathogen isolated from urine of UTI cases. *Escheriachia coli* and bacteria in the genus *Pseudomonas* and *Klebsiella* were the most frequently identified in this population. In addition, this study revealed the high proportion of ESBLs- and carbapenemase-producer among gram-negative bacteria associated with UTIs. Future study is required to refine the epidemiology of bacteria that cause UTI in developing countries or limited-resource region, especially in Yogyakarta.

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Characteristics, management, and major adverse cardiac events of ST-elevation myocardial infarction (STEMI) patients in rural area: a Jember acute coronary syndrome medical records study

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ABSTRACT

The mortality and morbidity of ST-elevation myocardial infarction (STEMI) remain high, and it is still the leading cause of death worldwide. Timely reperfusion lowers the risk of MACE and improves survival. However, reperfusion delay is still a significant issue in developing countries, particularly rural area. This study aimed to determine the characteristics, management, and major adverse cardiac events of STEMI patients in Jember Distric to enhance reperfusion therapy in rural areas. It was an analytical observational cross-sectional study with a quantitative method. A total of 108 medical records of STEMI patients of the Dr. Soebandi District Hospital, Jember in period of 2019 to 2020 were included using the consecutive sampling technique. As much as 78 (62%) of the patients did not receive reperfusion therapy while the remaining patients (41 or 38%) received the therapy, namely percutaneous coronary intervention (20 or 18.5%), fibrinolytic therapy (19 or 17.6%), and both (2 or 1.9%). Multivariate analysis for reperfusion was statistically significant (p=0.016; OR 3.688 95% CI: 1.274-10.679). The level of education, health funding, and distance to the hospital did not affect the delay of the reperfusion therapy. Reperfusion was highly associated with the incidence of MACE and this study discovered a threefold benefit of reperfusion in lowering the occurrence of MACE (*p*=0.016). In conclusion, the majority of STEMI patients does not get reperfusion therapy. Delays in reperfusion treatment are not related to the patient's level of education, health funding, or distance to the hospital. Nonetheless, the delay my be attributed to the Jember community's lack of awareness of STEMI symptoms.

ABSTRAK

Mortalitas dan morbiditas *ST-elevation myocardial infarction* (STEMI) masih tinggi, dan menjadi penyebab kematian tertinggi di dunia. Reperfusi yang tepat waktu menurunkan risiko MACE dan meningkatkan kelangsungan hidup. Namun, keterlambatan reperfusi masih menjadi masalah nyata di negara-negara berkembang, khususnya di daerah pedesaan. Penelitian ini bertujuan untuk mengetahui karakteristik, penatalaksanaan, dan kejadian jantung buruk mayor pasien STEMI di Kabupaten Jember untuk meningkatkan terapi reperfusi di daerah pedesaan. Penelitian ini merupakan penelitian analitik *observasional cross-sectional* dengan metode kuantitatif. Sebanyak 108 rekam medis pasien STEMI tahun 2019-2020 diikutsertakan dengan menggunakan teknik sampling konsekutif. Sebanyak 78 (62%) pasien tidak mendapatkan terapi reperfusi sedangkan pasien lainnya (41 atau 38%) mendapatkan terapi, yaitu: intervensi koroner perkutan (20 atau 18,5%), terapi fibrinolitik (19 atau 17,6%), dan keduanya (2 atau 1,9%). Analisis multivariat untuk reperfusi secara statistik signifikan (p=0,016; OR 3,688; 95% CI: 1,274-10,679). Tingkat pendidikan, dana kesehatan, dan jarak ke rumah sakit tidak mempengaruhi keterlambatan terapi reperfusi. Reperfusi sangat terkait dengan kejadian MACE dan penelitian ini menemukan manfaat tiga kali lipat reperfusi dalam menurunkan kejadian MACE (p=0,016). Dapat disimpulkan bahwa mayoritas pasien STEMI tidak mendapatkan terapi reperfusi. Keterlambatan perawatan reperfusi tidak berhubungan dengan tingkat pendidikan pasien, dana kesehatan, atau jarak ke rumah sakit. Meskipun demikian, keterlambatan tersebut mungkin disebabkan oleh kurangnya kesadaran masyarakat Jember terhadap gejala STEMI.

Keywords:
acute coronary syndrome; major adverse cardiovascular events (MACE); reperfusion; ST-elevation myocardial infarction

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INTRODUCTION

Cardiovascular disease is a global health issue, accounting for 31% of both morbidity and mortality. In 2025, the mortality rate of coronary heart disease (CHD) was 20 million, and it is expected to rise to 23.6 million by 2030.[1] Acute myocardial infarction (AMI) is one of the CHD is still the leading cause of mortality worldwide, with a prevalence of 12.2 % or around 7.2 million individuals. The prevalence of acute myocardial infarction with ST elevation has jumped from 25 to 40%. According to the Basic Research Data statistics from 2018, the STEMI mortality rate is also the primary cause of cardiovascular death in Indonesia.[2]

ST-elevation myocardial infarction (STEMI) is one of the spectra of acute coronary syndromes due to the sudden decrease of coronary artery blood flow by thrombus occlusion following a rupture of pre-existing atherosclerotic plaque. The American College of Cardiology/American Heart Association and the European Society of Cardiology recommend reperfusion therapy as the first-line therapy of STEMI, either fibrinolytic therapy or percutaneous coronary intervention (PCI). The therapy of PCI has a success rate of 99 %, while fibrinolytic therapy has a success rate of 20-30 %.[3] Those patients who do not have reperfusion therapy are more likely to have complications. Late-onset presentation (> 12 hr), the patient's source of financing, low education level, psychosocial factors, and other relative contraindications are possible reasons for not conducting reperfusion treatment.[3] Major adverse cardiovascular events (MACE) are the term for complications of the reperfusion treatment. This includes heart failure, cardiogenic shock, arrhythmias, and death.[4]

Very few data regarding the characteristics, management, and MACE in STEMI patients in Jember, East Java, Indonesia. Therefore, registry data study is required in order to improve reperfusion treatment. This study aimed to determine the characteristics, management, and major adverse cardiac events of STEMI patients in Jember to enhance reperfusion therapy in the rural area.

MATERIAL AND METHODS

Design of study

It was an analytical observational cross-sectional study using the quantitative method conducted at Dr. Soebandi General Hospital, Jember, East Java, Indonesia from February until March 2021 after ethical clearance approval by the Ethics Committee of the Faculty of Medicine, University of Jember, Number /H25.1.11/KE/2021/456.

Procedure

The study used secondary data from STEMI patient's medical records. All subjects were recruited using the consecutive sampling technique. A total of 119 STEMI patients at Dr. Soebandi General Hospital, Jember in period of 2019 to 2020 were selected in this study. As much as 108 patients met the inclusion and exclusion criteria, while 11 others were excluded. The inclusion criteria of the study were the following: 1) STEMI patients both receiving and not receiving reperfusion therapy for 5 d of evaluation in 2019-2020; 2) Complete medical record data. The following data were also obtained from the medical records: age, sex, the onset of chest pain, comorbidities, level of education, health funding, distance to hospital, and evaluation length. Patients with incomplete data were excluded.

Data analysis

Data were presented as frequency or percentage and analyzed by descriptive, bivariate, and multivariate analysis. Univariate analysis (descriptive analysis) was aimed to describe the characteristics

of each study variable. The Chi-square test was used to compare categorical parameters to evaluate the association between each parameter and MACE (bivariate analysis) in STEMI patients followed by a multivariate analysis logistic regression test with a 95% confidence interval (95%CI). A p value of < 0.05 was defined as statistically significant.

RESULTS

Among 119 STEMI patients who were selected in this study, 108 patients who met the inclusion criteria, while eleven others were excluded. The characteristics, management, and major adverse cardiac events of STEMI patients are presented in TABLE 1.

TABLE 1. Distribution of study parameters variables

Characteristic	[n (%)]
Reperfusion therapy	
Yes	41 (38.0)
PCI	20 (18.5)
Fibrinolytic	19 (17.6)
Both	2 (1.9)
No	78 (62.0)
MACE	
Yes	70 (64.8)
None	38 (35.2)
Age	
<50 y.o.	20 (18.5)
≥50 y.o.	88 (81.5)
Sex	
Males	83 (76.9)
Females	25 (23.1)
Chest pain onset	
<12 hr	51 (47.2)
>12 hr	57 (52.8)
Comorbidities	
Yes	72 (66.7)
None	36 (33.3)
Level of education	
Complete compulsory education	52 (48.1)
Incomplete compulsory education	56 (51.9)
Health funding	
Universal health coverage	0 (0.0)
Dues assistance recipients (PBI)	31 (28.7)
Premium paid	74 (68.5)
Personal funding	3 (2.8)
Distance to the hospital	
Near	14 (13.0)
Far	94 (87.0)

A significant relationship between reperfusion therapy and the occurrence of MACE (p=0.008; OR = 3.345) was observed (TABLE 2). It was demonstrated that STEMI patients who does not get reperfusion treatment has a 3.345 times higher risk of MACE than those who does. TABLE 2 also revealed a significant relationship between MACE with age (p = 0.010; OR=3.577), gender (p=0.022; OR=3.643), onset of pain (p=0.004; OR=3.254), comorbidities (p=0.046; OR 2.5), and level of education (p=0.033; OR 2.419), however no significant relationship with health funding (p=0.946; OR= 0.919).

TABLE 2. Bivariate analysis between reperfusion & parameters with MACE

Variable	MACE [n (%)]		Total	OR	95%CI	p
	Yes	No				
Reperfusion therapy						
Yes	33 (30.6)	8 (7.4)	41 (38.0)	3.345	1.346-8.311	0.008
No	37 (34.3)	30 (27.8)	67 (62.0)			
Total	70 (64.8)	38 (35.2)	108 (100)			
Age						
<50 y.o.	8 (7.4)	12 (11.1)	20 (18.5)			
≥50 y.o.	62 (57.4)	26 (24.1)	88 (81.5)	3.577	1.309-9.772	0.010
Total	70 (64.8)	38 (35.2)	108 (100)			
Sex						
Males	49 (45.4)	34 (31.5)	83 (76.9)			
Females	21 (19.4)	4 (3.7)	25 (23.1)	3.643	1.147-11.566	0.022
Total	70 (64.8)	38 (35.2)	108 (100)			
Onset pain						
<12 hr	26 (24.1)	25 (23.1)	51 (47.2)			
>12 hr	44 (40.7)	13 (12.0)	57 (52.8)	3.254	1.423-7.442	0.004
Total	70 (64.8)	38 (35.2)	108 (100)			
Comorbidities						
Yes	42 (38.9)	30 (27.8)	72 (66.7)			
None	28 (25.9)	8 (7.4)	36 (33.3)	2.500	1.001-6.241	0.046
Total	70 (64.8)	38 (35.2)	108 (100)			
Level of education						
Complete compulsory	39 (36.1)	13 (12.0)	52 (48.1)			
Incomplete compulsory	31 (28.7)	25 (23.1)	56 (51.9)	2.419	1.066-5.490	0.033
Total	70 (64.8)	38 (35.2)	108 (100)			
Health funding						
Universal health coverage	68 (63.0)	37 (34.3)	105 (97.2)	0.919	0.081-10.476	0.946
Personal funding	2 (1.9)	1 (0.9)	3 (2.8)			
Total	70 (64.8)	38 (35.2)	108 (100)			

TABLE 3. Bivariate analysis between distance to the hospital and reperfusion therapy

Distance to hospital	Reperfusion therapy [n (%)		Total	OR	95% CI	p
	Yes	No				
Near	7 (6.5)	7 (6.5)	14 (13.0)	0.567	0.183-1.752	0.320
Far	34 (31.5)	60 (55.6)	94 (87.0)			
Total	41 (38.0)	67 (62.0)	108 (100)			

TABLE 4. Multivariate analysis of the incidence of MACE

Variable	Coefficient	OR	95% CI	p
Reperfusion therapy	1.305	3.688	1.274-10.679	0.016
Age	1.271	3.564	1.115-11.393	0.032
Sex	1.420	4.136	1.154-14.816	0.029
Pain onset	1.021	2.776	1.068-7.218	0.036
Comorbidities	-0.268	0.765	0.593-0.987	0.039
Constant	-5.724	0.003	-	0.001

TABLE 3 presented no significant relationship between the distance to the hospital and reperfusion (p=0.320; OR=0.567). Multivariate analysis using logistic regression test (TABLE 4) confirmed these variables to be significantly associated with MACE: reperfusion (p=0.016), age (p=0.032), sex (p=0.029), onset since pain (p=0.036), and comorbidities (p=0.039) (TABLE 4). The overall percentage value in this study was 77.8%. Education level was not significantly related with p = 0.301.

DISCUSSION

The delay in reperfusion treatment was unaffected by the level of education, health funding, or distance to the hospital. Patients did not receive reperfusion treatment for a variety of reasons, including presenting late pain onset (>12 hr), declined reperfusion therapy, and poor level of education (38%) that might fuel the inadequacy of knowledge on STEMI symptoms. Nonetheless, multivariate studies indicated they were not statistically significant. The lack of funding (no universal health coverage), as well as contraindications in some patients, such as a history of stroke (0.9%) and post cardiopulmonary resuscitation (2.8%), and decreased consciousness, patient's fear, and the lack of understanding of the indications and symptoms of STEMI were the reasons of not receiving therapy. Moreover, the referral mechanism was ineffective. The distance to Dr. Soebandi General Hospital, Jember also did not affect reperfusion therapy.

The total number of patients who did not receive therapy was 62% of patients, while 38% of patients received reperfusion therapy. There were more patients receiving PCI therapy (20 patients or 18.5%) than fibrinolytic therapy (19 patients or 17.6%), while 2 patients received both PCI and fibrinolytic therapy (1.9%). Multivariate analysis indicated that reperfusion therapy significant effected the incidence of MACE with an OR of 3.688 (95%CI: 1.274-10.679). In other words, patients

who did not get reperfusion therapy had an increased risk of MACE 3.688 higher than patients with reperfusion therapy. These results are consistent with the study conducted by Yang *et al.* that reperfusion therapy is associated with decreased risk of MACE than the patients who does not get reperfusion therapy.[5]

Reperfusion aims to fix the myocardial blood flow, saving myocardium, maintaining the function of the left ventricle, and reducing the mortality level.[6] A successful reperfusion therapy greatly relies on the period passing between the symptom appearance and the therapy. Early reperfusion with a short period between "symptom-to-needle" and "door-to-needle" in patients with myocardial infarction is the main goal of reperfusion.

The key factor in STEMI treatment is the ischemic time, or when the symptoms appear until the reperfusion therapy.[7] The longer the artery is exposed to occlusion, the wider the ischemic wave which extends from the endocardium to the epicardium so an immediate reperfusion act must be made.[8] Percutaneous coronary intervention is the treatment of choice in the management of patients with acute STEMI which significantly reduces mortality and morbidity compared to fibrinolytics as a reperfusion strategy.

Clinical outcomes in STEMI patients are influenced by the occurrence of complications known as MACE, which consists of left ventricular dysfunction, recurrent ischemia, early reinfarction, severe coronary disease, stroke and malignant arrhythmias.[5] According to the data and result of studies, reperfusion therapy can lower the risk of MACE.

A study by Parung *et al.* stated that the number of patients who did not get reperfusion treatment was higher than the number of patients who did.[9] The number of STEMI patients who received reperfusion therapy at Dr. Soetomo General Hospital, Surabaya in 2013 was 41%, where 28% of them received PCI while 72% received fibrinolytic therapy. About 59% of STEMI patients did not receive reperfusion therapy. This occurred because the National Health Insurance (Jaminan Kesehatan Nasional/ JKN) had yet to begin in 2013, and most patients were still unable to afford reperfusion therapy.[9] This condition increases the incidence of MACE in patients with no reperfusion therapy. As recommended by the European Society of Cardiology, individuals with clinical symptoms of STEMI and persistent ST-segment elevation or new LBBB on the ECG should be treated as soon as possible within 12 hr.[10]

According to Huber *et al.*[11] three factors contributed to the time delay between the onset of chest pain and the start of reperfusion therapy: delayed patient's decision to get to the hospital (1.5 to 3 hr), delayed prehospital transportation (30 to 130 min) and delayed STEMI management. Furthermore, a referral system might lengthen the time it takes for patients to get reperfusion treatment. Another factor affecting the delay was the patient's inaccuracy in perceiving the symptoms of chest pain.[12] About 41% of the patients perceive that the symptoms of chest pain experienced were not heart disease and 64.1% of them had delayed treatment. The patient's ability to correctly perceive the symptoms would determine the patient's response.[13,14]

The patient's level of education has an impact on their knowledge and decision-making. Patients who did not complete obligatory education (low education) had a limited understanding of symptoms and decision-making, whereas patients who completed their compulsory education (higher education) had sufficient information for considering options and clear judgments.[15] Furthermore, as the level of education affected the reperfusion therapy, it might also indirectly affect the

incidence of MACE. Multivariate analysis in this study showed that education level does not influence reperfusion treatment delay. This might be due to other important factors: local cultural and the size of the study's sample. Local culture practice might cloud patient's judgment of STEMI symptoms.[16]

The National Health Insurance covered treatment costs for the majority of STEMI patients in Dr. Soebandi General Hospital. Patients with premium-paid the National Health Insurance participants (non-PBI) presented more STEMI cases than those with dues assistance recipients (PBI) participants. The PBI patients accounted for 31 patients (28.7%), while the non-PBI group consisted of 74 patients (68.5%). The personal decision to whether to receive reperfusion treatment or not was heavily influenced by their health insurance coverage. Patients who received reperfusion therapy were often residents covered by the National Health Insurance, while those who were not covered by the National Health Insurance were found to receive less reperfusion therapy. A total of 19 out of 108 patients (17,6%) received fibrinolytic reperfusion therapy with streptokinase as a fibrinolytic drug. This is due to the low cost of streptokinase.[9,10]

CONCLUSION

In conclusion, reperfusion is proven to be effective in reducing MACE with an OR of 3.688. The delay in reperfusion therapy is not related to the level of education, health funding, and distance to the hospital. Lack of awareness of STEMI is suspected to be the major cause of the delay in reperfusion therapy. This can be seen from late-onset presentation (> 12 hr) and psychosocial factors. Late-onset STEMI patients (>12 hr) have a 3.254 times higher risk of MACE than patients presented with early onset STEMI.

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The development and use of artificial intelligence (AI) in dermatology: a narrative review

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ABSTRACT

Artificial intelligence (AI) is defined as a computer science involving program development aiming to reproduce human cognition to analyze complex data. Artificial intelligence has rapidly developed in the medical field. In dermatology, its development is relatively new and is generally used in the diagnostic, especially for skin imaging analysis and classification, and also for risk assessment. The greatest advances have been primarily in the diagnosis of melanoma, followed by the assessment of psoriasis, ulcers, and various other skin diseases. The use of AI has shown good accuracy and is comparable to dermatologists in various studies, especially related to melanoma and skin tumors. However, several obstacles exist in the application of AI to daily clinical practice, including generalizability, image standardization, the need for large data quantities, and legal and privacy aspects. In current developments, AI should be aimed at helping enhance the decision-making of clinicians.

ABSTRACT

Artificial intelligence (AI) adalah ilmu komputer yang terlibat dalam pembuatan program yang bertujuan untuk mereproduksi kognisi manusia dan menganalisis data yang kompleks. *Artifical intelligence* berkembang pesat, namun di bidang dermatologi masih tergolong baru dan umumnya digunakan untuk diagnostik yaitu analisis, klasifikasi gambar dan penilaian risiko. Kemajuan paling besar adalah pada penegakan diagnosis melanoma, diikuti penilaian psoriasis, ulkus dan berbagai penyakit kulit lainnya. Penggunaan AI telah menunjukkan akurasi yang baik dan sebanding dengan dokter spesialis dermatologi dalam berbagai studi, terutama terkait melanoma dan tumor kulit. Meskipun demikian, terdapat beberapa hambatan dalam penerapan AI, meliputi kemampuan generalisasi, standardisasi gambar, kebutuhan akan kuantitas data yang besar, aspek legal, privasi dan lainnya. Sebaiknya, AI digunakan untuk membantu pengambilan keputusan oleh klinisi.

Keywords:
artificial intelligence;
deep learning;
dermatology;
machine learning;
machine learning

INTRODUCTION

Intelligence represents the mental ability to think, plan, solve problems, understand complex ideas, and learn from experiences.[1] Artificial intelligence (AI) is a scientific understanding of the underlying mechanisms of intelligent behavior and its embodiment in a machine. In other words, AI is defined as a branch of computer science involving program development aiming to reproduce human cognition to analyze complex data.[2] The term “augmented intelligence’ was often used rather than artificial intelligence to emphasize systems that enhance and augment human decision-making rather than an attempt to replicate human intelligence.[3]

In the medical field, AI is well-acknowledged and supported by the rapid technological development. AI in dermatology is fast emerging, especially for image classification and

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risk assessment.[4] We are entering an era of AI for dermatology; hence, a proper understanding is needed regarding this automated system and how it should be implemented in future clinical settings to deliver better skin care. This review aimed to discuss the basic concept of AI, AI development, and AI applications in dermatology.

DISCUSSION

Basic concept

The term AI was first coined by John McCarthy during the Dartmouth College Conference in 1956, though the concept of human behavior simulating machine was proposed by Alan Turing in 1950.[5,6] In 1970, AI began to be applied in life science, yet the development was hindered by technological limitations.[2] Rapid technology development in the last two decades provides an opportunity to apply AI in medical practices.[7] Artificial intelligence is divided into strong AI and weak AI. The former refers to a machine with human-level intelligence, capable of learning independently and performing several different tasks. Meanwhile, the latter refers to a machine that learns to fulfill a single task, thus requiring several programs to run several different tasks.[2,8]

Machine learning

Machine learning (ML) differs from classic programming, in which a computer is supplied with a dataset and an algorithm. Classical programming uses an existing algorithm to process the dataset into outputs. In contrast to classical programming, ML uses the dataset and its output, allowing the model to learn and generate an algorithm linking the data and the output. The generated algorithm can be used to process the new dataset.[9] In other words, ML represents a model's learning ability to find the pattern in a large dataset.[1]

There are several methods of machine learning, including supervised, unsupervised, semi-supervised, and reinforcement learning.[9] The model generates an algorithm from the training dataset to predict the outputs of the new dataset. In supervised ML, the data and target output have been labeled, while in unsupervised ML, the model finds the pattern of the data and categorizes them into target output on its own. The semi-supervised ML is positioned between the supervised and unsupervised ML, in which some data have been labeled while others are not labeled. Reinforcement learning employs a system of "reward and punishment" to generate the problem-solving strategy.[9,10]

Deep learning

The deep learning (DL) term was coined by Geoffrey Hinton, known as the father of deep learning.[11] Deep learning is a part of ML that uses artificial neural networks (ANN). Figure 1 shows the interrelationship of terms related to AI. DL has several processing layers, and each layer possesses the ability to recognize and learn a specific feature of the given data. These processing layers are built sequentially and can be unlimited in number. The complexity of the layered structure allows the model to perform more complex tasks.[7,12] The ANN is inspired by human biological neurons. Each ANN has nodes (similar to nerve cell bodies) communicating with other nodes (similar to axons and dendrites).[7] The data are analyzed using an algorithm in each layer. The output of the first layer is analyzed by the algorithm of the next layer until generating the final output.[5] The most common training method used in DL is the supervised method, in which the dataset (e.g., skin lesion) has been labeled (e.g., benign or malignant). The reinforcement learning method can be used in the learning process that requires demonstration, e.g. robotic surgical assistant.[12]

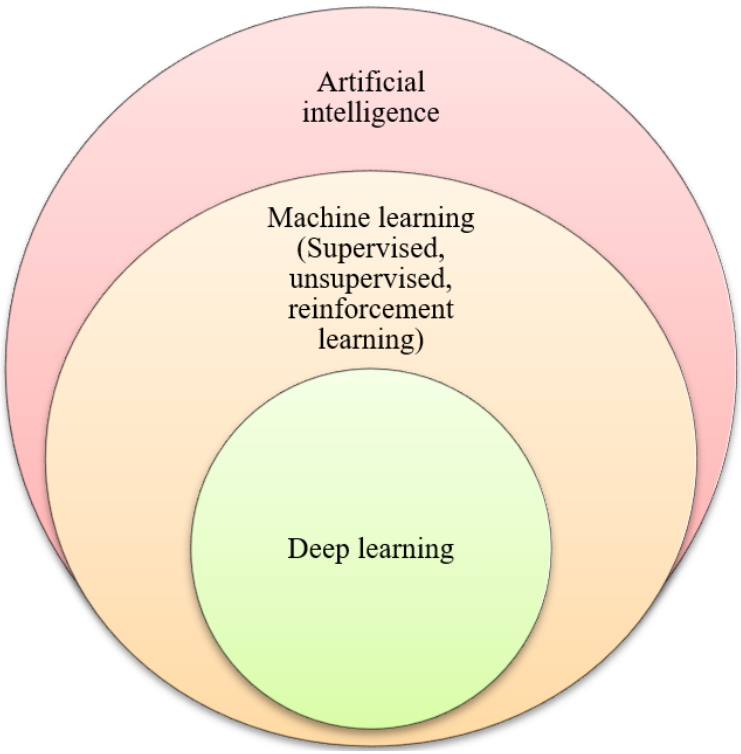

FIGURE 1. Diagram showing interrelationship of terms related to artificial intelligence.

A convolutional neural network (CNN) is a subtype of ANN that is commonly used to process grid-pattern data such as images. CNN is designed to recognize the spatial hierarchy of the image features. The CNN is composed of three types of layers: a convolutional layer and a pooling layer to extract features of an image, and fully connected layer that maps the extracted feature into final output such as image classification.[13] Thus, CNN is highly efficient for image processing since the feature could be anywhere in the image.

AI application in dermatology

Dermatology is a field involving high visual aspects with a large number of patients; hence, studies on medical image interpretation are highly required.[14] Image classification becomes the most frequent target of AI development in dermatology. Before 2016, most studies applied conventional computer assisted diagnosis (CAD) for automatic diagnosis based on the given medical images. CAD is composed of several tasks, including image quality enhancement, segmentation, feature extraction, and classification.[15]

Preprocessing

This process aims to enhance the image quality and eliminate noise and artifacts (hair, shadow, etc.) The frequently used methods include color transformation, illumination correction, contrast improvement, and artifact elimination. A proper preprocessing stage is important for facilitating the segmentation stage and improving classification accuracy.[16,17]

Segmentation

The segmentation process aims to determine the region of interest (ROI) in the images. This stage represents

the most complex phase due to the numerous diagnoses of skin lesions.[15] The segmentation is continuously performed until the ROI is successfully isolated from the rest of the unimportant parts of the image.[18] Previous studies have compared or combined several image segmentation methods. Some of the frequently used methods include edge-based (using information from sudden changes at the lesion edge, such as discontinuity and pixel intensity changes), thresholding and region-based (using similarity criteria to identify the skin lesion), AI-based (neural network, k-means clustering, fuzzy logic, and evolutionary computation), active contour-based, etc.[16] The accuracy of the segmentation method is evaluated by comparing it to the ground truth established by manual segmentation.[17]

Feature extraction

After determining ROI, the feature extraction stage aims to identify the features with discriminating characteristics to classify the image into certain categories.[15] For instance, in melanoma diagnosis, the most frequently used feature extraction algorithm is the ABCDE criteria. Asymmetry (A) is measured by dividing the segmented area into two subregions based on the X and Y axes. Border (B) is classified into regular and irregular borders. Color (C) denotes the number of colors found in a lesion. Diameter (D) is determined by measuring the greatest distance between two edges.[19] Other extractable features include shapes (area, asymmetry, diameter, density), color, texture, and histogram color.[18]

Image detection and classification

This process classifies the image set into suitable categories. There are several types of classifiers. Some frequently used models include logistic regression, support vector machine (SVM), decision tree, random forest, Naive Bayes, and K-nearest neighbor. Proper classifier selection is pivotal to generating a satisfactory result.[15,20]

In conventional CAD, the process of determining and extracting features requires considerable time and resources. In contrast, when using DL, the learning model can independently determine the important features for image classification. As a result, a process that previously took years to complete can now be completed in a few months.[11] Some AI studies used dermoscopic and non-dermoscopic image sets to segment and classify melanocytic, keratinocyte tumors, ulcers, psoriasis, and other inflammatory diseases. Some models even exhibit diagnosis capacity comparable to dermatologists.[21]

AI application in skin malignancy classification

Artificial intelligence has been developed to classify melanoma and non-melanoma skin cancers using digital images.[22] The CNN model can be used for binary or multiclass classification.[23] Nasr-Esfahani *et al*.[24] used CNN to distinguish melanoma from the benign lesion with a sensitivity and specificity of 0.81 and 0.80, respectively. Fujisawa *et al*.[25] employed the deep convolutional neural network (DCNN) GoogleNet to classify skin tumor images into fourteen types of diagnosis and compared them to the diagnosis of certified dermatologists. Their study used 4,867 images from 1,842 skin tumor patients obtained from the institution's database with the three-level assessment. The first level aimed to differentiate benign from malignant lesions; the second level aimed to classify the images into certain tumor categories; and the third level aimed to classify lesions into specific diagnoses. The sensitivity and specificity of the model were 96.3% and 89.5%. The most accurate diagnosis

was malignant epithelial tumor (95.7%), followed by benign melanocytic tumor (90.9%), malignant melanoma (72.6%), and benign epithelial tumor (62.8%). In the first level assessment, DCNN and dermatologist classification accuracy was 92.4 ± 2.1%, and 85.3 ± 3.7%; while in the third level, the accuracy was 74.5 ± 4.6% and 59.7 ± 7.1%. In general, a large number of labeled images is required to achieve high accuracy, yet this study used less than 5,000 training images.[25]

Han *et al.*[26] used pretrained CNN MicrosoftResNet-152 to classify medical images into 12 types of skin tumor diagnosis. The validation results showed sensitivity and specificity of 85.1% and 81.3% on the Caucasian dataset, and 85.4% and 85.5% on the Asian dataset respectively.[26] Brinker *et al.*[27] evaluated the CNN ResNet50 to distinguish between melanoma and nevus based on dermoscopic images using histopathology as the gold standard. Their study used 4,204 training images and 804 test images. The sensitivity and specificity of certified and junior dermatologists were 67.2% (95% CI: 62.6-71.1%) and 62.2% (95% CI: 57.6-66.9%), respectively, while the sensitivity and specificity of the CNN model were 82.3% (95% CI: 78.3-85.7%) and 77.9% (95% CI: 73.8-81.8%), respectively.[27]

Haenssle *et al.*[28] used pre-trained CNN Google's Inception v4 model to classify 100 dermoscopic images into melanoma and benign nevus, with dermatologists' diagnoses were used as the reference standard. Dermatologists were given two-level of assessment. In the first level, they were only given dermoscopic images, while in the second level, they were given dermoscopic images, clinical data, and clinical images. The study reported the sensitivity, specificity, and area under the curve (AUC) of dermatologists in the first level was 86.6 ± 9.3%, 71.3 ± 11.2%, and 71.3%, respectively, while in the second level, they was 88.9 ± 9.6%, 75.7 ± 11.7%, and 75.7%, respectively. Meanwhile, those of CNN were 63.8%, 86%, and 95%, respectively. This study showed that additional clinical information improves dermatologists' diagnosis accuracy. However, the specificity and AUC of dermatologists in this study were inferior compared to those of the CNN.[28]

AI application in classifying non-neoplastic skin disease

Gustafson *et al.*[29] developed natural language processing (NLP) to build a registry of atopic dermatitis from the electronic medical record. The data included coding of diagnosis (ICD9 and ICD10) and the narrative data from medical history. A group of keywords was determined based on Hanifin Rajka and The United Kingdom Working Party's (UKWP) criteria to build a dictionary concept. The concept found in the medical history was then extracted and converted into a group of features which will be analyzed using Lasso logistic regression. The model's sensitivity in this study was 75.0%, with a positive predictive value of 84.0%.[29]

A systematic review conducted by Yu *et al.*[14] evaluates the use of ML for psoriasis. It has been used in many studies to identify psoriasis lesions, calculate psoriasis area and severity, and predict outcomes.[14] Shrivastava *et al.*[30] developed a psoriasis risk assessment system (pRAS) to classify 670 skin lesion images into five categories: healthy skin, mild, moderate, severe, and very severe psoriasis. The support vector machine (SVM) and Fisher discriminant ratio (FDR) classifications exhibited 99.84% accuracy and 99.99% reliability.[30]

Zhao *et al.*[31] developed a CNN to diagnose psoriasis from more than 8,000 images consisting of nine diagnoses (four diagnoses that mimic psoriasis and five diseases significantly different from psoriasis) into binary classification, psoriasis, and non-psoriasis. The CNN

model was reported to exhibit an AUC of 0.981 ± 0.015 with 96% accuracy, higher than the accuracy of 25 dermatologists (87%).[31] The severity of psoriasis was clinically measured by the psoriasis area and severity index (PASI), consisting of erythema, scales, and induration criteria. Some studies have implemented ML to automatically assess the psoriasis severity from medical images. George reported the model accuracy to assess the degree of erythema and scales to be 70.1% and 80.81%.[32,33]

Several recent studies developed AI for multiclass image classification. Liu *et al*.[34] used a deep learning system (DLS) to distinguish the twenty-six most common skin conditions in primary care, with the reference standard was a panel consisting of three certified dermatologists. The DLS provided three differential diagnoses and achieved top-1 and top-3 accuracies of 0.71 and 0.93, respectively.[34] Meanwhile, Zhu *et al*.[21] applied CNN Google's Efficient Net-b4 model to classify dermoscopic images into fourteen diagnosis categories and reported an overall accuracy of 94.8%, a sensitivity of 93.4%, and a specificity of 95%.[21]

AI application in ulcer assessment

A wound analysis system is frequently implemented to capture high-quality images, determine the wound border and area, classify wound tissue, and assess wound recovery.[35] Mukherjee *et al*.,[36] employed Bayesian and SVM classification to recognize different tissues in chronic wounds, such as granulation tissue, slough, and necrotic tissues compared to clinicians' assessment. The accuracy of SVM was reported to be 87.84, 90.90, and 79.78% in classifying granulation tissue, slough, and necrotic tissue, respectively, with the overall accuracy of 86.13%.[36] Wang *et al*.[35] used SVM to determine the wound border of 100 leg ulcer images taken using smartphones. The study reported sensitivity of 73.3% and and and specificity of 94.6%.[35] Dhane *et al*.[37] applied AI to determine the area of ulcers with an unclear border and reported a sensitivity of 87.3% and specificity of 95.7%.[37] Another study used ML to predict the risk of developing pressure injuries in postoperative patients based on data extracted from electronic medical records. The prediction model was reported to exhibit an AUC of 0.79.[38]

AI application in dermatopathology

Deep learning (DL) has been implemented to improve the precision of breast and lung cancer diagnosis. Hekler *et al*.[39] used a pre-trained CNN RestNet50 model to identify melanoma or benign nevus from histopathological images and compared it to the classification of a certified histopathologist. The study reported that the discordance of melanoma and nevus classification were 18% (95% CI: 7.4-28.6%) and 20% (95% CI: 8.9-31.1%), respectively.[39] The discordance between expert histopathologists in the classification of melanoma and nevi, as described in the literature, is 25%.[40] This is on par with the discordance between CNN and the histopathologists in this study. Digital pathology has the potential to enhance the accuracy of melanoma histopathological diagnosis.

Other applications of AI in dermatology

Artificial intelligence has also been used for diagnosing onychomycosis. Even though the evidence is limited, AI has the potential to assist clinicians in deciding whether further tests should be performed. Artificial intelligence can also be used to help patients evaluate their nails and to seek further assessment for nails that are suspicious for onychomycosis.[41] It can be used to monitor and predict disease outcomes.

Veredas *et al.*[42] used neural networks, random forest decision trees, and SVM to classify the types of wound tissue to monitor wound improvement. Their study reported an average accuracy of 81.87% (95% CI: 80.03-83.61%); 87.37% (95% CI: 85.76-88.86%); 88.08% (95% CI: 86.51-89.53%), respectively. Zang *et al.*[43] used ML to classify the risk of skin sensitization of some substances based on their chemical structure and reported the prediction accuracy was 78% in animal trials and 75% in humans.[43]

In cosmetic dermatology, AI is used to provide skin and hair care recommendations, analyze skin conditions, and assist cosmetic procedures. One examples of an AI applications is the VISIA® skin analysis system, which can analyze skin parameters, such as wrinkles, texture, pores, spots, and redness using face images. In the most recent VISIA® model, the TruSkin age feature provides information on wrinkle degree, UV damage, and discoloration based on the patients' age and compares them to a range of variables in the database.[44] Another skin analysis system is Janus-III, which uses high-resolution images to analyze pores, wrinkles, sebum, porphyrin, skin pigmentation, and skin color. The skin pigmentation parameter was found to be associated with dermatologist assessment (Pearson correlation coefficient = 0.869).[45] Another application is FotoFinder, which facilitates total body photography, dermoscopy, and trichoscopy. This system is equipped with AI to quantify hair falls, density, and anagen hair proportion.[44]

During the Covid-19 pandemic, research that focused on utilizing AI increased. Computational techniques, information and communication technologies, AI, and big data can handle a huge amount of data from public health surveillance, real-time epidemic outbreaks monitoring, trend forecasting, updating from governmental institutions, and others.[46] Big data is defined by three Vs: velocity (the unprecedented speed of data acquisition, processing, and manipulation), volume (the high amount of information), and variety (the number of different sources and channels releasing big data). It is a massive dataset that exceeds the computational capacity of conventional database systems to capture, store, manage, and analyze.[46,47]

AI has been immensely helpful in telemedicine, such as in providing systems to analyze medical information and assist in diagnosis. During the Covid-19 outbreak, AI was implemented in telemedicine for various diseases. AI can be used to make early diagnosis and contact tracing, monitoring symptoms and treatment, clinical management, and virtual and remote treatment.[48] Artificial intelligence in telemedicine can also be used as a method of triaging patients with potential skin cancer who require in-person evaluation by dermatologists.[49] Medical professionals need to adapt to AI advances to provide better healthcare delivery.[48]

AI implementation and interpretation in dermatology

Although some previous studies have reported the superiority of machine learning over dermatologists, it is necessary to highlight the presence of bias commonly found in the study design and bias that puts clinicians at a disadvantages.[50] Machine learning is trained and tested using the same data sources, thereby limiting their generalizability. Model learning is basically a reflection of the training data. Bias in the training data is likely to affect the model's performances and will be apparent when they are tested on a completely different dataset.[50]

In previously published studies, most learning models used binary classification, which does not reflect

clinical practice, where numerous differential diagnoses are taken into account.[27] Moreover, AI models are often tested by comparing them to dermatologists without considering the clinical context and the limitation of diagnosis based on images alone.[12] Automated diagnosis using AI is beneficial as a tool to assist and enhance dermatologists' diagnosis, but not as an independent decision-maker without clinicians' supervision.[27]

Limitation in the development and application of AI

Despite its significant development in dermatology, AI still faces several limitations. Artificial intelligence development requires solid multidisciplinary collaborations, such as computer science, biomedical, and medical staff. Massive skin image resources are extremely important. Currently, skin disease images data is still inadequate, information-sharing among hospitals is low, and the quality of skin images varies.[51] The most critical factor in developing a predictive model is the dataset. A supervised DL model requires a large and labeled dataset. Obtaining a few labeled datasets is possibly easy but may result in poor performance on a new dataset. Meanwhile, an unlabeled dataset can only be useful for semi-supervised or unsupervised model.[12] Small datasets potentially lead to bias and lower accuracy, especially for neural networks.

The uneven proportion of certain disease categories in the training dataset also results in bias and affects the model's generalizability.[34] Navarrete-Dechent *et al.*[52] conducted external validation on the learning model developed by Han *et al.*[26] on a Caucasian population dataset. The study reported that Han's algorithm sensitivity was lower when tested on a different population. This result indicates that developing and training the DL model requires a large dataset covering the full spectrum of the human population and clinical variation. Detection of skin lesion is also affected by several factors, including skin color variation, redness level, severity, etc.[53]

Another challenge in dermatology comes from image standardization, including variability in technology (camera type), and image-capturing technique (lighting, angle, body position, etc.). Unlike in radiology, there is no standardization for taking images in dermatology.[54] Navarrete-Dechent *et al.*[52] attempted to manipulate medical images by changing the magnification, contrast, brightness, and rotation from a previous dataset, which resulted in some different diagnoses. Although AI can enhance medical service accuracy, access, and efficiency, it carries a risk of misdiagnosis. This risk increases if the AI system is provided directly to patients.[52]

The current AI diagnosis also involves legal, ethical, and patient privacy aspects that have not yet been fully resolved. Certain data may violate patients' privacy and in the case of an adverse event, the matter of accountability is yet to be addressed.[55] Furthermore, establishing a diagnosis requires various clinical information in addition to clinical images or photographs. These data should be integrated to determine the patient's working diagnosis, treatment, and prognosis. Future research integrating AI diagnosis and clinical data will provide better information on how to implement them in clinical settings. Lastly, the development of AI in medicine does not aim to substitute doctor-patient communication, holistic approach, and other humanistic care.[51]

CONCLUSION

Artificial intelligence can significantly contribute to medical clinical practice, including dermatology.

Existing studies have shown that AI-assisted diagnosis has comparable accuracy to dermatologist, especially in skin cancer screening. However, the models need to be trained on a large dataset with the full spectrum of the human population and clinical manifestations to obtain better generalizability, and further study integrating AI diagnosis and clinical data is necessary. Artificial intelligence is a beneficial tool to assist clinicians' decision-making processes and improve health services in the future healthcare system.

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Autoimmune manifestation in splenic atrophy presented with toxic shock syndrome: a case report

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ABSTRACT

Splenic atrophy is an uncommon diagnosis, associated with autoimmune gastrointestinal disorders and other well-characterized connective tissue diseases. We would like to contribute a case report to support the association evidence of the real-world data. To our best knowledge, there is no similar case of splenic atrophy with the presentation of streptococcal toxic shock syndrome reported. Our patient was initially detected with atrophic spleen via CT scan and subsequently diagnosed with systemic sclerosis. Hyposplenism should be suspected in patients with adult-onset infections caused by encapsulated bacteria, especially if autoantibodies are present. Our patient received her pneumococcal vaccination before discharge and was followed in the clinic for further vaccination education and health check-up. Learning points: 1) Acquired splenic atrophy is a rare condition that may be suspected from persistent isolated thrombocytosis after the resolution of sepsis and Howell-Jolly bodies from peripheral blood film; 2) The pathophysiological mechanism of splenic atrophy in the context of autoimmune disorders remains unknown; 3) A high index of suspicion towards the evaluation of splenic function is required if a patient presented with community-acquired encapsulated organism bacteraemia; 4) Vaccination against encapsulated bacterial agents should be performed in patients with hyposplenism.

INTRODUCTION

The most frequent causes of adult-onset recurrent infections in Asia are primarily secondary states of immunodeficiency, such as human immunodeficiency virus (HIV) infection, Hepatitis infection, malignancy, and autoimmune diseases. Acquired, non-surgical, functional asplenia is a rare cause.[1] By definition, functional asplenia or hyposplenism occurs when splenic tissue is present but does not work well, and splenic atrophy is where there's evidence of acquired diminution of the size of the spleen which can lead to functional asplenia.

This condition is characterised by the impairment of the reticuloendothelial and immune functions of the spleen. Consequently, patients with hyposplenism are at risk of developing life-threatening infections. Defective spleen function has already been reported in several haematological, immune-mediated, infectious and gastrointestinal disorders, including sickle cell disease, coeliac disease (CD), inflammatory bowel disease, systemic lupus erythematosus, Sjögren's syndrome and other primary eosinophilic disorders. To the best of our knowledge, no case of toxic shock syndrome in a patient with acquired splenic atrophy and autoimmune disease has previously been reported in Asia.

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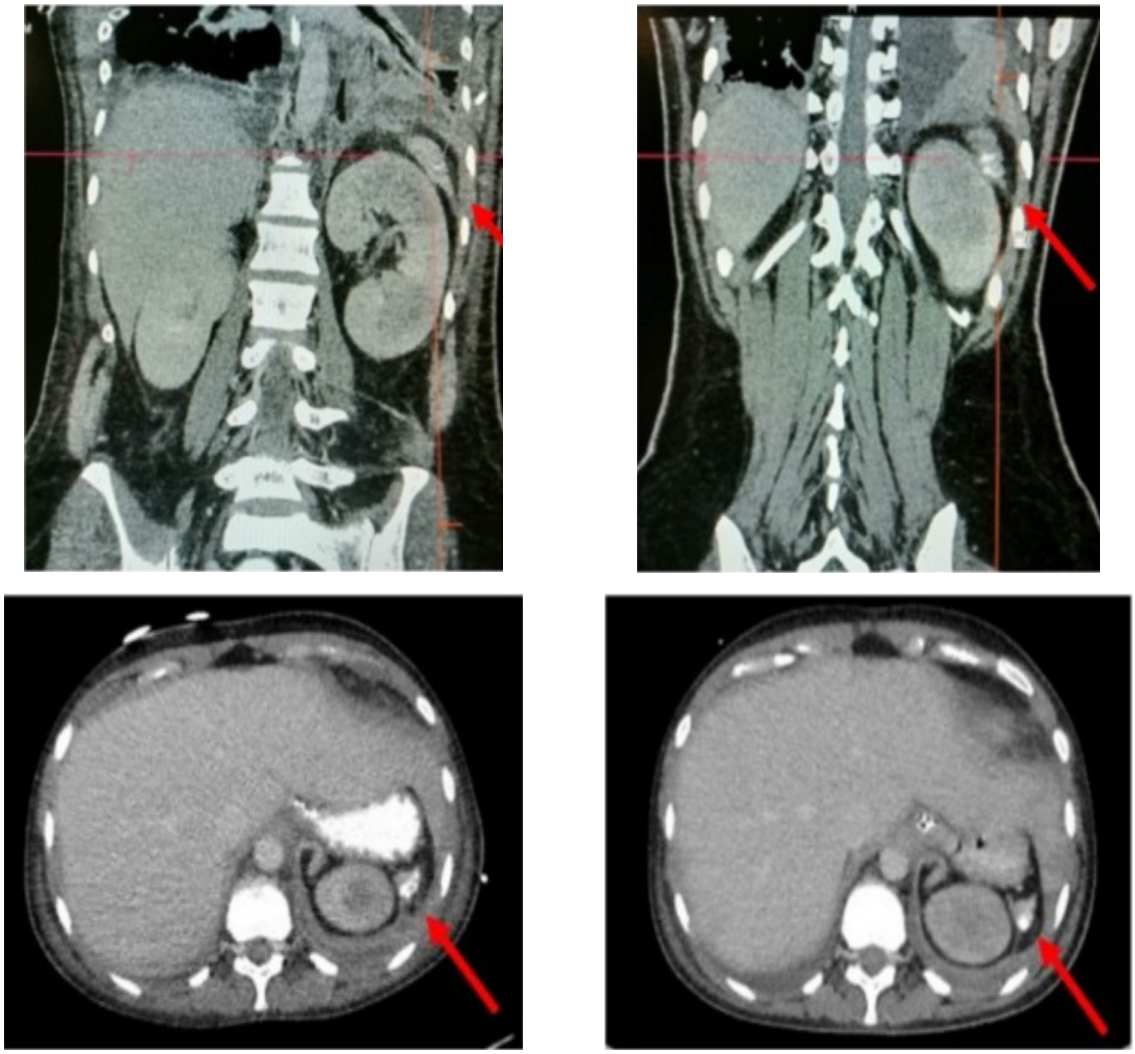

FIGURE 1. Coronal and axial view of patient's CT abdomen pelvis

CASE

We report a case of a 25 y.o. Indian woman, Miss I with no known medical illness, who presented to us with progressive worsening of lower abdominal pain for 3 d associated with clinical sepsis of fever, lethargy, vomiting and diarrhoea. Initial assessment and investigations showed that she had septic shock secondary to leaking tubo-ovarian abscess. She was rushed for early surgical source control of intraabdominal sepsis after prompt resuscitation. Intraoperative findings include drainage of 100mL turbid yellowish peritoneal fluid, pus drainage from Tubo-ovarian site with Fitz-Hugh-Curtis syndrome. Post-surgery, she was nursed in the intensive care unit. Primary microbiology assessment from blood and peritoneal pus cultures consistently revealed *Streptococcus pyogenes* as the culprit organism.

Initial and subsequent CT scans revealed the finding of the atrophic spleen (FIGURE 1) which prompted us for further evaluation. Her autoimmune panel of antinuclear antibody (ANA) and AntiSc1 70 were positive on day 14 of ICU admission and was subsequently referred to the rheumatology team with the impression of systemic sclerosis. Other autoimmune serology markers including antineutrophil cytoplasmic antibodies (pANCA & cANCA), anti-

cardiolipin antibody (IgM & Ig G), anti-beta 2- glycoprotein-1 (IgG & Ig M) were negative. Her blood trends in ICU revealed persistent thrombocytopenia ranging from 410-520 (10^9/L) and serial peripheral blood picture showed Howell-Jolly Bodies.

Her stay in ICU was complicated with distal right upper limb compartment syndrome likely secondary to septic emboli of *S. pyogenes*, left pyelonephritis with lobar nephronia and left pectineus intramuscular abscess. Despite her arduous journey in ICU, she was able to be discharged from the hospital and subsequently decannulated from tracheostomy. She received a dose of PPV23 vaccination prior to discharge.

The summary timeline of patient's progress is presented in TABLE 1.

TABLE 1. Summary timeline of our patient's progress

Date	Events and progress
26/4/2022	Miss I presented to our Emergency Department with progressive worsening of lower abdominal pain for 3 d associated with clinical sepsis of fever, lethargy, vomiting and diarrhoea.
27/4/2022	She was rushed to operation theatre for source control of intraabdominal sepsis where initial CT abdomen pelvis has reported left tubo-ovarian abscess with evidence of free fluid collection. Intraoperative findings include drainage of 100mL turbid yellowish peritoneal fluid, Fitz-Hugh-Curtis syndrome, and significant pus drainage from Tubo-ovarian site. Post op, patient is admitted to ICU.
1/5/2022	She developed progressive swelling of right distal upper limb complicated by compartment syndrome likely secondary to septic emboli from *Streptococcus* bacteraemia. Orthopaedic and vascular surgery teams were onboard timely for surgical decompressive management.
10/5 /2022	Rheumatological consult was obtained in view of the physical examination suggestive of cutaneous scleroderma at bilateral hands MTP joints with suspicious features of sclerodactyly (FIGURE 2). Autoimmune panel workup positive for ANA and AntiSc1 70. A preliminary impression of systemic sclerosis was made by the rheumatological team.
14/5/2022	A repeated CT thorax abdomen pelvis was performed due to the persistence of patient's clinical and laboratory sepsis where disseminated infectious seeding is our major concern. Left pyelonephritis with lobar nephronia and left pectineus intramuscular abscess were detected. Interventional radiology and surgical team was consulted for source control of targeted percutaneous drainage. At this time, the recurrent term of atrophic spleen which was reported has caught our interest of critical thought and management (FIGURE 1).
3/6/2022	She underwent tracheostomy in view of recurrent intubation in ICU, prolonged weaning.
28/6/22	She was discharged from ICU to the general ward after successfully weaned off from mechanical ventilation and remained stable for 1 week. Rehabilitation, tracheostomy care and post-ICU recovery interventions were main goals of management in the GW.
1/8/22	Miss I received her vaccination dose of PPV23 prior to discharge. Follow-up appointments has be given for tracheostomy decannulation, outpatient rehabilitation and respective clinical subspecialties which included rheumatology, infectious diseases, and obstetrics & gynaecology.

DISCUSSION

Throughout history, the spleen has been regarded as a fascinating and mysterious organ with distinctive functions. The anatomical study of spleen was first discovered by Malpighi[1] in De liene in 1965, which reported the "splenic cap" and of the trabeculae "that accompany the distributions of vessels, collected in bundle to tube shape". The spleen is a secondary lymphoid organ, located in the upper left part of the abdomen, sheltered by the ribcage. The spleen possesses two main functions, which are blood filtering, which is necessary for removing old erythrocytes (haemocatheresis) and other blood cells in the red pulp, and mounting immune responses against pathogens through both the innate and adaptive immune system branches in the white pulp.[2,3]

Acquired splenic atrophy is a rare condition that is usually detected incidentally via abdominal scans. Although spleen size does not necessarily correlate with spleen function, hyposplenism should be excluded in patients with incidentally found small spleen (length <8 cm in men and <7.5 cm in women).[4] In our patient who presented with sepsis, reactive thrombocytosis was our initial explanation to her high platelet count. However, her baseline platelet count has consistently been more than 410 (10^9/L) from the day of post-surgery till the day of ICU discharge opposing the decreasing trend of inflammatory markers (C-reactive protein & Pro-calcitonin), which prompted to us evaluate other causes of reactive thrombocytosis aside of sepsis- related. In our case of hyposplenism, decreased platelet sequestration is the main pathophysiology for thrombocytosis.[5]

Our patient also fulfilled the ACR-EULAR Criteria for the classification of systemic sclerosis which includes bilateral hands sclerodactyly with right-hand fingertip lesion which results in gangrenous transformation and positive AntiScl 70 (FIGURE 2).[6] The pathophysiological mechanism of splenic atrophy in the context of autoimmune disorders remains uncertain.[7,8] Severe lymphocyte depletion in the spleen in association with severe fibrosis has been reported. It was hypothesized that self-reactive lymphocytes produce factors that directly or indirectly induce splenic fibrosis, which results in lymphocyte depletion and atrophy.[9] Alternatively, functional hyposplenism secondary to Fc-receptor blockage by circulating immune complex saturation has been described in SLE and systemic vasculitis.[10,11] Acquired asplenia or hyposplenism increases an individual's susceptibility to infections with encapsulated bacteria such as *S. pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis*. In our case of *S. pyogenes* bacteraemia, most clinical isolates of *S. pyogenes* elaborate a capsular polysaccharide, which is composed of hyaluronic acid, a high-molecular-mass polymer of alternating residues of N-acetyl glucosamine and glucuronic acid.[12] Therefore, a high index of suspicion towards the evaluation of splenic function is required if a patient presented with community-acquired encapsulated organism bacteraemia.

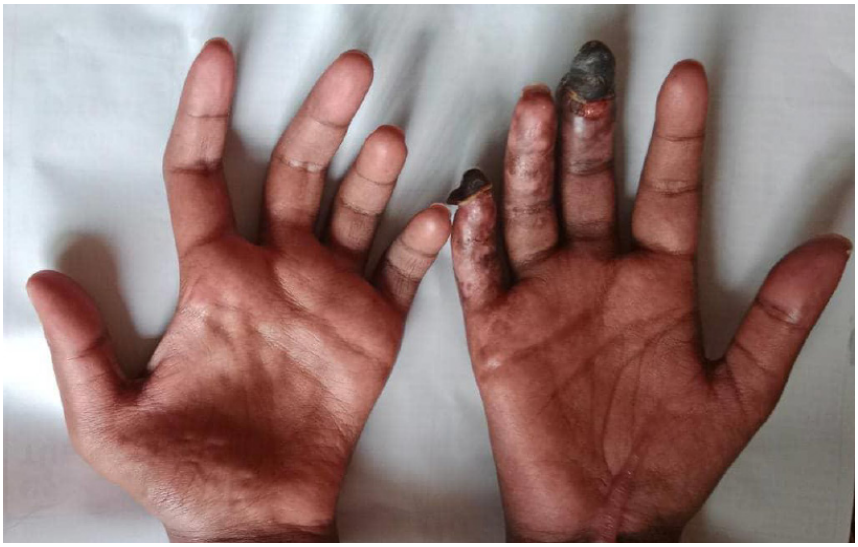

FIGURE 2. Bilateral hands sclerodactyly with fingertip lesion.

Thromboembolism is another clinical manifestation that may occur in patients with asplenia or hyposplenism, but available data on epidemiology and risk factors are very limited.[13] An increased risk of thromboembolic events has been noted in patients who have undergone splenectomy, and this increased risk appears to be at least partially independent of the typical reactive thrombocytosis noted in these patients.[14] In patients with hyposplenism, the contribution of impaired splenic function to thrombotic events is still unclear.

Prophylactic measures with immunizations are necessary for individuals with asplenia to prevent disastrous infection. It is recommended to ensure patients with functional asplenia to receive their *Pneumococcal, Meningococcal, Haemophilus influenzae* (HiB), tetanus, diphtheria, pertussis (DTaP), measles, mumps, rubella, varicella (MMRV) and Influenza vaccinations.[15,16] Individuals with hyposplenism or asplenia should be advised to present to their nearest hospital for prompt treatment in the event of fever.[17]

CONCLUSION

We describe a case of a patient with splenic atrophy and its possible association with systemic sclerosis. Hyposplenism should be suspected in patients with adult-onset infections caused by encapsulated bacteria, especially if autoantibodies are present. Early diagnosis and prompt vaccination can help to prevent potentially life-threatening sepsis. Puzzling associations between splenic atrophy and autoimmune disorders still requires more evidence and pathophysiology studies.

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Juvenile ossifying fibroma accompanied with low-grade central osteosarcoma in sinonasal: a rare case report

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ABSTRACT

Sinonasal osteosarcoma is comparatively rare and accounts for 6.5% of all osteosarcomas. The five-year survival rate is less than 25% and may be improved to 60% when chemotherapy is initiated earlier. The diagnosis of low-grade central osteosarcoma requires a meticulous histopathological examination because histopathologically the tumor may mimic fibro-osseus neoplasm. We report a 12 y.o. male patient who complained of a lump on the face for 4 yr with symptoms of nasal discharge, congestion, epistaxis, and a feeling of fullness in the ears. Sinonasal biopsy was later performed and revealed an inverted papilloma. Two months after the biopsy procedure, mass extirpation and medial maxillectomy were performed. Histopathology examination confirms the diagnosis of ossifying fibroma accompanied by low-grade central osteosarcoma. Low-grade central osteosarcoma is an exceptionally rare variant, and the diagnosis is occasionally difficult. It can be misdiagnosed as a benign lesion, especially fibrous dysplasia or ossifying fibroma. Histomorphological, the discovery of atypical tumor cells producing osteoid matrix can be used to confirm that the lesion is a malignant lesion of low-grade central osteosarcoma. As demonstrated in our case, the tumor can consist of a trabecular and curvilinear arrangement of immature bone, at the edges of which there is an osteoblastic rimming appearance with a background of connective tissue stroma which is a histopathological feature of ossifying fibroma.

ABSTRAK

Osteosarkoma sinonasal relatif jarang terjadi dan berkontribusi sekitar 6,5% dari seluruh kasus osteosarkoma. Angka kesintasan 5 tahun kurang dari 25%, dan dapat ditingkatkan hingga 60% ketika kemoterapi diberikan lebih awal. Diagnosis *low-grade central osteosarcoma* memerlukan pemeriksaan histopatologi secara menyeluruh serta teliti karena tumor ini secara mikroskopis dapat menyerupai gambaran neoplasma fibrooseus. Kami melaporkan seorang pasien anak laki-laki berusia 12 tahun dengan keluhan benjolan di wajah selama 4 tahun dengan gejala nasal discar, kongesti, epistaksis, dan rasa penuh pada telinganya. Hasil biopsi sinonasal menunjukkan suatu *inverted papilloma*. Dua bulan kemudian dilakukan maksilektomi dan pengangkatan massa keseluruhan. Secara histopatologi disimpulkan suatu *ossifying fibroma dengan low-grade central osteosarcoma. Low-grade central osteosarcoma* sendiri merupakan varian dari osteosarkoma yang jarang terjadi dan diagnosisnya seringkali sulit. Penyakit ini dapat salah didiagnosis sebagai lesi jinak, terutama *fibrous dysplasia* dan *ossifying fibroma*. Secara histomorfologi ditemukannya sel tumor atipik yang memproduksi matriks osteoid dapat digunakan untuk konfirmasi lesi tersebut merupakan lesi ganas *low-grade central osteosarcoma*. Seperti terlihat pada kasus kami, tumor dapat terdiri atas anyaman tulang imatur tersusun trabekular dan kurvilinear, yang ditepinya dijumpai gambaran *osteoblastic rimming* dengan latar belakang stroma jaringan ikat yang mana merupakan gambaran histopatologi dari suatu *ossfiying fibroma*.

Keywords:
low-grade central osteosarcoma; ossifying fibroma; sinonasal ostesarcoma; juvenile

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INTRODUCTION

Osteosarcoma is a highly malignant bone tumor that typically develops from the metaphysis of long bones.[1] In children and adolescents, the incidence rate of the tumor is five cases per million people.[2] Less than 10% of osteosarcoma occurs in craniofacial bone.[2] Sinonasal osteosarcoma is rarer, accounting for roughly 6.5% of all osteosarcomas.[3] Moreover, only 5–6% of all osteosarcomas are low-grade osteosarcomas.[4] This rarity and its histopathological similarity with benign lesions can lead to underdiagnosis. Osterosarcoma is an aggressive tumor that is prone to both local and distant damage. Five-year survival is less than 25%, which may be improved to 60% when chemotherapy is initiated early.[5] Compared to other head and neck malignancies, like squamous cell carcinoma, sinonasal osteosarcoma has a worse prognosis rate.[6] A history of ionizing radiation exposure, fibrous dysplasia, retinoblastoma, or previous exposure to thorium oxide, a radioactive scanning agent, has all been linked to the development of osteosarcoma. Four percent of all osteosarcoma patients have a history of prior radiation therapy for other tumors or conditions.[7,8]

Osteosarcomas can be divided into several subtypes according to the degree of differentiation, location within the bone, and histological variants. These subtypes vary in imaging findings, demographics, and biological behavior. The subtypes include intramedullary which is the most common encompassing 80% of the cases and includes conventional high-grade, and low-grade central osteosarcoma.[1,8] Among paranasal subsite involved by tumor, maxillary sinus (63.8%) was the most often affected paranasal subsite by tumors, followed by the ethmoid sinuses (52%), nasal cavity (46%), sphenoid sinus (28%), and frontal sinus (20%).[9]

The low-grade central osteosarcoma diagnosis requires a meticulous histopathological examination because, histopathologically, the tumor may mimic fibro-osseus neoplasm.[5,10] The problem arises when the classic histological appearance of osteosarcoma overlaps with that of a benign fibro-osseus lesion, making the diagnosis difficult.

Ossifying fibroma is a fibro-osseus tumor of the craniofacial skeleton. It affects individuals ranging from 3 mo to 70 y.o. (frequently in the 3 to 4th decade). The tumor can produce clinical features such as facial enlargement, nasal obstruction, pain, sinusitis, proptosis, and exophthalmos. A juvenile type of ossifying fibroma is more common in the paranasal sinuses and periorbital bone, with a more aggressive clinical course and a recurrence rate of 30-58%.[11] Lee *et al.*[12] reported the case of low-grade osteosarcoma arising from cemento-ossifying fibroma in the mandible. Due to the rarity of such cases, we report a case of low-grade sinonasal osteosarcoma accompanied by juvenile ossifying fibroma in the sinonasal region.

CASE

A 12 y.o. boy presented with progressive facial enlargement in the last 4 yr. He also experienced nasal congestion, discharge, epistaxis, and fullness with ear pain. Three years earlier, the doctor in primary health care recommended surgery for the patient. The patient refused and tried herbal medication (propolis) for about 2.5 yr, but the symptoms did not improve.

The patient finally agreed to medical intervention. A head CT scan revealed a mass measuring 6x4x5 cm, occupying the nasal cavity. There was thickening and deformity of the nasal and ethnoid bone, extending to the basis cranii. There was also effacement of the hard palate. No intracerebral invasion was observed (FIGURE 1). The sinonasal biopsy was later performed and revealed an inverted papilloma. Two months after the biopsy

procedure, mass extirpation and medial maxillectomy was performed in Dr. Sardjito General Hospital, Yogyakarta and the specimen was sent to the Department of Anatomical Pathology.

Macroscopic examination reported that the tumor was pieces of ragged tissue, approximately 200 cc. The biggest specimen was 6x3x0.6 cm, and the smallest specimen was 0.4 cm in diameter, white to tan, and brittle (FIGURE 2). Microscopic examination showed woven bone with a curvilinear, trabecular arrangement, like a Chinese letter lined by an osteoblastic rimming between cellular connective tissue stroma (FIGURES 3-4). In another part, there were also infiltrating tumor cells into the connective tissue and bone around them. The tumor cells were polymorphic, spindle-to-oval, with scanty cytoplasm. Nuclei were oval to spindle, with coarse chromatin; some were hyperchromatic (FIGURES 3-4). Osteoid matrix was also found. The histopathology examination confirmed the diagnosis of low-grade central osteosarcoma accompanied by ossifying fibroma. Immunohistochemical staining showed positivity of osteocalcin with a proliferation index of Ki-67 at 10% (FIGURE 4).

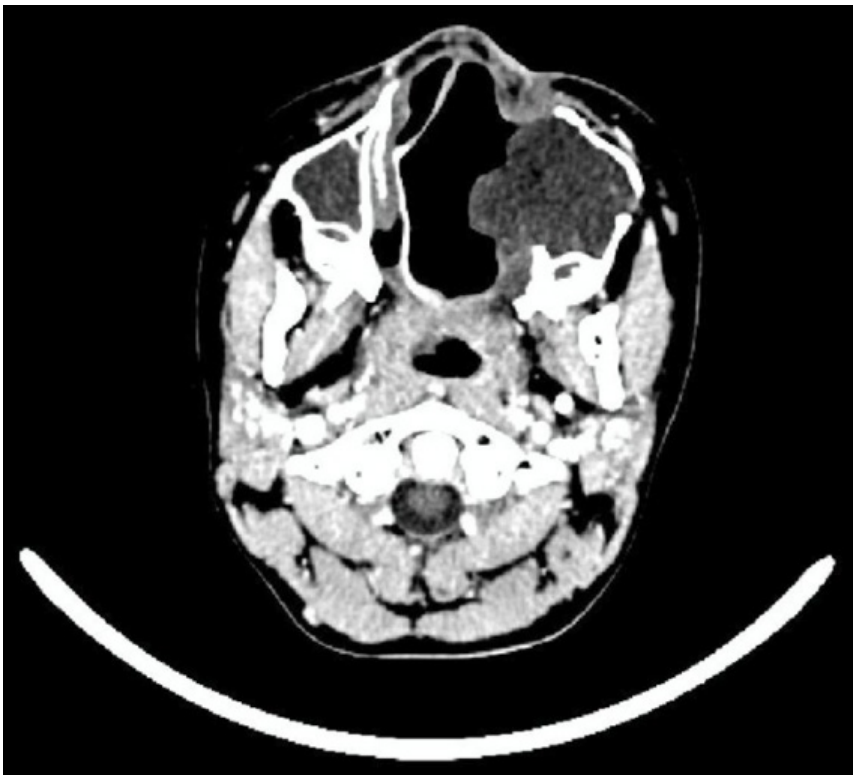

FIGURE 1. CT scan revealed isodens lesion with extension to nasal cavity and left maxillary sinus

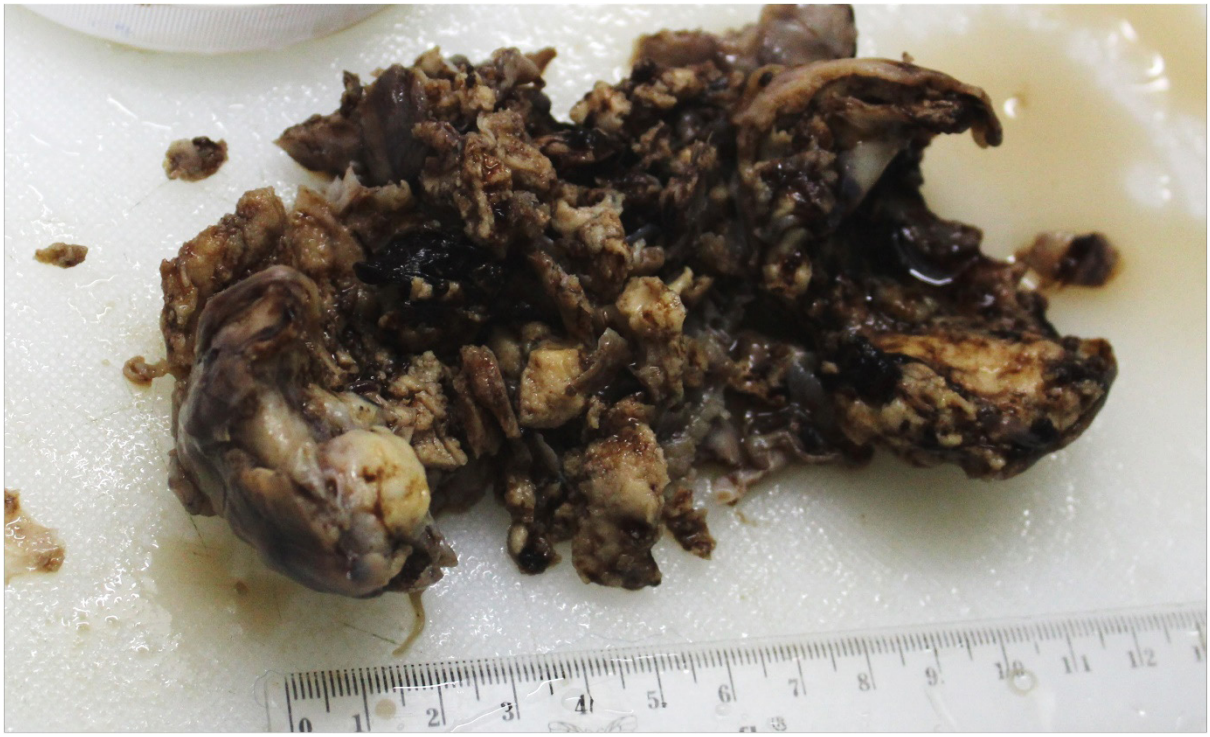

FIGURE 2. Gross examination of mass extirpation and medial maxillectomy.

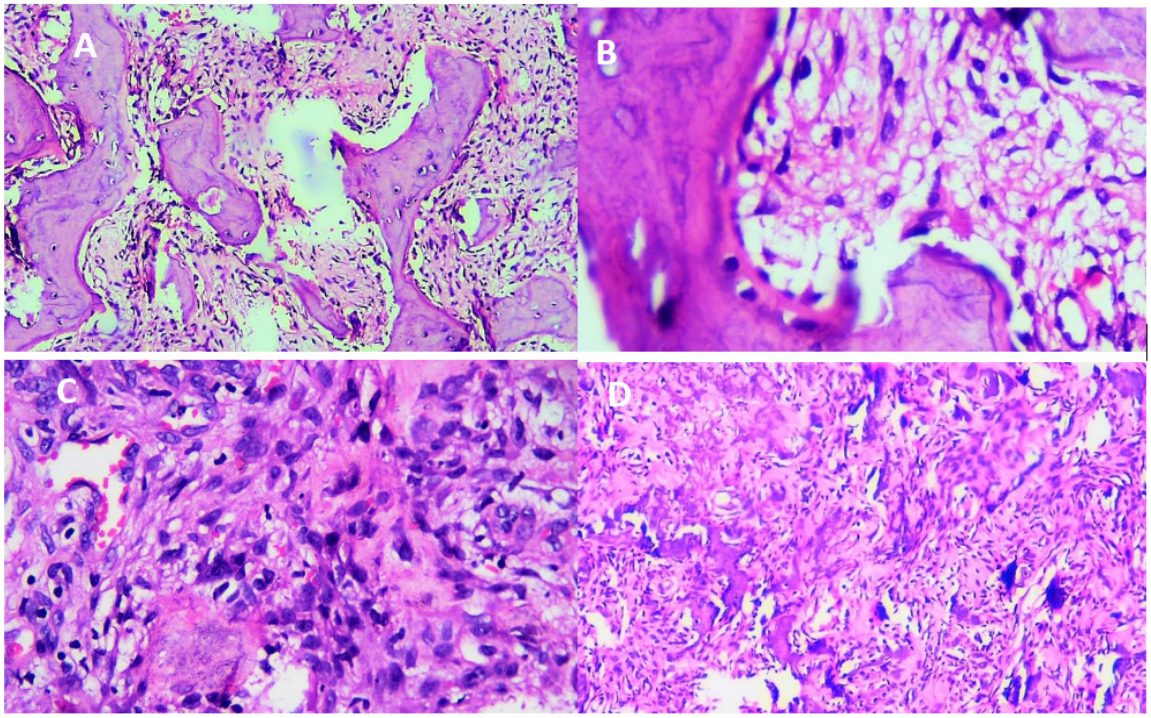

FIGURE 3. HE 100x. The part of ossifying fibroma (A &B). Curvilinear woven bone with osteoblastic rimming between cellular fibrous stroma. Tumor cells are relatively monomorphic, bland nuclei, with smooth chromatin. The part of low-grade central osteosarcoma (C&D). HE staining showed infiltrating tumor cells to connective tissue and bone around them some tumor cells are polymorph, spindle to oval, with scanty cytoplasm. Nuclei are oval to spindle, coarse chromatin, with prominent nucleoli.

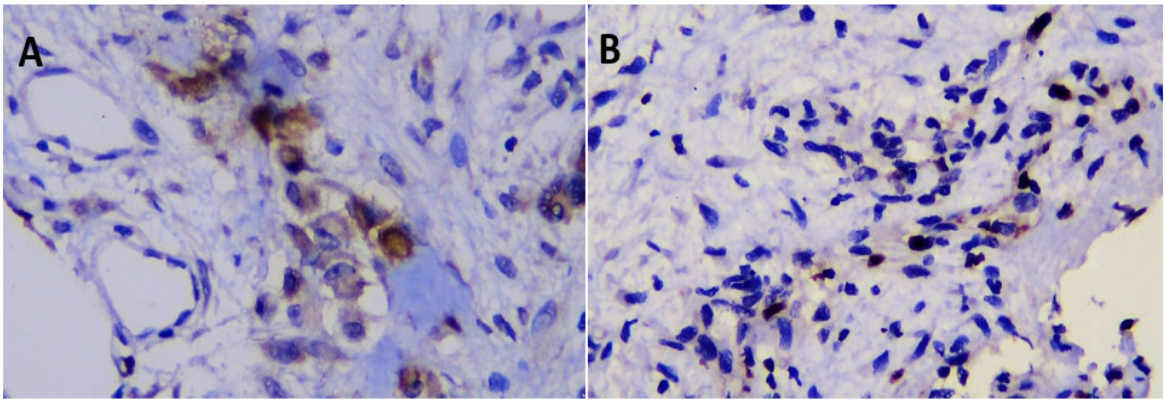

FIGURE 4. A. Positive expression of osteocalcin staining on tumor cell cytoplasm. B. Positive expression of Ki-67 staining on tumor cell nuclei.

DISCUSSION

Primary osteosarcoma in the head and neck region mostly occurs in the maxilla and mandibula, and sinonasal is an unusual site for craniofacial osteosarcoma.[1] In contrast to classical osteosarcoma of the long bones, which primarily affects adolescents and young adults, craniofacial osteosarcomas most commonly occur in the third or fourth decade of life. There has been no recognition of gender predominance.[1,8] Pediatric craniofacial osteosarcoma is extremely rare, as Hadley *et al.*[13] report in their article, with only 23 cases of cranial osteosarcoma in pediatric patients reported in the literature between 1945 and 2012, and they found that the mean age of the patients was 12.2 y.o. Our patient was 12 y.o. when diagnosed.

The term osteosarcoma refers to a heterogenous group of primary malignant neoplasms affecting bone-forming or mesenchymal tissues that have histopathologic evidence of osteogenic differentiation.[5] Histopathologic appearances of osteosarcoma, osteomyelitis, and fibrous dysplasia occupy a spectrum that may have considerable overlap. In some cases, a classic histopathologic appearance makes the diagnosis clear; however, when the picture is that of new bone formation in a background of cellular fibrous connective tissue, the diagnosis is more difficult.[5,10]

The subtypes of osteosarcoma include conventional high-grade with fibroblastic, osteoblastic, or chondroblastic differentiation, high grade surface osteosarcoma, parosteal, periosteal, and low grade central osteosarcoma. Low-grade central osteosarcoma is extremely rare and requires a meticulous histopathological examination, because, histopathologically the tumor may mimic fibro-osseus neoplasm. Histopathology features of atypical sarcoma cells that produce an osteoid matrix can be used to confirm the malignant lesion of low-grade central osteosarcoma.[5,10]

Histopathology revealed two distinct tumor entities, as demonstrated in our case. Some parts of the tumor area were suitable for ossifying fibromas, while others represent malignant atypical sarcoma cells from an osteosarcoma. Osteosarcoma may destroy connective tissue and bone boundaries. Tumor cells vary in size and shape and frequently have large hyperchromatic nuclei; bizarre tumors and giant cells are common, as are mitotic figures. The production of mineralized or unmineralized bone (osteoid) by malignant cells is essential for the diagnosis of osteosarcoma. This

finding was also seen in our case.[10,12]

Ossifying fibroma histologically consists of fibrous connective tissue of low to moderate cellularity with a trabecular pattern containing irregular bony trabeculae. A psammomatoid pattern composed of spheroid bony islands may be found. Osteoblasts rimming woven bone are inconspicuous, with occasional mitotic figures. Osteoclast-like giant cells can also be found. This finding was also seen in our case.[5]

Osteocalcin immunohistochemistry may be helpful in distinguishing osteosarcoma from other malignancies and has been proven to be sensitive but lacks specificity.[14] Very low expression of Ki-67 immunohistochemistry (< 10%) is observed in this tumor, in contrast with the cases of high and intermediate grades of osteosarcoma, that showed strong to moderate positivity of >50% and 25-50%, respectively.[15] From a therapeutic point of view, the most important factor in determining prognosis is resection with wide surgical margins, which has an 80% 5-year survival rate. In cases of low-grade central osteosarcoma, adjuvant chemotherapy or radiotherapy appear to be ineffective.[1,8]

The nasopharynx, orbit, and cranium are some of the anatomical systems that sinonasal osteosarcomas can invade. Although lung and and lymph node metastases have been recorded, haematogenous spread is less common in sinonasal osteosarcoma than in its long-bone counterpart. This tumor needs special attention because, although it is well differentiated, low-grade osteosarcomas can dedifferentiate into high-grade osteosarcomas and lead to more aggressive clinical significance.[16]

Osteosarcoma, as a secondary tumor, may arise from a preexisting benign bone disease such as Paget's disease, bone infarcts, osteomyelitis, or trauma. In our case, there is a possibility that ossifiying fibroma may trans` form into low-grade osteosarcoma, despite insufficient data for evaluating this malignant transformation. Ossifiying fibroma is a benign lesion, but sometimes it may behave in an aggressive manner and cause extensive bone destruction. In addition, there is only one reported case of low-grade osteosarcoma arising from cemento-ossifying fibroma.[12]

Current treatments for osteosarcoma include wide excision, neoadjuvant therapy, radiation therapy, and chemotherapy. Intramedullary and surface tumors of low-grade osteosarcoma with no metastasis need wide excision alone, whereas tumor located in the periosteal region need neoadjuvant therapy before wide excision. A high-grade tumor with no metastasis needs neoadjuvant therapy, restaging, wide excision of the resectable, evaluation of the chemotherapy response, and chemoradiotherapy. If there is an adequate response to chemotherapy, the same neoadjuvant therapy can be continued, whereas if there is an inadequate response to neoadjuvant chemotherapy, consider a new chemotherapy regimen, additional surgical resection, and radiation therapy. A tumor with metastasis needs metastasectomy and chemoradiation in addition to the above procedures.[2] On the patient, tumor location in the sinonasal made it more difficult to achieve a clear margin excision; therefore, close surveillance is warranted.

The NCCN guidelines suggest surveillance every 3 mo for post-op years 1 and 2, every 4 mo in post-op year 3, every 6 mo in post-op years 4 and 5, and yearly for post-op years 6 and beyond. Surveillance should include physical examination, imaging of the post-op site and chest, a PET/CT scan, and additional laboratory tests as clinically indicated.[2]

CONCLUSION

Juvenile ossifying fibroma with part of low-grade central osteosarcoma is a

rare disease. We should be aware of the rare possibility that ossifiying fibroma may transform into osteosarcoma, as shown by the presence of the two different tumor entities in our case. Careful diagnosis and regular follow up are needed for an optimum clinical outcome.

Patients with low-grade sinonasal malignancy need careful attention, especially in obtaining a representative sample, biopsy procedure, diagnosis, and treatment. Optimal clinic-radio-pathological correlation and effective communication between clinician, radiologist, and pathologist were needed to diagnose low grade osteosarcoma that occurs at less frequent sites, for example, in the sinonasal region. Early, appropriate diagnosis will prevent wasting time, resources, and opportunities to treat patients with optimal results. Close surveillance after treatment is needed in tumor cases that are located close to vital organs, since wide resection and a clear margin are difficult to achieve.

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Differences in the clinical presentations of anti-NMDAR (anti-N-methyl-D-aspartate receptor) encephalitis with status epilepticus: a retrospective case series

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ABSTRACT

Literature on anti-NMDAR (anti-N-methyl-D-aspartate receptor) encephalitis is limited in developing countries, including Indonesia. This retrospective observational case series explored the impact of early diagnosis and treatment on patient outcomes in four distinct cases of anti-NMDAR encephalitis with status epilepticus, and other related conditions, of patients referred to Dr. Sardjito General Hospital, Yogyakarta. Clinical data from May 2021 to August 2023 were collected through the review of medical records, encompassing demographic information, clinical presentation, history, laboratory results, imaging studies, EEG reports, interventions, and the progression of the disease. Four cases were reported, three of whom were diagnosed with anti-NMDAR and one with bacterial encephalitis, each presenting a variety of neuropsychiatric clinical symptoms, leading to hospitalization, extensive testing, and interventions to establish the definitive diagnosis. Cases 1 and 4 have a childhood history of seizures. The cases analyzed factors including the impact of childhood versus adulthood onset and the adherence to taking medicine regularly leading to exacerbation symptoms and relapses. Distinguishing anti-NMDAR encephalitis from related conditions, such as bacterial encephalitis, was further complicated in patients with varied neuropsychiatric presentations (seizures, hallucinations, irritable behavior, headaches) and responses to the treatment. Supporting investigation finds positive NMDAR testing and abnormal CT, MRI, and EEG results, contributed to definitive diagnoses. It could be concluded that comprehensive diagnostic investigations are important for prompt recognition of clinical characteristics, and early initiation of immunomodulatory therapy in managing anti-NMDAR encephalitis and related conditions in Yogyakarta, Indonesia.

ABSTRAK

Pustaka mengenai ensefalitis anti-NMDAR (anti-N-methyl-D-aspartate receptor) masih terbatas di negara-negara berkembang, termasuk Indonesia. Beberapa kasus observasional retrospektif ini ditujukan untuk mengetahui pengaruh diagnosis awal dan terapi terhadap luaran pasien yang terbagi dalam empat kasus berbeda tentang encephalitis anti-NMDAR dengan status epilepticus, dan kondisi terkait lainnya, pada pasien yang dirujuk ke RSUP Dr. Sardjito, Yogyakarta. Data klinis yang dikumpulkan dari rekam medis antara bulan Mei 2021 hingga Agustus 2023 mencakup informasi demografis, presentasi klinis, riwayat, hasil laboratorium, penunjang foto, hasil EEG, intervensi, dan progresifitas penyakit. Empat kasus dilaporkan, tiga di antaranya didiagnosis dengan anti-NMDAR dan satu dengan encephalitis bakteri, masing-masing menunjukkan berbagai gejala klinis neuropsikiatri, hingga rawat inap, sehingga membutuhkan pemeriksaan yang intensif, dan intervensi untuk mendapatkan diagnosis definitif. Sedangkan untuk kasus 1 dan 4 memiliki riwayat kejang pada masa anak-anak. Kasus ini menganalisis beberapa faktor termasuk dampak onset yang terjadi pada masa anak-anak dibandingkan dengan dewasa, dan kepatuhan dalam mengonsumsi obat secara teratur atau tidak yang mengakibatkan gejala memburuk dan kambuh. Encephalitis anti-NMDAR dibedakan dari kondisi terkait lainnya, seperti encephalitis bakteri, sehingga semakin rumit variasi presentasi klinis neuropsikiatri pasien (kejang, halusinasi, perilaku yang mudah tersinggung, sakit kepala) dan respon terhadap terapi. Pelacakan lebih lanjut menemukan hasil yang positif pada pengujian NMDAR dan hasil CT, MRI, dan EEG yang abnormal, memberikan kontribusi untuk menegakkan diagnosis definitif. Sehingga dapat disimpulkan bahwa pelacakan lebih lanjut yang komprehensif merupakan suatu hal yang esensial untuk deteksi dini terhadap karakteristik klinis dan inisiasi terapi imunomodulator dalam mengelola encephalitis anti-NMDAR dan kondisi terkait di Yogyakarta, Indonesia.

Keywords:
anti-NMDAR encephalitis;
autoimmune encephalitis;
bacterial encephalitis;
status epilepticus;
seizure

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INTRODUCTION

Anti-N-methyl-D-aspartate receptor (anti-NMDAR) encephalitis is the most recognized form of autoimmune encephalitis, predominantly affecting children and young adults. Despite its recognition, its rarity persists, affecting approximately 1 out of 1.5 million people annually.[1] Misdiagnosis is common, and diagnostic delays often occur due to its highly variable clinical presentation.[2] Patients typically present with seizures, encephalopathy, cognitive decline, and neuropsychiatric symptoms followed by behavioral changes, movement disorders, autonomic dysfunction, and in severe cases, coma.[3,4] Cerebrospinal fluid testing is typically more reliable than blood serum for detecting NMDAR antibodies.[5]

It is hypothesized that early diagnosis, prompt treatment initiation, and consistent medication adherence can significantly reduce seizure frequency. Identifying the underlying autoimmune etiology of epilepsy is important, especially for patients who are resistant to conventional anti-seizure medications (ASM).[6] Plasma exchange, steroids such as methylprednisolone, as well as immunomodulatory therapies including intravenous immunoglobulin (IVIG), cyclophosphamide, and rituximab, have led ASM-resistant patients to attain seizure control.[6] A previous study by Titulaer *et al.*,[7] of 577 patients with anti-NMDAR encephalitis found early treatment being a significant predictor for recovery.

Existing literature on anti-NMDAR encephalitis and related conditions is notably sparse in developing countries, including Indonesia. This retrospective case series seeks to address this gap by presenting four cases treated at Dr. Sardjito General Hospital, Yogyakarta between 2021 and 2023. These cases involved individuals with varied clinical characteristics, each diagnosed with anti-NMDAR encephalitis or related conditions, with examination findings confirming infection. It occurred at different stages of life, ranging from early childhood to adulthood. It is hoped that this case series can contribute valuable insights into the manifestations and management of these conditions within the distinctive healthcare context of developing nations.

CASE SERIES

This retrospective observational case series examines four distinct cases of patients who were referred to Dr. Sardjito General Hospital, Yogyakarta, Indonesia. The inclusion criteria were patients presenting neurological symptoms indicative of anti-NMDAR encephalitis or related conditions. Clinical data from May 2021 to August 2023 were collected and reviewed for demographic information, clinical presentation, history, laboratory results, imaging studies, EEG reports, diagnostic investigations, interventions, and progression of the disease.

The findings of each case were presented individually to elucidate clinical nuances and diagnostic workup details. These results offer a comprehensive view of the varied nature of anti-NMDAR encephalitis, highlighting the unique challenges encountered in each case. Challenges in diagnosis and management, shared characteristics or disparities across cases, and the efficacy of treatment strategies, considering the timing of administration, and its influences on outcomes, were analyzed.

Case 1

Ms. A, an 18 y.o. university student from Yogyakarta, was admitted to Dr. Sardjito General Hospital, Yogyakarta in an intubated and sedated state, reporting symptoms of visual and auditory hallucinations, irritable behavior, aggravating headaches, and seizures for the past 2 wk. Seizures were characterized by stiff spasms affecting her face and left limbs, occurring 10-15

times daily, each lasting 1-2 min. During the postictal and interictal phases, the patient remained unaware.

The patient's medical history revealed joint pain and refractory convulsive status epilepticus seizures dating back 8 yr to childhood that were initially triggered by a one-week-long fever and have since been managed with valproic acid. The patient faced challenges in complying to prescribe the therapy regimen. Before the patient's referral, she stayed 15 d in the Intensive Care Unit at Jogja International Hospital, followed by a 5 d stay at RSI Klaten (Klaten Islamic Hospital). During this period, the patient experienced a 3 d fever, coinciding with a reduction in the duration and frequency of seizures.

Behavioral issues improved after receiving an intravenous midazolam infusion at a rate of 5 mg/hr. Therapy which was given to the patient included methylprednisolone (i.v. 125 mg q.i.d), Cellcept (p.o. 500 mg b.i.d), acyclovir (p.o. 800 mg o.d.), valproic acid (p.o. 500 mg b.i.d.), and phenytoin (p.o. 100 mg t.i.d).

Diagnostic examination indicated cerebral edema with radiological findings interpreted as intracranial infection on a head CT scan without contrast and cardiomegaly on a thoracic X-ray. Abnormal EEG findings included delta theta rhythm background deceleration, supporting clinical general awakening and indicating possible diffuse cortical dysfunction. During treatment, the patient exhibited silent behavior, appearing blank but reported auditory and visual hallucinations in the form of hearing whispers and seeing human-like shadows. Neurological examination yielded normal results with no meningeal signs or lateralization.

Laboratory tests indicated leukocytosis,anemia,hypercoagulapathy, hypoalbuminemia, and electrolyte imbalances. A diagnosis of anti-NMDAR encephalitis was made. Treatment at Dr. Sardjito General Hospital, Yogyakarta included physiotherapy, methylprednisolone (inj. 31.25 mg o.d.), cefepime (inj. 2 g b.i.d), omeprazole (inj. 40 mg o.d.), Depakote (tab. 500 mg b.i.d), and phenytoin (tab. 100 mg t.i.d). Upon discharge, the patient continued taking the same treatment in tablet form.

Case 2

Mr. AR, a 22 y.o. store employee from Bantul, Yogyakarta, Indonesia, came to Dr. Sardjito General Hospital, Yogyakarta with a 4 d progressive acute onset headache and fever. The patient showed some symptoms including visual hallucinations, frequent crying, incoherent self-conversation, catatonic states, restlessness, and fits of anger. On the 5th and 6th days after being admitted, the patient had seizures throughout his body in which the patient remained unconscious, each lasting 2-3 min before stopping spontaneously. Clobazam (10 mg o.d.) and nopres (10 mg o.d.) were initiated, followed by an olanzapine injection on the 7 d given by the psychiatrist. The patient continued to have 7-10 generalized seizures daily. On the 9th day, the use of diazepam was necessitated to stop a 10 min seizure. The patient had declined consciousness, a high fever, and high blood pressure. The patient was referred to the Neurology Department on the 10th day. On the 12th day, an MRI with contrast yielded normal results, and the patient commenced MP therapy at a dose of 250 mg every 6 hr for 5 d. On the 14th day, a positive lumbar puncture showed a viral infection. Plasma exchange treatment was administered on days 22, 23, and 28. The 22nd and 23rd days were marked by an absence of seizures.

The patient was suspected of anti-NMDAR encephalitis with a differential diagnosis of neuroleptic malignant syndrome. On the 27th day, abnormal EEG results were characterized by diffuse slowing and triphasic waves, further supporting the presence of generalized seizures. On the 28th day, the patient experienced 3 generalized seizures in 24

hr, along with focal seizures affecting his right hand every 30 min. From the 34th to 36th day, the focal seizures in his right hand persisted at the 30 min interval, but the frequency of generalized seizures reduced to one per day, and the patient was transferred to the ICU. On the 35th day, the PDT confirmed the diagnosis of anti-NMDAR encephalitis. Post-discharge treatment included methylprednisolone (tab. 31.25 mg o.d.), cyclophosphamide (tab. 50 mg o.d.), and mycophenolate mofetil (tab. 500 mg b.i.d).

Case 3

Mr. TYS, a 19 y.o. employed in the private sector from Yogyakarta, was referred to Dr. Sardjito General Hospital, Yogyakarta due to suspected meningoencephalitis and recurrent seizures. The patient was first treated in Bethesda Hospital, Yogyakarta, where he presented with progressively worsening subacute headaches for 3 wk, and focal seizures in his left hand and leg for 2 wk before admission. These seizures had an unclear pre-ictal phase, with ictal duration of less than 5 min, and left him unconscious. A non-contrast head CT scan revealed cerebral edema.

Upon referral, the patient continued to experience recurrent seizures, accompanied by slurred speech, confusion, fever, and focal seizures in his left hand. Phenytoin (inj. 100 mg b.i.d) and haloperidol (inj. 5 mg b.i.d) were administered but seizures persisted. A lumbar puncture on the 2nd day was positive indicating a viral infection. The patient's condition worsened on the 6th day, necessitating intubation as his oxygen saturation dropped to 85-90%, while Diazepam proved ineffective in managing the seizures that continued. On the 8th and 9th days, he was transferred to the ICU, and NMDAR testing returned positive.

Plasma exchange treatment was administered on days 16, 18, and 19. On the 16th day, there was some improvement in consciousness, but focal seizures continued to manifest, occurring 3 times, each lasting less than 5 min. On the 18th day, abdominal distension was observed, and on the 19th day, seizures had become bilateral, occurring 3 times a day.

Diagnostic investigations indicated cerebral edema on a head CT scan without contrast, immunoserology testing was positive for anti-NMDAR, and urine analysis was positive for *Staphylococcus haemolyticus*. Chest X-ray, MRI, and CSF culture yielded normal findings. The patient was diagnosed with anti-NMDAR encephalitis and status epilepticus. A post-discharge treatment regimen was prescribed including phenytoin (tab. 100 mg t.i.d), levetiracetam (tab. 750 mg b.i.d.), acyclovir (tab. 800 mg quinq. die), and a ketogenic diet.

Case 4

Mr. FA, a 30 y.o. male printing office employee from Kapling Janan village, Magelang, Central Java, was referred to Dr. Sardjito General Hospital, Yogyakarta from Magelang Hospital, Central Java, because of prolonged seizures. Notably, Mr. FA's aunts have a history of primary epilepsy. The patient had his first seizure at the age of 6 mo, accompanied by a fever, and continued until he was 6 y.o., after which he became seizure-free. However, his seizures resurfaced 6 yr before his admission to Dr. Sardjito General Hospital.

These seizures presented with specific characteristics, typically lasting 1-2 min, occurring 3-4 times a month, and commencing with mouth babbling, upward eye deviation, stiffness, and clonic movements of the extremities. Postictally, the patient was left confused. As his irritability and visual hallucinations became pronounced, routine treatment was prescribed by a neurologist and psychiatrist consisting of phenytoin (tab. 200 mg o.d.), folic acid (tab. 1 mg o.d.), trihexyphenidyl (tab. 1 mg o.d.), haloperidol (tab. 1.5 mg b.i.d), and clobazam (tab. 10 mg o.d.). Five months before hospitalization, tremors

in his fingers appeared, impacting daily activities and his job.

A month before admission, the patient felt tired from taking his medication and instead turned to alternative medicine including herbs and drinking chicken blood, leading to fever and altered seizure semiology of uncontrolled movements of the left shoulder, upward eye deviation, stiffness, and clonic movements in extremities. The patient was admitted to the emergency room of Dr. Soeroyo Mental Hospital, Magelang where seizures became more frequent and prolonged, prompting a transfer to the ICU and intubation. Upon arrival at Dr. Sardjito General Hospital, Yogyakarta in a coma and on sedatives, laboratory tests revealed anemia, leucocytosis, elevated liver enzyme, hypoalbuminemia, and hyponatremia. A chest X-ray showed bilateral pneumonia, while CSF analysis detected *S. hemolyticus*. The EEG displayed abnormal slow, epileptiform spikes, unilateral waves on the right temporooccipital region, and occipital intermittent rhythmic delta activity. His non-contrast head CT showed brain atrophy of the cerebri and cerebellum as well as ventriculomegaly ex vacuo. The MRI findings added results of hypertrophy of the right inferior concha.

The patient was diagnosed with bacterial encephalitis and received treatment of methylprednisolone (inj. 250 mg q.i.d), phenytoin (inj. 100 mg b.i.d), linezolid (inj. 600 mg b.i.d), omeprazole (inj. 40 mg o.d.), clonazepam (tab. 2 mg b.i.d.), valproic acid (tab. 500 mg b.i.d), and levetiracetam (tab. 500 mg b.i.d). Treatment was continued post-discharge taken in a tablet regimen.

TABLE 1. Case summaries

Age (yr), gender	History	Presenting complaints	Additional neurological findings	Paraclinical findings	Diagnosis	Treatment post-discharge
18, Female	8 yr ago, a fever led to repetitive seizures and joint pain.	Aggravating headaches, seizures, irritable behaviour, visual and auditory hallucinations.	CT: cerebral edema, intracranial infection findings	EEG: δ, θ rhythm background deceleration Chest X-ray: cardiomegaly	Anti-NMDAR encephalitis	Methylprednisolone (tab. 31.25 mg o.d.), cefepime (tab. 2 g b.i.d), omeprazole (tab. 40 mg o.d.), depakote (tab. 500 mg b.i.d), phenytoin (tab. 100 mg t.i.d)
22, Male	Progressive acute onset headache and fever for 4 days.	Recurrent seizures, visual hallucinations, acute psychotic episodes.	MRI: normal	EEG: diffuse slowing and triphasic waves	Anti-NMDAR encephalitis	Methylprednisolone (tab. 31.25 mg o.d.), cyclophosphamide (tab. 50 mg o.d.), mycophenolate mofetil (tab. 500 mg b.i.d)
19, Male	Worsening subacute headache for 3 wk, focal seizures on the left hand and leg.	Suspected meningoencephalitis, recurrent seizures.	CT: cerebral edema Lumbar puncture: viral infection (+) MRI, CSF culture: normal	Urine analysis: *S. haemolyticus* (+) Immunoserology testing: anti-NMDAR (+)	Anti-NMDAR encephalitis	Phenytoin (tab. 100 mg t.i.d), levetiracetam (tab. 750 mg b.i.d.), acyclovir (tab. 800 mg quinq. die)
30, Male	First seizure at 6 mo.o. continued until 6 y.o. Prolonged seizures resurfaced 6 years before hospital admission.	Prolonged seizures, irritability, visual hallucinations, clonic movements in the extremities.	CT, MRI: cerebri et cerebellum atrophy, ventriculomegaly ex vacuo. CSF analysis: *S. haemolyticus* (+). Lumbar Puncture: viral infection (+)	Chest X-ray: pneumonia bilateral. EEG: epileptiform spike, unilateral wave, occipital intermittent rhythmic delta activity.	Bacterial encephalitis	Methylprednisolone (inj. 250 mg q.i.d), phenytoin (inj. 100 mg b.i.d), linezolid (inj. 600 mg b.i.d), omeprazole (inj. 40 mg o.d.), clonazepam (tab. 2 mg b.i.d.), valproic acid (tab. 500 mg b.i.d), levetiracetam (tab. 500 mg b.i.d)

DISCUSSION

Diagnostic challenges

The complexity of diagnosing and managing anti-NMDAR encephalitis arises from its diverse clinical manifestations, need for specialized tests, and response to treatment. Case 1's presentation of joint pain and refractory convulsive status epilepticus highlighted the necessity for comprehensive investigations as NMDAR testing and EEG revealed abnormal findings indicative of secondary epilepsy.

Case 2 presented with visual hallucinations and acute onset headache, leading to a range of treatments and tests to be carried out including MRI and lumbar puncture. Seizures persisted with diazepam notably needing to be administered to stop a 10 min seizure during day 9. Seizures stopped only after initiation of plasma exchange treatment on day 22. The diagnosis of anti-NMDAR encephalitis was confirmed on day 35 only after persistent seizures and abnormal EEG results.

Contrastingly, in Case 3 the seizures continued even after plasma exchange was initiated as diazepam was found to be ineffective in managing the seizures. Case 3, characterized by recurrent seizures, suspected meningoencephalitis, and progressively worsening subacute headaches, underwent diagnostic examinations from both Bethesda Hospital, Yogyakarta and Dr. Sardjito General Hospital, Yogyakarta. This included a lumbar puncture and plasma exchange, highlighting the complexities of establishing a conclusive diagnosis. It was only until positive findings in NMDAR testing, head CT indicating cerebral edema, and immunoserology testing when a conclusive diagnosis was made.

While, Case 4 introduced the challenge of distinguishing bacterial encephalitis from anti-NMDAR encephalitis which was complicated by the identification of *S. hemolyticus* in the CSF analysis, emphasizing the need to consider bacterial encephalitis in the differential diagnosis. Establishing a diagnosis was further complicated by the patient's history of primary epilepsy, irregular medication adherence, and alternative medicine.

Clinical manifestations varied and were inconsistent. While Cases 1, 3, and 4 had abnormal CT or MRI results ranging from cerebral edema to cerebral atrophy, Case 2's MRI results were normal. In contrast, Cases 1, 2 and 4 had abnormal EEG results. This research builds on a study by Quek *et al.*[8] which suggested general diagnostic characteristics to increase clinical suspicion for autoimmune epilepsy, which were similar to the characteristics found across the cases included in this case series, including high seizure frequency, ASM resistance, and history of autoimmunity or malignancy.

Past studies have documented EEG findings such as 'extreme delta brush' in patients with anti-NMDAR encephalitis,[9] which was consistent with what was found in Case 1 demonstrated abnormal EEG findings including delta theta rhythm background deceleration, while Case 4 had occipital intermittent rhythmic delta activity. Viswanathan *et al.*[10] analyzed 131 EEGs, finding the most common patterns to be diffuse slowing (n = 20), generalized rhythmic delta activity (n = 9), and focal spikes and slowing (n = 8 each), while delta brush patterns were observed in only 3 EEGs.

Furthermore, Case 3's CSF culture was normal yet immunoserology testing was positive for anti-NMDAR. This demonstrates how some patients are not always positive for autoimmune encephalitis antibodies in both serum and CSF. Mo *et al.*[11] showed that serum and CSF which were collected from all patients and tested for autoimmune encephalitis antibodies, revealed that 58

(96.7%) patients were positive for anti-NMDAR antibodies in their CSF, while only 39 (65.0%) patients were positive in their serum.

Early diagnosis and treatment and its influence on clinical outcomes

While Case 1 received prolonged valproic acid treatment for refractory convulsive status epilepticus over 8 yr before her anti-NMDAR encephalitis diagnosis, Case 4's history of luminal therapy from infancy resulted in seizure freedom until the age of 6 yr, after which seizures reappeared, leading to a bacterial encephalitis diagnosis at age 30 yr. Both cases highlight the limitations of long-term pharmacological treatment in controlling seizures, as symptoms eventually exacerbate into neurological and psychiatric manifestations, necessitating hospitalization.

In contrast, Cases 2 and 3, lacking a childhood seizure history, sought immediate medical attention for recurrent seizures and acute psychotic episodes. Comparing cases treated since childhood (Cases 1 and 4) and adulthood (Cases 2 and 3) offers insights into variations in disease progression. While all cases experienced recurrent seizures, neuropsychiatric symptoms such as aggravating headaches, irritability, and hallucinations varied. In a retrospective study of 106 anti-NMDAR encephalitis patients in China by Wang *et al.*,[12] 74.5% exhibited behavioral changes, 67% experienced seizures with 54.9% being focal seizures. According to Amugoda *et al.*,[13] during the prodromal phase 70-86% of patients experience unspecific viral-like symptoms, with fever and headache commonly occurring after the onset of neuropsychiatric symptoms, lasting up to 21 d. Seizures were observed in up to 82% of patients, and psychotic features including agitation, paranoid delusions, and hallucinations manifested two weeks following the prodromal phase.

In Case 3, despite early treatment with phenytoin, haloperidol, and diazepam during the early days of hospitalization, the increasing frequency of seizures only occurred after plasma exchange treatment was initiated following a positive NMDAR result. So far, since the final diagnoses of all four cases were made between 2021 and 2023, delayed diagnoses in those with a childhood history of seizures may be attributed to a lack of awareness among clinicians during the patients' early years as anti-NMDAR encephalitis was only first described in 2007.[3]

As a result, this study supports the value of early diagnosis and immunomodulation in reducing seizure frequency, especially in patients with autoimmune epilepsy resistant to conventional anti-seizure medications. Empiric treatment usually involves step-wise immunotherapy escalation including first-line therapy with steroids, plasma exchange, or IVIG, followed by second-line therapy with cyclophosphamide, rituximab, or combinations.[14,15] Armangue *et al.*[16] have studied 20 pediatric anti-NMDAR encephalitis patients all of whom received steroids, IVIG, or plasma exchange, and 7 rituximab or cyclophosphamide, found 85% of patients had substantial recovery after a median follow-up of 17.5 mo. Gong *et al.*[17] similarly found clinical improvements in 84.8% of 244 anti-NMDAR patients within 4 wk after beginning immunotherapy, and 80.7 and 85.7% exhibited substantial recovery at 12 and 24 mo. Titulaer *et al.*[7] noted that first-line immunotherapy in the initial episode of encephalitis resulted in symptom improvement in 53% of 577 patients within 4 wk of treatment, 97% of whom had a good outcome at 24 mo follow-up, and was observed to have a lower frequency of relapses. Among patients who received second-line immunotherapy, 84 out of 125 obtained symptom improvement.

Medication adherence

Medication adherence is a critical aspect of the management of seizures, as highlighted by the case of Mr. FA in Case 4 who had a history of seizures since infancy, then he was free from seizure at the age of 6 yr through luminal therapy but relapsed 6 yr before his admission to Dr. Sardjito General Hospital, Yogyakarta. His irregular medication adherence, starting one month before hospital admission, likely contributed to exacerbating symptoms, including prolonged seizures with a different semiology, now involving whole extremities, along with irritability, visual hallucinations, and hand tremors that impacted his job.

In Indonesia, factors such as affordability concerns, fear of adverse effects from pharmaceutical medicines, and alignment with cultural beliefs drive patients to seek alternative and traditional methods.[18-20] Unfortunately, this often leads to discontinuation of prescribed medications, and worsening symptoms, as observed in Mr. FA's case. Similar trends have been observed in studies related to neurological conditions, with Das *et al.*[21] reporting a 71% non-adherence rate to antiepileptic treatment which had a significant association with increased seizure severity and the complexity of annual treatment. Notably, 17.2% of patients with high drug adherence remained seizure-free in the past year, a marked contrast to the 1.4% among poorly adherent patients.[21] Moreover, a study by Gabliondo *et al.*[22] revealed an elevated relapse risk in patients who did not receive immunotherapy in the first episode of anti-NMDAR encephalitis ($p = 0.009$), thus further emphasizing the importance of adherence to immunomodulatory therapies post-discharge to minimize such risk of relapse episodes. In a cohort study by Gong *et al.*,[17] it was found that 15.9% of 244 anti-NMDAR encephalitis patients experienced a relapse episode, with 82.0% occurring within the first 24 mo. Consistent with these findings, other studies reported relapse rates ranging from 20 to 25%.[22,23]

Limitations of this study include its retrospective design, which is subject to bias of unmeasured factors, and its small study population. The included patients, largely referred to Dr. Sardjito General Hospital, Yogyakarta from surrounding medical facilities in Yogyakarta, may have introduced potential variations in the anamnesis recorded in our institution compared to the originating sources. The small study population, while intentionally selected for its unique clinical manifestations and patient demographics, is not accurately reflective of the true number of anti-NMDAR encephalitis cases in Dr. Sardjito General Hospital, Yogyakarta. Therefore, a multicenter study involving all diagnosed cases of anti-NMDAR encephalitis is needed to reflect the true number of anti-NMDAR encephalitis cases in Yogyakarta, which we believe to be higher than the current reported number, especially given the limited existing literature on the subject.

CONCLUSION

This study highlights the complexities of the clinical characteristics of anti-NMDAR encephalitis and related conditions in Yogyakarta, Indonesia, emphasizing the need for early recognition of common clinical and electrographic presentations, including abnormal EEG patterns such as 'extreme delta brush.' A retrospective analysis of Cases 1 and 4, treated since childhood, versus Cases 2 and 3, treated in adulthood, provides valuable insight into disease progression variations and the need for comprehensive examinations.

Examining the impact of early diagnosis and treatment on clinical outcomes, our study reinforces the

significance of prompt intervention of immunomodulatory agents to reduce seizure frequency. All cases received post-discharge prescriptions of immunomodulatory agents, antiepileptic drugs, and supportive care which thus showcases the need for tailored interventions based on individual presentations.

Future prospective studies will be necessary to determine the ideal immunomodulatory treatment regimen for patients based on clinical presentation and antibody type. As understanding of these conditions evolves, clinicians must stay up-to-date with the latest advances to provide optimal care. This research contributes to the ongoing dialogue surrounding anti-NMDAR encephalitis, emphasizing the importance of continued awareness, research, and patient-centered care.

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