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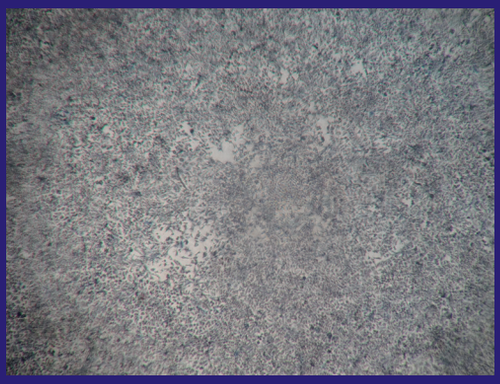

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Research Report

Correlation of Nutritional Status with Hookworm and *Strongyloides stercoralis* Infection in Children Under Five Years in Kokar Public Health Center, Alor Regency, East Nusa Tenggara

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ABSTRACT

Malnutrition can reduce immune response particularly in cytokine (IL-4, IL-5, IL-10) production and immune effector (eosinophil, IgE, and mast cell), thus increasing the probability of intestinal nematode infection. Through this study, intestinal nematode infections occurred among children under five years, at different nutrition status, in Kokar Public Health center, Alor Regency, East Nusa Tenggara was captured. Hookworm and Strongyloides stercoralis were studied as both of them have devastating impacts compare to other helminthes compare to other helminths. This study is a cross-sectional study with a quote sampling technique. As many as 238 children, aged 12-59 months living in Kokar's Public Health Center area, Alor regency were recruited in this study i.e. 7.7% severely underweight, 19.2% underweight, 70.5% normal and 2.6% overweight. Data were collected in August - October 2016. Hookworm and S. stercoralis infection were determined from collected fecal samples of all subjects using either Baermann test, Koga Agar Plate (KAP), or Harada-Mori culture method. The prevalence of hookworm and S. stercoralis infection was 8.82%, and 0,42%. Correlation between nutritional status and hookworm infection were analyzed by Mann-Whitney test with p value = 0.54 (p > 0.05). Prevalence of hookworm and S. stercoralis among children under five years in Kokar were 8.82% and 0.42%. There was no significant correlation between nutritional status with hookworm infection prevalence.

Keywords: *Hookworm infection prevalence, nutritional status, Kokar, Alor Regency*

ABSTRAK

Malnutrisi dapat menyebabkan penurunan pada sistem imun terutama pada produksi sitokin (IL-4, IL-5, IL-10) dan kinerja sel efektor pada respon imun (eosinofil, IgE, dan sel mast). Gangguan ini dapat berakibat pada meningkatnya risiko infeksi nematoda usus. Selain malnutrisi, faktor perilaku tidak higienis juga dapat meningkatkan risiko infeksi nematode usus. Infeksi nematoda usus pada balita dapat menyebabkan gangguan pertumbuhan dan perkembangan. Nematoda usus yang menyerang anak-anak usia balita umumnya disebabkan oleh soil-transmitted helminth (STH), diantaranya adalah hookworm dan Strongyloides stercoralis. Studi ini bertujuan untuk mengetahui hubungan status gizi terhadap prevalensi infeksi pada balita di Puskesmas Kokar, Kabupaten Alor. Rancangan penelitian bersifat cross-sectional, dengan teknik quote sampling. Sampel 238 balita di Puskesmas Kokar, kabupaten Alor. Pengumpulan data pada bulan Agustus - Oktober 2016. Subjek dan orangtua yang memenuhi kriteria penelitian diwawancara menggunakan panduan kuisioner. Sampel feses dikumpulkan dan diperiksa jenis infeksi menggunakan metode uji Baermann, Koga Agar Plate (KAP), dan Harada-Mori. Prevalensi infeksi hookworm dan S. stercoralis pada balita di Puskesmas Kokar adalah 8,82% dan 0,42%. Korelasi antara status gizi dan infeksi hookworm dianalisis menggunakan Mann-Whitney test didapatkan nilai p = 0,54 (> 0,05). Perilaku tidak higienis tidak memiliki korelasi terhadap infeksi hookworm. Prevalensi infeksi hookworm dan S. stercoralis pada balita di Kokar adalah 8,82% dan 0,42%. Tidak ada

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hubungan bermakna antara status gizi balita dan prevalensi infeksi hookworm. Tidak ada korelasi antara perilaku tidak higienis dengan prevalensi infeksi hookworm.

***Kata kunci**: prevalensi infeksi hookworm, status gizi balita, perilaku, Kokar, Alor*

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INTRODUCTION

Nutritional status is a health condition of children under five that is measured by age, body weight (BW) and body height (BH)[1]. Nutritional status could be influenced by nutrition intake in food, parenting style, children's health services, environmental health, economic factors, sociocultural factors, and parents' educational factors (knowledge). In 2012, the prevalence of severe-weight and underweight children in Indonesia is at 19.60% spread across all levels of the community's economy[2].

Malnutrition may lead to increasing risk of intestinal nematode infection. The relationship of malnutrition to intestinal nematode infection occurs through two pathways, namely: malnutrition causes an increased risk of infection and helminthiasis is caused malnutrition[3,4]. Increased risk of infection is probably due to reduced production of cytokines (IL-4, IL-5, IL-10) and effectors cell performance on immune (eosinophils, IgE, and mast cells)[5-7]. Thus low level of IL-4 is suggested to increase the probability of STH infection due to less immune system to prevent helminth infection. This study focused on hookworm and *S. stercoralis* infection as it may cause anemia and malabsorption syndrome due to haemorrhagic and desquamation of intestinal epithelium respectively[8]. Briefly, both infections may lead to a growth disturbance in children.

Approximately 1.4 billion of the world's population are estimated infected with Soil Transmitted Helminths (STH) with the highest number of cases occurring in developing countries. The prevalence of STH infection in Indonesia is ranging low to high, with the highest prevalence occurs usually in remote or underdeveloped areas. Study reports showed a high prevalence of STH helminthiasis in children, such as: in Jayapura (50%), Central Mollucas Regency (99.4%), Padang (51.3%), Nangroe Aceh Darussalam (59.2%), East Nusa Tenggara (27.7%), and West Kalimantan (26.2%)[9,10,11].

In 2013, the province of East Nusa Tenggara ranked second highest in malnourished children in Indonesia with a percentage of 11.50%[2]. The numbers are escalating in the work area of Kokar's public health center, with a percentage of severe-weight (6.00%), underweight (12.70%), normal weight (77.80%), and overweight (3.50%). Such condition had put Kokar to ranked third highest in percentage of severe-weight and underweight children after Apui and Kabir in Alor Regency. Kokar selected as study area due to complete category of nutritional status and sufficient facilities related to this study (electricity).

This study focused on finding correlation between nutritional status with hookworm and *S. stercoralis* infection. This study also try review about *S. stercoralis* prevalence due to lack of strongyloidiasis data in Indonesia until present time which perhaps due to difficulty in performing *S. stercoralis* detection method such as Baermann and Koga Agar Plate (KAP) tests. Results of this study might be useful for further action was taken by stakeholders and local government.

MATERIALS AND METHODS

A cross-sectional study with nutritional status as an independent variable and hookworm and *S. stercoralis* infection as a dependent variable. The reachable population of this study were children under 5 years for in Kokar totaling 631 people. Through Slovin formula[12], total samples in this study determined as 245 children of 12-59 months old. Samples were selected from population based

on inclusion and exclusion criteria as follows. Inclusion criteria include: (1) children aged 12 to 59 months and (2) children did not consume the anthelmintic drugs for the last 4 months. Exclusion criteria include: (1) feces contaminated by dirt, water, and urine, (2) feces were given for more than 24 hours after defecate, (3) children's parent did not approve inform consent.

Nutritional status data were secondary data based on *Kartu Menuju Sehat*/ Children Growth Chart (KMS/CGC) obtained from Kokar Health Center. Nutritional status data based on anthropometric measurements of body weight (kg) for age (month). Subjects recruited in four categories nutritional status, in line with the proportion in the population, i.e. 7.7% severely underweight, 19.2% underweight, 70.5% normal and 2.6% overweight.

Data Collection

Data collection was held in August - October 2016 in the working area of Kokar public health, Alor Barat Laut district, East Nusa Tenggara Province. Children's parent are given an explanation of how to collect and store stool samples e.g. stool sample collected must be fresh (no more than 24 hours) without any contamination from water, soil and urine, and the respondent was not given any anthelmintic treatment in the last four months. Stool samples collected in stool containers.

After accommodated, the sample was submitted The Public Health center staff and researcher will retrieve afterward. Each stool sample were examined using 3 diagnostic methods: Baermann test, Koga Agar Plate (KAP), and Harada-Mori culture methods to identify the presence or absence of infection.

Baermann Method (BM)

In this study, the Baermann Method composed of tea strainer, plastic funnel, gauze bandages, hose and hose clamp. Each tea strainers placed on funnel mouth with gauze bandage on strainer. Warm water used to check whether there is a bubble in the hose. Part of fresh feces (5 gram), wrapped with gauze bandages, then placed on strainer. A 40W bulb lighted at the bottom of hose for 2-3 hours. The filtrate poured as many as 15 ml, centrifuged at 2,500 rpm for at least 5 minutes. The supernatant discarded fast, the sediment examined under light microscope with a magnification of 100 and 400 times[13].

Koga Agar Plate (KAP)

KAP Method started with the making of agar medium consist of 15 gram of agar-agar gepulvert, MERCK, Art.1615; 5 gram of bacto-liver, DIFCO, Control 763182; 10 gram of tryptone peptone, DIFCO, 211705, and 5 gram solid NaCl MERCK, 1.06404.1000. All the ingredients were dissolved with 1 liter of hot aquades. Agar poured into petri dish as much as 10 ml. A 5 gram of feces were placed in the center of agar, and given an identification code number. The covered dishes incubated at room temperature for 48 hours. After 48 hours, agar medium cleaned with 10 ml of sodium acetate-acetic-formalin (SAF). The SAF solution retrieved then, poured into a centrifuge tube, and centrifuged at 2,500 rpm for 5 minutes. The supernatant discarded fast, the sediment examined under a light microscope with a magnification of 100 and 400 times[13, 14].

Harada-Mori Culture Method (HM)

Feces (0.5-1 gram) smeared in the center of the filter paper. Smeared filter paper entered in a plastic bag filled with ±5 ml water, with a record the smeared section that is not submerged in the water. Plastic bag attached with paper clips and hung up for 7 days at room temperature, avoid exposure to direct sunlight. Water retrieved from the plastic bags by way of cut and placed in a centrifuge tube. The volume of water added up to 15 ml before centrifuged at a speed of 2,500 rpm for 5 minutes. The supernatant was discarded fast, the sediment examined under a light microscope with a magnification of 100 and 400 times[15].

Data Analysis

Results were analyzed statistically and descriptively. Descriptive analysis was meant to determine the frequency distribution of measurement results independent variables and

the dependent variable. Correlation between hookworm and *S. stercoralis* infection with nutritional status were analyzed by Mann-Whitney's test.

Ethical Considerations

The study was approved by the Ethics Committee for Medical and Health Research, Medical Faculty, Gadjah Mada University with reference number Ref: KE/FK/892/EC/2016.

RESULT AND DISCUSSION

One of the criteria for children to participate in this study did not anthelmintic drugs for the last 4 months due to STH reinfection processes. Reinfection time varies on the helminth species, *A. lumbricoides* takes 2 months, *T. trichiura* takes 3 months, and hookworm takes 35 days to reach sexually mature inside the human body[8,16]. Another condition is that the feces should not be contaminated by dirt, water, and urine. Contamination by dirt may lead to false positive due to contamination by hookworm rhabditiform larva live in soil. Condition of sample should not be contaminated by water and urine due to fecal sample in this study were used together with Lalangpuling[17] which used Kato-Katz method. Contamination by water and urine can lead feces become inconvenient to be mold in Kato-Katz due to feces low consistency.

Table 1 shows that from total of 238 children under 5 years fecal samples, 21 were found positive in hookworm infection and only 1 subject found positive in *S. stercoralis* infection. Table 1 also shows that the KAP method has a better sensitivity with success rate (efficacy) 68.18% of detect hookworm infection compares to Harada Mori method with success rate of 50%. This result agrees with Reiss et al[18] which found that KAP were superior to Harada Mori methods in hookworm larvae detection. Baermann test were the only method to reach its goal to detect *S. stercoralis* infection, with only on 1 subject. Examination in multiple diagnostic methods needed to reach a true prevalence due to absence of a gold standard for S. stercoralis detection[14]. Filariform larvae which found in Harada Mori method identified as *Necator americanus* from its appearance of gap between esophagus and intestinum[31] (see Figure 1).

Table 1. Identification of fecal samples from children under 5 years in Kokar public health center

	Total Infection (n =22)	
	Strongyloides stercoralis n(%)	*Hookworm* n(%)
KAP	-	15 (68.18%)
Harada Mori	-	11 (50 %)
Baermann test	1 (4.54%)	-
KAP + Harada Mori	-	21 (95.45%)

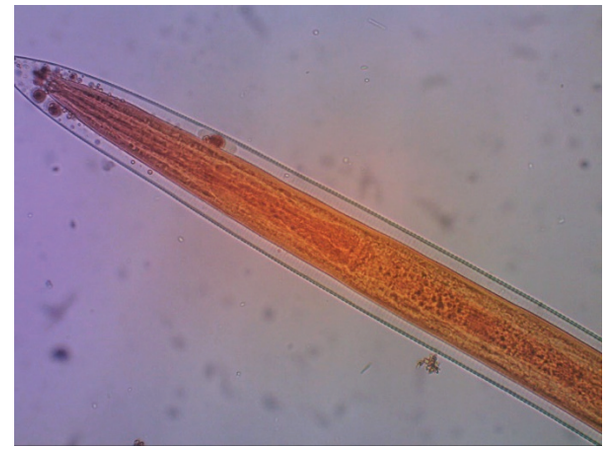

Figure 1. *Necator americanus* filariform larvae, 400 X magnification

Figure 2. Rhabditiform larvae tracks in Koga Agar Plate

A study of hookworm claims that KAP is better than Harada-Mori in hookworm detection due to diversity in stool weight requirement in both methods, which also found in this study that Harada-Mori requires a lesser than 5 gram of faeces[18]. Other study held in the same period and with the same subjects reveals that there were only a light infection of hookworm (1-1999 epg) in children under 5 years in Kokar[17]. A low concentration in a few stools can lead to the decrease in the probability of hookworm eggs to be carried in filter paper of Harada-Mori culture method.

One of KAP method's results is a larva track in agar surface (see Figure 2), though not all positive KAP were found with larva tracks on the agar (21). This occurrence also happen in this study The tracks might be made by rhabditiform larvae and become obvious due to growing of bacterial colonies that grow along path[19,20]. In KAP with larva tracks, it is advised to use a dissecting microscope to search for larvae directly. Compare to another study of hookworm and *S. stercoralis* in other regions of Indonesia and from other countries, the prevalence of hookworm and *S. stercoralis* in this study are considered low. Many studies of hookworm infection in many regions in Indonesia found a higher prevalence like in Jayapura (14.3%) and in Maluku Tengah Regency (56.8%)[9,10]. Prevalence of *S. stercoralis* are found higher in Bali (1.6%)[21].

This study found that the prevalence of hookworm and *S. stercoralis* infection obtained in this study is at 8.82% and 0.42% respectively (see Table 2). Hookworm infection occurs in almost all categories of respondents, except in overweight children. The highest score was in children under five years with normal nutritional status (7.14%). *Strongyloides stercoralis* infection only found in 1 child from overall 238 children.

The low prevalence of hookworm might be due to MDA (mass drug administration) with DEC (diethylcarbamizine) and albendazole as an anthelmintic drug for a brugian filariasis and STH infections. The program was held in Alor regency from 2002 to 2007, with a target of Alor citizens from 3-50 years old. In post evaluation, 3 years after the program was held, hookworm (*N. americanus*) prevalence showed a 75% decrease, from 28% become 7%[12]. A study by Pion *et al.*[22] and Supali *et al.*[23] found that filariasis treatment for 12 months with ivermectin and albendazole could diminish STH infection to 91%. This low prevalence of infection could lead to low risk of transmission from parents to their children.

Children under 5 years with normal status have the highest score, both in percentage and quantity of hookworm infection (see Table 3). The relationship between nutritional status and hookworm infection were analyzed using Mann Whitney test with p-value = 0.54, which shows that nutritional status has no correlation with

Table 2. Distribution of hookworm and *S. stercoralis* cases in children under 5 years old based on age and nutritional status in Kokar public health center, Alor Regency

Characteristic of Respondent	Hookworm n(%)	*S. stercoralis* n(%)	N
Age (month)			
12-23 months	4 (1.68)	0	84
24-59 months	17 (7.14)	1 (0.42)	154
Nutritional Status			
Severe-weight	1 (0.42)	0	18
Underweight	3 (1.26)	0	46
Normal weight	17 (7.14)	1 (0.42)	168
Overweight	0	0	6
Total	21 (8.82)	1 (0.42)	22 (9.24)

Table 3. Correlation between variable of nutritional status and hookworm infection in children under 5 years in Kokar Public Health Center, Alor Regency, August-October 2016

Nutritional Status, n (%)	Hookworm Infection (n=238)			p
	Negative (n=217)	Positive (n=21)	Total (n=238)	
Severe weight	17 (7.14)	1 (0.42)	18 (7.56)	0.54
Underweight	43 (18.07)	3 (1.26)	46 (19.33)	
Normal	151 (63.44)	17 (7.14)	168 (70.58)	
Overweight	6 (2.52)	0 (0)	6 (2.52)	

hookworm infection. This study was in line with another study of elementary school children in Purus, Padang[11].

The absence of correlation might be due to the adequacy of protein requirements. This condition leads to body capable in normal production of IL-4, which is the main cytokine in IgE production. IgE antibody is an adaptive immunity response against helminth infection[5,24]. Other reason might be due to other confounding variables such as children's behavior. A study by Alemu *et al.*[25] shows that hookworm infection has an association with children's habit of shoe wearing. Outdoor activities are the main cause of hookworm infection due to the practice of open defecation in society[14,26-28]. Therefore playing with soil and walking barefooted in outdoor activities may lead children to increase the risk probability of filariform infection. In addition to behavior, social-economic status may also have an effect on STH infection. In Kokar most of children's parents are farmer (71,4%) with low monthly household income (95,3%)[17]. Some studies reveal that social- economic status such as family income were significant risk factors for STH infection[23,29].

Our results do not display a correlation between nutritional status and *S. stercoralis* infection due to quantity of subjects with positive infection were too few, therefore cannot be used as a reference for data processing.

CONCLUSION

Our result found that the prevalence of hookworm and *S. stercoralis* among children under 5 years old in Centre of public health in Kokar, Alor Regency, is 8.82% and 0.42% respectively. There was no correlation between nutritional status and hookworm infection among children under 5 years. This study recommended collaborating KAP and Harada-Mori to detect the presence of hookworm infections as it is able to increase the detection level up to 95.45%.

CONFLICT OF INTEREST

There is no conflict of interest of this study.

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Research Report

The Epidemiological Pattern and Risk Factor of ESBL (*Extended Spectrum B-Lactamase)* Producing *Enterobacteriaceae* in Gut Bacterial Flora of Dairy Cows and People Surrounding in Rural Area, Indonesia

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ABSTRACT

Livestock would be a risk factor of resistant bacteria that impact on human health. Rural area with farms as major economic source has become a risk of the spread of the ESBL producing Enterobacteriaceae The aim of the study was to explore the distribution and risk factor of ESBL (extended-spectrum β-lactamase) producing Enterobacteriaceae in the gut bacterial flora of dairy cows and people surrounding farming area. Total of 204 fecal swab samples were collected, 102 from dairy cows and 102 from farmers. Samples were sub-cultured by streaking on MacConkey agar supplemented with 2 mg/L cefotaxime. The growing colonies were confirmed of the ESBL producer by Modified Double Disk Test (M-DDST) and identification of Enterobacteriaceae by biochemical test. ESBL genes were identified by PCR. ESBL producing bacteria were found 13.7% in dairy cows and 34.3% in farmers. ESBL producing Enterobacteriaceae in dairy cows were 6.9% and in farmers of 33.3%. Statistical analysis showed: Distribution of ESBL producing Enterobacteriaceae strain were insignificant among dairy cows and farmers while bla_{TEM} distribution was significantly different (p= 0,035) and use of antibiotic was identified as a risk factor of colonization of ESBL producing Enterobacteriaceae in farmers (p= 0,007). Farmers had suspected as the source of ESBL producing Enterobacteriaceae based on higher prevalence. Further education of appropriate use of antibiotic need to enhance to control risk factor and prevent the colonization of ESBL producing Enterobacteriaceae.

Keywords: *Enterobacteriaceae, ESBL, gut flora, dairy cow, farmer, rural*

ABSTRAK

Hewan ternak diduga sebagai faktor risiko kejadian bakteri resisten yang berdampak terhadap kesehatan manusia. Area rural dengan potensi ekonomi di sektor peternakan merupakan area yang berisiko terhadap penyebaran Enterobacteriaceae penghasil ESBL. Penelitian bertujuan mengeksplorasi pola distribusi dan faktor risiko Enterobacteriaceae penghasil ESBL pada bakteri flora usus sapi perah dan penduduk sekitarnya. Total 204 sampel swab feses, terdiri dari 102 swab feses sapi perah dan 102 swab feses peternak. Swab feses ditanam pada media MacConkey yang ditambahkan 2 mg/L cefotaxime. Koloni yang tumbuh dikonfirmasi sebagai penghasil ESBL dengan metode Modified Double Disk Test (M-DDST) and diidentifikasi dengan uji biokimia. Identifikasi gen ESBL menggunakan metode PCR. Prevalensi bakteri penghasil ESBL di sapi perah sebesar 13.7% dan di peternak sebesar 34.3%. Distribusi Enterobacteriaceae penghasil ESBL pada sapi perah 6.9% dan pada peternak 33.3%. Analisis statistik menunjukkan: Tidak ada perbedaan signifikan antara distribusi bakteri Enterobacteriaceae penghasil ESBL pada sapi perah dan peternak, distribusi bla_{TEM} pada sapi perah dan peternak berbeda signifikan (p = 0,035), dan penggunaan antibiotik sebagai faktor risiko kolonisasi Enterobacteriaceae penghasil ESBL pada peternak (p= 0,007). Peternak diduga sebagai sumber Enterobacteriaceae penghasil ESBL. Penyuluhan

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tentang penggunaan antibiotik secara tepat perlu ditingkatkan untuk mengendalikan faktor risiko dan mencegah kolonisasi Enterobacteriaceae penghasil ESBL.

Kata kunci: *Enterobacteriaceae, ESBL, flora usus, sapi perah, peternak, rural*

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INTRODUCTION

The inappropriate use of antibiotics in human and animal health is a major cause of pathogenic bacterial resistance.[1] Resistant pathogenic bacteria that have increased significantly over the past few decades are ESBL-producing bacteria (extended-spectrum β-lactamase).[2] ESBL mainly distributed among gram-negative bacilli of the *Enterobacteriaceae* group.[3]

The use of antibiotics as growth promotion and the prevention of disease in veterinary [4] was correlated with the increase of ESBL producing Gram-negative bacteria.[5] It thus, the livestock are identified as a risk factor for ESBL producing *Enterobacteriaceae* (ESBL-E).[6] In 2018, East Java Province was identified has the highest population of dairy cows in Indonesia, about 283,311 cows.[7] Most of the dairy farms in East Java Province are located in rural areas to empowering the community's economy. Kalipucang Village in District of Pasuruan, East Java was established as the first center of public dairy farming in Indonesia at 2016.[8]

ESBL producing *Enterobacteriaceae* (ESBL-E) bacteria cause various infections in humans, such as: bacteremia, gastroenteritis, respiratory infections, urinary tract infections, and infections of the central nervous system.[9] In dairy cows, *Escherichia coli* and *Klebsiella spp* are identified as an agent that causing inflammation of mammary gland and udder tissue (mastitis) which impact on decreasing quantity and quality of milk production, increasing the rejected prematurely, and death.[10] ESBL-E becomes a serious challenge in therapy for infection includes prolong of diagnosis and expensive, a longer duration of treatment, limited antibiotic choices that impact on higher cost of therapy for an infection, as well as increased morbidity and mortality.[11] Multiple resistance to fluoroquinolones, aminoglycosides, and trimethoprim are commonly found in ESBL-E.[3] It also causes carrier in both humans[12] and livestock.[6]

Since ESBL-E has been identified as one of the causes of mastitis in dairy cows in 2000,[6] dairy farming was suspected to be at risk as a source of ESBL-E transmission. It thus the epidemiological profile of ESBL-E in farm needs to be explored. This study is the first study to analyze the epidemiological patterns of ESBL producing *Enterobacteriaceae* in livestock and humans in rural areas in Indonesia.

The aim of the study was to identify and analyzed the distribution and risk factor of ESBL producing *Enterobacteriaceae* in gut bacterial flora of dairy cows and people/farmer who have close contact with dairy cows.

MATERIALS AND METHODS

Design

This study was conducted a cross-sectional design. This study was approved by Research Ethics Committee in Faculty of Medicine of Universitas Airlangga, no: 82/EC/KEPK/FKUA/2019.

Samples Collection

Fecal samples were collected from April until July 2019 from dairy cows and farmers. Samples collected using Amies transport medium (Deltalab, Spanyol). The total dairy farming in the District of Pasuruan, East Java, are 648 clusters, of which as many as 102 were randomly included as the samples in this study, consisting

of 102 samples from dairy cows and 102 from humans that living around and have close contact with dairy cows. These clusters were located in Kalipucang village. The samples transportation were using a cool box and ice pack (4-8˚C). Samples were processed within 24 hours after taken from the sample source.

Bacterial and ESBL Identification

The isolation, confirmation, and identification of ESBL-E were conducted in The Clinical Microbiology Laboratory of Dr. Soetomo Hospital, Surabaya. Amies swab was streaked on MacConkey selective medium supplemented by cefotaxime 2 mg/L and incubated for 18-24 h at 37˚C. The growing colonies were ESBL confirmed by Modified Double Disk Synergy Test (M-DDST). Colonies which grow were inoculated in Mueller Hinton medium (0,5 MacFarland) with five (5) antibiotics disk : amoxicillin/clavulanic (AMC) 30 ug, ceftazidime (CAZ) 30 ug, cefotaxime (CTX) 30 ug, ceftriaxone (CRO) 30 ug, and aztreonam (ATM) 30 ug which placed within 15 mm of distance from edge to edge of AMC disk.[13] Incubated for 18-24 hours at 37˚C. The inhibition zone which show synergy zone between one of cephalosporin disk or aztreonam disk with amoxicillin/clavulanic disk was confirmed as ESBL producer. ESBL positive strain were bacteriologically identified using the biochemical test: Triple Sugar Iron (TSI) test, Indol test, Methyl Red (MR) test, Voges Proskauer (VP) test, Urease test, and Motility test. All bacterial isolates were then stored in deep freeze minus 80˚C.

Genotypic Examination

Genotypic examination was held in Institute of Tropical Diseases, Universitas Airlangga, Surabaya.

DNA Extraction

DNA extraction was conducted by boiling method. The identified ESBL producing bacteria were re-cultured on Mueller Hinton medium, incubated at 37˚C for 18 – 24 h. Four to five colonies were taken and suspended in sterile distilled water in 1,5 ml Eppendorf tube. The suspension was homogenized with vortex for 15 seconds and immersed in a thermostat at 95˚C for 10 minutes, then centrifuged at 14.000 rpm for 1 minutes. The supernatant was used as DNA template in PCR and stored in -20˚C.[14]

DNA Amplification

Three ESBL gene primers are used to amplify and identify the ESBL gene, as follow (Table 1) : [15]

PCR reaction was run in volume of 20 µl: 10 ul of GoTaq Green Master Mix 2x (Promega),1 ul for each of forward and reverse primers, 3 µl nuclease free water, and 5 µl DNA template. PCR was run as follow: for $bla_{\text{CTX-M}}$: denaturation on 94ºC for 7 minutes and the following 35 cycles on 94ºC for 50 seconds, annealing on 50ºC for 40 seconds, extension on 72ºC for 1 minute, and final extension on 72ºC for 5 minutes; for bla_{SHV}: denaturation on 96ºC for 5 minutes and the following 35 cycles on 96ºC for 1 minute, annealing on 60ºC for 1 minute, extension on 72ºC for 1 minute, and final extension on 72ºC

Table 1. Primers of ESBL Genes

Gen	Sekuens Primer (5'-3')	Amplicon size (bp/base pair)
$bla_{\text{CTX-M}}$	F : 5' ATGTGCAGYACCAGTAARGT 3' R : 5' TGGGTRAARTARCTSACCAGA 3'	593
bla_{SHV}	F : 5' GGTTATGCGTTATATTCGCC 3' R : 5' TTAGGTTGCCAGTGCTC 3'	867
bla_{TEM}	F : 5' ATGAGTATTCAACATTTCCG 3' R : 5' CTGACAGTTACCAATGCTTA 3'	867

for 10 minutes; and for bla_{TEM}: denaturation on 96ºC for 5 minutes and the following 35 cycles on 96ºC for 1 minute, annealing on 58ºC for1 minute, extension on 72ºC for 1 minute, and final extension on 72ºC for 10 minutes. PCR amplicon were visualized in 2% gel electrophoresis.

Questionnaire to Find Risk Factors

Information about risk factor of ESBL producing *Enterobacteriaceae* in dairy cows and farmers were obtained through interview and questionnaires. *Enterobacteriaceae* strain and genotype distribution among dairy cows and farmers and risk factors were analyzed by Chi Square/Fisher Exact Test on SPSS 22 version program.

RESULTS

Distribution of ESBL Producing *Enterobacteriaceae* in Dairy Cows and Farmers

Prevalence of ESBL producing bacteria in dairy cows was 13.7% (14/102) and in farmers 34.3% (35/102). ESBL producing *Enterobacteriaceae* in dairy cows were 6.9% (7/102) and in farmers 33.3% (34/102) (Table 2). The ESBL producing *Enterobacteriaceae* in dairy cows were mostly: *Escherichia coli* 85.7% (6/7) and *Enterobacter spp* 14.3% (1/7), whereas among 34 ESBL-E in human were *Escherichia coli* 82.4% (28/34), *Enterobacter spp* 14.3% (3/34), *Klebsiella pneumoniae* 5.9% (2/34), and *Klebsiella oxytoca* 2.9% (1/34). There were no significant differences in distribution of ESBL producing *Enterobacteriaceae* strain in dairy cows and in farmers (Table 2) and Fig.1.

Table 2. Distribution of ESBL producing *Enterobacteriaceae* strain in dairy cows and farmers

ESBL Producer	Dairy Cows (n=102)	Farmers (n=102)	*p* value
ESBL producing bacteria	14 (13.7%)	35 (34.3%)	
Non-*Enterobacteriaceae*	7 (6.9%)	1 (0.9%)	
Enterobacteriaceae	7 (6.9%)	34 (33.3%)	
Escherichia coli	6 (85.7)	28 (82.4)	$p = 1{,}000$
Enterobacter spp	1 (14.3)	3 (8.8)	$p = 0{,}542$
Klebsiella pneumoniae	0 / 0	2 (5.9)	$p = 1{,}000$
Klebsiella oxytoca	0 / 0	1 (2.9)	$p = 1{,}000$

Note: ESBL-E: ESBL producing *Enterobacteriaceae*

Figure 1. The Double Disk Synergy Test (DDST) for identifying ESBL producer bacteria.
Note: The increasing of inhibition zone in area between cephalosporin disk and clavulanic acid disk was marked as positive ESBL producer.

Escherichia coli were identified as dominant strain of ESBL producing *Enterobacteriaceae* in dairy cows and farmers (85.7% vs. 82.4%).

Distribution of ESBL Gene Among Dairy Cows and Human (Farmers)

Among seven ESBL producing *Enterobacteriaceae* in dairy cows, six isolates were harbored $bla_{CTX\text{-}M}$ (85.7%) and one an unidentified gene (14.3%). Among 34 isolates of ESBL producer in farmers, 26 isolates harbored $bla_{CTX\text{-}M}$ (76.5%), 15 isolates $bla_{TEM,}$ and three

Table 3. ESBL genes distribution of ESBL producing *Enterobacteriaceae* in dairy cows and farmers

ESBL Gene	Dairy Cows (n=7)	Farmers (n=34)	*p* value
$bla_{CTX\text{-}M}$	6 (85.7)	26 (76.5)	$p = 1{,}000$
bla_{SHV}	0 (0)	3 (8.8)	$p = 1{,}000$
bla_{TEM}	0 (0)	15 (44.1)	$p = 0{,}035$
Unidentified gene	1(14.3)	0 (0)	$p = 0{,}171$
$bla_{CTX\text{-}M,}$ bla_{TEM}	0 (0)	8 (23.5)	-
$bla_{CTX\text{-}M}$, bla_{SHV}, bla_{TEM}	0 (0)	1 (2.9)	-

Note: *Unidentified gene*: gene of ESBL producing *Enterobacteriaceae* which couldn't detected with specific primer used in this study

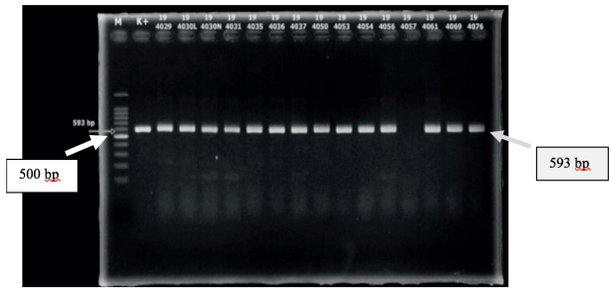

Figure 2. Electrophoresis of amplified gene of bla_{CTX-M} (593 bp)

isolates bla_{SHV} (8.8%), respectively. There was a significant difference of bla_{TEM} distribution in dairy cows and in farmers (p = 0, 035) (Table 3).

Combination of two and three of ESBL genes were found in *Enterobacteriaceae* producing ESBL isolates in farmers. Eight isolates harbored bla_{CTX-M} dan bla_{TEM} (23.5%) and one isolate harbored bla_{CTX-M}, bla_{SHV}, bla_{TEM} (2.9%) (Table 3).

Risk Factor for Colonization of ESBL Producing *Enterobacteriaceae*

Age, origin of dairy cows, history of illness during the last 3 months, history of drug use, type of drug given last 3 month, and type of feed were not risk factors for colonization of ESBL-producing *Enterobacteriaceae* in dairy cows. Risk factor for ESBL producing *Enterobacteriaceae* colonization in farmers was the use of antibiotics (p = 0.007). Gender, age, education level, household, hygiene sanitation of environment (location of dairy cow shed and type of toilet), personal hygiene sanitation (frequency and how to wash hands), and frequency of going out of the city during the last 3 months were not a risk factor.

DISCUSSION

Colonization of ESBL producing *Enterobacteriaceae* in dairy cows by 6,9% in this study is similar with study in healthy ruminant (cows and buffaloes) in rural areas in Cambodia by 7%[16] and lower than in cattle farm in German by 54.5%.[6] Colonization of ESBL producing *Enterobacteriaceae* in farmers by 33,33% lower than the colonization of ESBL-producing bacteria in healthy individuals in rural areas in Thailand by 65.7%,[17] in China by 73.9%,[18] and workers in cattle farms in Germany: 12.5%.[6]

Human and animal gut were the natural habitat of many bacterial especially *Enterobacteriaceae* and become a reservoir of various infections.[12] Non-appropriate and overuse of antibiotic caused selective pressure that supports the growth of resistance bacteria.[9] Colonization of resistance bacteria in human and animal gut causing transmission of resistance genes in gut flora bacterial through horizontal gene transfer by conjugative plasmid.[12] ESBL mostly encoded by genes in plasmids.[19] *Enterobacteriaceae* was identified as having plasmid carrying resistant genes. IncFII plasmid group known as a plasmid group that encoded ESBL genes and it widely distributed in *Enterobacteriaceae*. It called epidemic resistant plasmid group.[20]

This study identified *Escherichia coli* as the dominant ESBL producing bacteria in dairy cows (85.7%) and farmers (82.4%). Distribution of *Escherichia coli* as an ESBL producer in dairy cows in this study was 85.7%, higher than in cattle farms in Mecklenburg-Western Pomerania, Germany by 54.5%.[6] At farmers, distribution of ESBL producing *Escherichia coli* by 82.4% is lower than the distribution of *Escherichia coli* in healthy individuals in rural areas in Thailand by 85.4% [17] and in China 88%,[18] but higher than workers in cattle farms in Germany: 12.5%.[6]

Escherichia coli is the main organism that produces ESBL in communities[21] and associated with urinary tract infections (UTI). It is related to their role as gut bacterial flora and are pathogenic to humans and animals.[12] The resistance of commensal *Escherichia coli* to antimicrobial agents has been found in healthy individuals.[22] This bacterium also acts as an indicator of 'acquired antibiotic resistance genes' in the community.[23]

Distribution of bla_{CTX-M} in the ESBL producing *Enterobacteriaceae* in dairy cows by 85.7% is higher than the distribution of bla_{CTX-M} in cattle farms in Germany 80%.[6] At farmers,

distribution of bla_{CTX-M} in the ESBL-producing *Enterobacteriaceae* by 76.5% higher than in healthy individuals in rural areas in China by 68.1%[18] and in Thailand by 65.7%.[17] bla_{CTX-M} that mostly integrated with conjugative plasmid, and conjoint with the other resistant gene, has a higher transferability among bacteria, and impact on higher prevalence epidemiologically.[24]

bla_{CTX-M}, bla_{SHV}, and bla_{TEM} are dominant ESBL genes in various regions worldwide and found from isolates of humans, animals and the environment. bla_{TEM} and bla_{SHV} mainly found in *Escherichia coli* and *Klebsiella pneumoniae*.[25] The study identified bla_{TEM} in *Escherichia coli* and *Enterobacter spp* isolates and bla_{SHV} in *Klebsiella pneumoniae* and *Escherichia coli* isolates. bla_{CTX-M} is the dominant ESBL gene worldwide, especially in community and has increased in incidence since 2000.[12] It were identified in livestock and pets with *Escherichia coli* as the main producing bacteria.[26] Our study showed that bla_{CTX-M} was identified in *Escherichia coli*, *Enterobacter spp*, and *Klebsiella oxytoca*.

Dissemination of bla_{CTX-M} occurs rapidly, extensive, and significantly. Plasmids are known to carry genes which encode resistance for antibiotic.[27] Conjugative plasmids play an important role in facilitating the horizontal dissemination of bla_{CTX-M} among bacteria.[28] bla_{CTX-M} was identified in various epidemic resistant plasmid groups, including: groups IncF, IncN, IncI1, IncL / M, and IncHI2.[24] These plasmid group are able to capture and transfer resistant genes among bacteria.[29] The IncFII Group is the largest plasmid group encoding bla_{CTX-M} and widely found in *Enterobacteriaceae*[24] and isolates from human and animal.[20]

IS*Ecp*1 is the genetic element which associated with all variants of bla_{CTX-M}, play a role incoding transposase and inducing bla_{CTX-M} expression. Transposase is an enzyme that mobilizes bla_{CTX-M} in certain plasmids.[28] Other types of IS include: IS*CR*1 plays a role in bla_{CTX-M} group 2 and 9 expression, IS*10* in bla_{CTX-M} group 8 expression,[24] and IS*26* in bla_{CTX-M} group 1 and 9 expression. IS*Ecp*1 and IS*CR*1 play a role in the mobilization of class 1 integron that encodes various types of resistant genes (MDR cassettes).[28]

Clones of *Escherichia coli* were identified having a significant role in the dissemination of bla_{CTX-M}, among others, such as ST131, ST38, ST393, ST405. ST131 serotype 025: H4 phylogenetic group B2 is an extra-intestinal pathogenic *E. coli* strain and was mainly involved in bla_{CTX-M} dissemination especially $bla_{CTX-M-15}$ in worldwide.[24] It identified having IncFII plasmid group and found in isolates originated from the animal, environment, and especially human.[28]

Combination of two or three ESBL genes in one bacterial isolate is due to integron and plasmid that carry several resistant genes. *Enterobacteriaceae* would be harboring of 5 to 6 plasmids in one isolate.[30] Class 1 integron which related to bla_{CTX-M} were identified encoding several types of resistant genes (MDR cassettes).[28] The unidentified gene is thought to be an ESBL gene in addition to bla_{CTX-M} (group 1), bla_{SHV}, and $bla_{TEM.}$

The finding of antibiotic use as risk factor of ESBL producing *Enterobacteriaceae* in farmers in this study ($p= 0,007$) related according to the study of Luvsansharav et al,[17] which identified the use of antibiotics in the last 3 months (OR 1,883; 95% CI 1,221-2,903) as a risk factor for colonization of ESBL producing bacteria in healthy individuals in rural area in Thailand and Zang et al[18] that identified antibotic use in the previous 6 months (OR 1,892; 95% CI 1,242–2,903; $p = 0.034$) as a risk factor for colonization of ESBL-producing bacteria in healthy individuals in rural area in China.

In dairy cows in this study, there was not any antibiotic use detected based on data on questionnaires. The total of 52% of dairy cows were given anthelmintic every three months. Risk factors for colonization of ESBL producing *Enterobacteriaceae* in dairy cows was not identified. Dissemination of ESBL producing bacteria occurs from animals to humans or vice versa.[6] ESBL producing Gram negative in dairy cows have the potential as a zoonotic risk,[31] especially through close contact during daily care.[6]

The results of the study showed that the rural community could act as a reservoir of ESBL-producing *Enterobacteriaceae*. The finding of *Escherichia coli* and bla_{CTX-M} as the dominant strain and ESBL gene epidemiologically indicated alarming sign. *E. coli* ST131 consider as virulent strain,[24] multiple resistance, easily colonize and spread between humans, animals and environment isolates.[27] It contributes to the spread of bla_{CTX-M} globally through horizontal gene transfer.[24] ESBL-producing *E. coli* and bla_{CTX-M} have driven the spread of ESBL gene in the community. Colonization of ESBL-producing *Enterobacteriaceae* is a risk factor for infection of ESBL-E.[3] The colonization of ESBL-producing *Enterobacteriaceae* in community were predicted increasingby about 5% annually.[24] This certainly becomes a challenge in therapy of infectious disease.

CONCLUSION

Farmers had suspected as the source of ESBL producing *Enterobacteriaceae* based on higher prevalence. The use of antibiotic in human, was identified as risk factor for colonization of ESBL producing *Enterobacteriaceae* while not identified in dairy cows.

CONFLICT OF INTEREST

There is no conflict of interest of this study.

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Research Report

GeneXpert MTB/RIF and *Mycobacterium tuberculosis* Sputum Culture in Establishing the Diagnosis of Pulmonary Tuberculosis and Rifampicin Resistance in Suspected Childhood Pulmonary Tuberculosis in Soetomo Hospital

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ABSTRACT

The diagnosis of childhood tuberculosis remains a challenge worldwide. The GeneXpert MTB/RIF test, a rapid Mycobacteria tuberculosis diagnostic tool, was recommended for use in children. No pediatric studies of GeneXpert MTB/RIF assessing pulmonary tuberculosis within a hospital setting has been done in Indonesia. We evaluated the performance of the GeneXpert MTB/RIF test compared with sputum culture on Lowenstein-Jensen (LJ) for the diagnosis of childhood pulmonary tuberculosis. This study was conducted in pediatric respirology inpatient and outpatient Dr. Soetomo Hospital, a tertiary care facility in Surabaya between June and August 2015 with a cross-sectional design. We consecutively enrolled 27 children aged 3 months to 14 years who had history of close contact with adult tuberculosis patients and showed symptoms of pulmonary tuberculosis. Sputum collection was performed by induced sputum and three examination methods were performed (microscopic, GeneXpert MTB/RIF and sputum culture) simultaneously followed by a drug sensitivity test for specimens detected with MTB growth. The GeneXpert MTB/RIF test had a sensitivity of 100% (95% CI 100-100) and a specificity of 95% (95% CI 85-100). The positive predictive value for diagnosing pulmonary TB was 89% (95% CI 68-100), the negative predictive value was 100% (95% CI 100-100) and positive likelihood ratio was 20 (95% CI 2.82-128). The GeneXpert MTB/RIF test on one sputum sample rapidly and correctly identified all children with culture-confirmed pulmonary tuberculosis with high specificity. Similar results were obtained between GeneXpert MTB/RIF and sputum culture based on age groups and clinical manifestations. Rifampicin resistance were both detected in GeneXpert MTB/RIF and MTB sensitivity test.

Keywords: *Childhood pulmonary tuberculosis; Sensitivity, Specificity; GeneXpert MTB/RIF*

ABSTRAK

Menegakkan diagnosis tuberkulosis (TB) pada anak sampai saat ini masih sulit dikerjakan. GeneXpert MTB/RIF adalah suatu metode diagnostik baru yang dapat mengidentifikasi Mycobacterium tuberculosis (MTB) dengan cepat. Walaupun metode ini telah direkomendasikan pada anak-anak, namun penelitian tentang GeneXpert MTB/RIF dalam mendiagnosis TB paru anak di lingkungan Rumah Sakit (RS) belum pernah dikerjakan di Indonesia. Kami membandingkan hasil pemeriksaan GeneXpert MTB/RIF dengan kultur dahak MTB pada media Lowenstein Jensen (LJ) dalam menegakkan diagnosis TB paru pada anak yang diduga TB paru. Penelitian ini dilakukan di poli dan bangsal respirologi anak RSUD Dr. Soetomo antara Juni sampai Agustus 2015 secara cross sectional. Dengan sampling konsekutif mengumpulkan 27 anak usia 3 bulan sampai 14 tahun yang mempunyai kontak erat dengan penderita TB dewasa dan menunjukkan gejala TB paru. Pada setiap anak dilakukan pengambilan dahak dengan cara induksi dahak kemudian dilakukan tiga metode pemeriksaan sekaligus yaitu secara mikroskopis, GeneXpert MTB/RIF dan kultur yang dilanjutkan dengan uji kepekaan MTB bagi spesimen yang terdeteksi ada pertumbuhan MTB. Sensitivitas GeneXpert

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MTB/RIF adalah 100% (95% CI 100-100) dan spesifisitas 95% (95% CI 85-100). Nilai duga positif GeneXpert MTB/RIF adalah 89% (95% CI 68-100), sedangkan nilai duga negatifnya adalah 100% (95% CI 100100) dan Likelihood positive nya adalah 20 (95%CI 2,82-128). GeneXpert MTB/RIF mampu mendeteksi semua spesimen yang terdeteksi positif MTB oleh kultur dahak MTB namun dalam waktu yang lebih singkat dan dengan spesifisitas yang tinggi. Kesepadanan hasil antara GeneXpert MTB/RIF dan kultur dahak didapatkan berdasarkan kelompok umur dan manifestasi klinis TB. Selain Dalam mendeteksi resistensi Rifampicin, GeneXpert MTB/RIF memberikan hasil yang sama dengan uji kepekaan MTB.

Kata kunci: *Tuberkulosis paru anak, Sensitivitas, Spesifisitas, GeneXpert MTB/RIF*

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INTRODUCTION

Difficulty to diagnose tuberculosis in children can lead to under and over diagnosis of TB which can cause higher morbidity and mortality. Confirming the diagnosis of childhood TB is a major challenge. However, research on childhood tuberculosis in relation to better diagnostics is often neglected because of technical difficulties, such as the slow growth in culture, the difficulty of obtaining specimens, and the diverse and relatively nonspecific clinical presentation of TB in this age group. While the classic presentation of childhood TB is prolonged cough and weight loss, HIV infection, with its chronic pulmonary manifestations and wasting, may confound the diagnosis of childhood TB. These difficulties are worsened by the increased incidence of multiple drug resistance.[1,2] Therefore, early diagnosis of TB in children is very important in order to control the incidence of TB disease.

Tuberculosis remains a major problem for the health of mankind. In 2012, the estimated incidence of TB cases were 8.6 million cases / year and 58% of these cases occur in Southeast Asia and the western Pacific. Approximately 50-60% of children living with adult pulmonary tuberculosis (PTB) patients who have positive acid-fast bacilli (AFB) sputum results will be infected with TB as well and about 10% of them will get TB disease.[3] World Health Organization (WHO) in 2012 estimated that there were 530,000 new cases of TB in children with a mortality rate of 74,000.[4] Indonesian TB data in 2012 showed the proportion of TB cases in children among all TB cases was 8.2%.[5]

The diagnostic approaches that exist today are less sensitive. Although conventional examination with a microscope has a high positive predictive value for detecting Mycobacterium tuberculosis (MTB), its sensitivity is low. Examination using media culture with Lowenstein-Jensen (LJ) is still the gold standard for diagnosis but this test is difficult and requires a long time (± 6-9 weeks) to get the results with positive results obtained only in 10% - 15% culture examination.[6,7] Polymerase chain reaction (PCR) test provides high sensitivity by multiplying deoxyribonucleic acid (DNA) of bacteria, and has been extensively evaluated in order to detect the DNA of MTB. GeneXpert MTB/RIF is an integrated and automated test with molecular approaches. Sample preparation, amplification and detection is done automatically by PCR. GeneXpert MTB/RIF is able to detect MTB as well as diagnosing resistance to Rifampicin. The results will be obtained in less than 2 hours.[8,9,10,11]

In December 2010, WHO has encouraged the use of GeneXpert MTB/RIF as a tool for the diagnosis of TB due to high sensitivity and specificity but studies on the use of GeneXpert MTB/RIF in children are still rare.[11,12] The aim of this study is to compare the GeneXpert MTB/RIF with MTB sputum culture examination in the diagnosis of PTB and rifampicin resistance in children with suspected PTB in Dr. Soetomo Hospital Surabaya.

MATERIALS & METHODS

This study was analytical observational to compare the GeneXpert MTB/RIF assay with MTB sputum culture for detection of pulmonary tuberculosis and Rifampicin resistance in new pediatric inpatients and outpatients at the Department of Pediatric and Child Health, Dr. Soetomo Hospital, Surabaya, Indonesia, a tertiary referral center. This study was approved by the research ethics committee of Dr. Soetomo Hospital Surabaya. The parents of all study participants provided written informed consent.

Between June 2015 and August 2015, new outpatients and inpatients children, aged 3 months old - 14 years old, with the diagnosis of suspected tuberculosis were eligible for enrollment in the study. A patient with suspected tuberculosis was defined as having a symptom and risk factor screening (one or more of five factors: tuberculosis contact, cough for more than 3 weeks, weight loss, malnutrition, or fever for more than 2 weeks with unknown origin) according to the Indonesian National TB Program. Patients were excluded if they were deemed to have a poor prognosis, congenital heart defects, severe congenital abnormalities, acute hemodynamic disturbances (hypotension, shock, heart failure, decreased consciousness), critical illness (sepsis, renal failure, impaired liver function severe), have received TB treatment > 1 month and patients with HIV infection or if parents or guardians refused the informed consent.

Procedure of sample collection started with history taking and physical examination taken when administered. The clinical manifestations observed in this study were in accordance with TB scores commonly used in Indonesia, including a history of close contact with adult TB patients, the results of TST, fever ≥ 2 weeks are not unexplained, coughing ≥ 3 weeks, enlarged neck lymph nodes, inguinal and axillary, nutritional status, swelling of bones/joints and chest X-rays. Sputum samples were collected from children who could expectorate. In children who could not spontaneously expectorate, sputums were collected by induced sputum procedure. Smear microscopy, culture with LJ media and

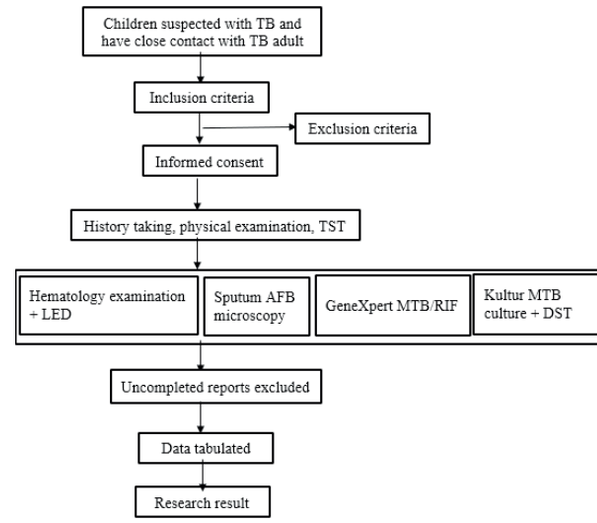

Figure 1. Operational Framework

GeneXpert MTB/RIF were done simultaneously on all samples as described previously. Cultures were classified as negative when no growth were detected after 8 weeks of incubation. Contaminated samples were retreated and re-cultured, and excluded if still contaminated. Drug susceptibility testing was done on LJ media. Sputum was added to the GeneXpert MTB/RIF sample reagent in a 1:1 ratio (1 mL of sputum to 1 mL of the sample reagent). Two mL of this mixture was added to the GeneXpert MTB/RIF cartridge and run in the machine in accordance with manufacturer's instructions.

All clinical and laboratory data were compiled in databases. Selected variables were exported to SPSS (version 21) for analysis. Comparisons of GeneXpert MTB/RIF assay and sputum culture assay were done with Pearson χ^2 or Fishers exact test. The sensitivity, specificity, and predictive values of the assays with 95% CIs were calculated. The equivalence between GeneXpert MTB/RIF assay and sputum culture were analyzed with McNemar test and Kappa. All statistical tests were two-sided with alpha of 5%.

RESULTS

Twenty seven children were recruited and had sputum for analysis. Table 1 shows about half (51.9 %) of the children enrolled were younger than 5 years. The most common clinical

Table 1. Baseline Characteristics

Characteristics	N(%)
Age < 5 years old	14 (51.9)
≥ 5 years old	13 (48.1)
Gender Male	14 (51.9)
Female	13 (48.1)
Contact Identified	18 (66,7)
Not Identified	9 (33,3)
Contact MDR	5 (18.5)
Non MDR	12 (44.4)
Scar BCG Present	21 (77,8)
None	6 (22,2)
Cough > 3 weeks	18 (66.7)
Fever > 2 weeks	19 (70.4)
Lymph node enlargement	9 (33.3)
Nutritional Status Normal	12 (44.4)
Poor	10 (37.0)
Malnutrition	5 (18.5)
TST Positive	18 (66.7)
Negative	9 (33.3)
Bone destruction	1 (3.7)
Chest X-ray Suggestive TB	16 (59.3)
Not Suggestive TB	11 (40.7)
TB Score ≥ 6	23 (85.1)
< 6	4 (14.8)

Table 2. Sputum Results

Sputum Examination	N (%)
MTB culture Positive	8 (29.7)
Negative	19 (70.3)
GeneXpert MTB/RIF Positive	9 (33.3)
Negative	18 (66.7)
Smear microscopic Positive	6 (22.2)
Negative	21 (77.8)

Table 3. Drug Sensitivity Test of Positive Results (8 LJ Culture and 9 GeneXpert MTB/RIF)

Rifampicin Sensitivity Test	N (%)
Drug sensitivity test with LJ	
Rifampicin Sensitive (+)	8 (100)
Rifampicin Resistance	0 (0)
INH Sensitive	7 (87.5)
INH Resistance	1 (12.5)
Etambutol Sensitive	8 (100)
Etambutol Resistance	0 (0)
Streptomycin Sensitive	7 (87.5)
Streptomycin Resistance	1(12.5)
GeneXpert MTB/RIF	
Rifampicin Sensitive (+)	9 (100)
Rifampicin Resistance	0 (0)

manifestation reported was fever with no apparent cause lasting more than 2 weeks. Eighteen subjects (66.7%) showed positive TST and 9 (33.3%) had negative result.

Molecular examination with GeneXpert MTB/RIF provides the highest positive results of 33.3%, followed by microbiological culture (29.7%) and microscopic examination (22.2%) (Table 2). There was no false negative results for GeneXpert MTB/RIF examination.

GeneXpert MTB/RIF correctly identified all 9 rifampicin-sensitive on specimen analysis (Table 3). All 8 LJ culture positive specimens were also analyzed with the LJ drug-sensitivity test and none were Rifampicin resistance. Therefore MTB drug sensitivity cannot be analyzed statistically since the LJ results on solid media were equivalent with the results by the GeneXpert MTB/RIF (Table 3).

Table 4 shows that only 5 (27.8%) of 18 patients with positive TST results were confirmed positive by GeneXpert MTB/RIF, whereas 13 (72.2%) others showed negative results by GeneXpert MTB/RIF. There were 4 (44.4%) of 9 children who showed negative TST result, but confirmed positive on GeneXpert MTB/RIF, and 5 (55.5%) of 9 children showed a negative result on both TST and GeneXpert MTB/RIF.

Table 4. TST Result VS GeneXpert MTB/RIF

		GeneXpert MTB/RIF		Total
		Positive (%)	Negative (%)	
TST	Positive	5 (27,8)	13 (72,2)	18
	Negative	4 (44,4)	5 (55,5)	9
Total		9	18	27

Table 5 shows the equivalence results of GeneXpert MTB/RIF and MTB sputum culture in the age group ≥ 5 years old as many as 12 samples. There is only one sample which showed a positive result on GeneXpert MTB/RIF but confirmed

Table 5. GeneXpert MTB/RIF vs MTB Sputum Culture in Age Group

GeneXpert MTB/RIF		MTB culture (+)	MTB culture (-)	Agreement (%)	McNemar	Kappa
< 5 years old	(+)	2	0	100	p=1,000	1,000 p=0,00
	(-)	0	12			
≥ 5 years old	(+)	6	1	92,4	p=1,000	0,847 p=0,002
	(-)	0	6			

Table 6. GeneXpert MTB/RIF vs MTB Sputum Culture in Clinical TB Group

GeneXpert MTB/RIF		MTB culture (+)	MTB culture (-)	Agreement (%)	McNemar	Kappa
Clinical TB	(+)	8	1	94,2	1,00	0,883 p=0,000
	(-)	0	8			
Non Clinical TB	(+)	0	0	100	-	-
	(-)	0	10			

negative on MTB sputum culture. McNemar test shows no significant difference. Kappa test results shows significant reliability between the results of the GeneXpert MTB/RIF with MTB sputum culture MTB in the age group ≥ 5 years. The agreement between GeneXpert MTB/RIF and MTB sputum culture examination was 92.4%.

Table 6 shows the total positive clinical TB assessment in 17 (63.0%) of 27 children. There were 9 (53.0%) clinical TB children who have positive results of GeneXpert MTB/RIF. All children with clinically negative TB showed negative result on GeneXpert MTB/RIF. McNemar test showed no significant difference between the results of GeneXpert MTB/RIF and MTB sputum culture with positive clinical manifestations of TB. Kappa test showed significant equivalence between GeneXpert MTB/RIF and MTB sputum culture with positive clinically manifestation of TB.

This study showed that GeneXpert MTB/RIF had 100 % sensitivity (95% CI 100-100), specificity of 95% (95% CI 85-100), PPV 89% (95%CI 68-100), NPV 100% (95% CI 100-100), LR +20 (95% CI 2.82-128), LR - 0.

DISCUSSION

In our study, more than 50% of samples had a history of close contact to adult patients with positive AFB smear, cough > 3 weeks, fever of unknown origin > 2 weeks, positive TST results and X-rays which showed PTB process. More than 50% of the sample had a value of TB score > 6. Positive TST results were not always found in children suspected of pulmonary TB. In our study, Positive TST results obtained in 66.67% of the samples, of which only 55.5% of the samples were GeneXpert MTB/RIF positive. Sekadde et al (2013) and Nicol et al (2011) obtained positive results only at ± 30% of the samples[13,14], while Nataprawira et al (2001) get positive TST results only in 9.7% of children who had close contacts with adult TB patients or adults suspected of having TB in Bandung.[15] There are several factors that influence the results of TST, such as malnutrition which effect on phagocytosis, cellular immunity and cytokine production.[16] Malnutrition leads to lymphoid tissue atrophy, thus affecting the development, differentiation and cause a decrease in lymphocytes. Moderate and severe malnutrition lead to decreasing delayed-type hypersensitivity reactions and recall process.[17]

The positive results of microscopic and molecular examination in this study is quite high when compared to previous studies. Giang et al reported positive results of GeneXpert MTB/RIF on 8.6% of samples,[18] Nicol et al reported 12.8%,[14] Sekadde et al reported 14%,[13] Singh et al and Nhu et al reported a respective 16.9% and 16.2%.[19,20] This condition is likely due to several factors, such as the number of MTB in children (paucibacillary) and sputum production capabilities that are lacking in children. Different inclusion criteria with previous studies may also cause these differences. In our study, most of the sample had more than 4 clinical manifestations as well, while these other studies established inclusion criteria of children aged ≤ 14 years with at least 2 clinical manifestations of cough ≥ 2 weeks and one of the symptoms of weight

loss or fever ≥ 2 weeks with unknown origin, or a history of contact with adult TB patients, or a positive result on TST or positive X-ray for TB process.[13,14,20]

Microscopic examination (Olympus CH-20, Olympus Corp., Japan; 1000x magnification) is able to detect 66.7% of specimens with positive GeneXpert MTB/RIF and 75% of specimens positive by MTB sputum culture. Positive smear cases were found mainly in the > 5 years old age group. This occurs because children > 5 years old or adolescents have pathological features of "adult-type" TB that is not paucibacillary with more bacilli accumulated and generally give more positive results on microscopic examination.[21,22] Previous studies reported the same result. Marlowe et al in the US collected 217 sputum specimens and showed that microscopic examination is able to detect 73% of GeneXpert MTB/RIF positive results. Lawn et al (2011) only reported 45% positive smear result of TB cases. This can be explained due to the colony of microscopic detection capabilities is less sensitive than the other two examination modalities. The detection capability of the colony smear microscopy is $5x10^3$ to $5x10^4$ bacilli/ml, the detection capability of GeneXpert MTB RIF is 102-107 CFU/ml and the culture detection capability is 10-100 CFU/ml.[12,23] However, microscopic examination is not specific to diagnose TB because there are several other bacteria that are resistant to acid staining which are Rhodococcus spp, Nocardia spp, Legionella micdadei, cysts and isospora of Crytopsoridium spp, that will give a false-positive smear result.[24]

In this study, the sensitivity of GeneXpert MTB/RIF is 100% and specificity was 94.7%. There is one GeneXpert MTB/RIF positive result that is not detected by MTB sputum culture. PCR concept used in GeneXpert MTB/RIF sequence all of MTB DNA without the capability to detect the viability of the MTB. GeneXpert MTB/RIF false-positive may result from patients who had been treated as well.

Systematic reviews conducted by WHO in 2013 among 13 studies involving 2,603 participants mention pooled sensitivity of GeneXpert MTB/RIF TB was 66% (95% CI 52-77) and pooled specificity was 98%.[25] Meta-analysis conducted in 2012 of 18 studies involving 10,224 specimens reported sensitivity of GeneXpert MTB/RIF amounted to 90.4% (95% CI 89.2 to 91.4) and specificity of 98.4% (95% CI 98-98, 7).[26] Recent meta-analysis of the ability of GeneXpert MTB/RIF in the diagnosis of childhood PTB reported pooled sensitivity of 62% (95% CI 51-73) and a pooled specificity of 98% (95% CI 97-99).[27]

Bates et al reported no significant differences between specimens derived from sputum or liquid gastric washings in the GeneXpert MTB/RIF examination and concluded the use of liquid gastric washings can replace sputum specimens if they are not available.[28] In the study conducted by Nhu et al and Singh et al, stored sputum specimens were used instead of fresh sputum specimens.[19,20] In a sputum that was kept frozen and then thawed, the DNA will be damaged and affect the viscosity of sputum, thus giving bias.[29] Performing GeneXpert MTB/RIF examination twice on one specimen reportedly do not increase the rate of case detection. Repeated examination of GeneXpert MTB/RIF will increase the cost, even though there are still other supporting diagnostic examination. BBLK Surabaya's policy is to do single sputum GeneXpert MTB/RIF examination for each patient.[14,20]

The existence of GeneXpert MTB/RIF machines is not widely available in primary and secondary health facilities, therefore Sekkade et al conducted a study in Uganda and analyze the clinical characteristics associated with GeneXpert MTB/RIF positive results. It is intended to help health workers in limited medical care facilities to predict the likelihood of TB in a suspected TB children. Researchers reported some characteristics of the sample that has a tendency to get positive result of GeneXpert MTB/RIF, such as age group of > 5 years, a positive TST result and a positive TB contacts.[13]

All sputum specimens with positive result of GeneXpert MTB/RIF and MTB sputum culture show sensitive result to rifampicin in this study. A study by Carriquiry et al on 130 patients aged > 18 years in Peru in 2012 reported 100% (95% CI 61-100) and 91% (95% CI 88.7 to 100) for sensitivity and specificity respectively. Predictive result were

66.7% (95% CI 35.4 -87.9) and 100% (95% CI 88.7 to 100) for PPV and NPV respectively.[29] Some researchers have assessed the ability of GeneXpert MTB/RIF in detecting MTB with Rifampicin resistance, but the samples were too small and therefore cannot be assessed.[14,20,30]

Tuberculosis is more progressive and fatal in children aged < 5 years old, while those aged ≥ 5 years old was associated with disease progression being "adult-type TB". Other than that this age group is the most common group of contracting TB in countries with high TB prevalence. This type of "adult-type TB" has the potential to cause extensive damage to lung parenchyma due to calcification and formation of cavities, and this age group is potentially infectious to the community.[21] In our study, the statistical test shows significant equivalence between GeneXpert MTB/RIF and MTB sputum cultures in both age groups. This means that GeneXpert MTB/RIF can be used interchangeably with MTB sputum culture to diagnose PTB in both age groups if there is no MTB sputum culture examination facilities. Beside, molecular methods with GeneXpert MTB/RIF also gives advantage of reading the results quickly (± 2 hours) so clinical decisions to initiate TB treatment can be accelerated. Sekadde et al and Nhu et al reported the same result for sensitivity and specificity of GeneXpert MTB/RIF for > 5 years old age group higher than group age < 5 years old.[19,20]

In our study, statistical analysis suggested there is a link between clinical manifestations and GeneXpert MTB/RIF or MTB sputum culture results. There is equivalence between GeneXpert MTB/RIF results and MTB culture result in the clinical TB group. GeneXpert MTB/RIF and MTB sputum cultures had lower sensitivity in diagnosing TB children clinically than molecularly. This is because children naturally had paucibacillary MTB although demonstrated clinical manifestations of TB and have symptoms improvement after treatment.

Symptoms of TB in children are not specific and more than 50% of children with TB are asymptomatic. Children with TB exhibiting clinical symptoms mostly will experience lung disorders, while 25% - 35% have extra-pulmonary disorders. Systemic disorders such as fever, night sweats, anorexia may also occur. The most common clinical symptoms are cough, body fatigue and weight loss. Specificity of clinical symptoms depends on the tightness of operational definitions used. However there is no cut-off for clinical symptoms that have been validated until now.[22,31,32]

The diagnosis of PTB in children cannot be established by clinical symptoms alone. Laboratory tests need to be done in children with or without clinical symptoms of PTB. However, the negative results of bacteriological examination does not exclude the possibility of TB disease.[22] Patients aged < 5 years, with a positive TST result and history of close contact with adult TB patients but do not show symptoms of PTB, were given INH prophylaxis of 7-15 mg / kg / day, once daily for 6 months, while patients that show symptoms of PTB were given TB drugs according to standard procedures.[33]

CONCLUSION

GeneXpert MTB/RIF has a good sensitivity and specificity to diagnose pulmonary tuberculosis (PTB) in children which give parallel results with MTB sputum culture methods in aiding the diagnosis of PTB in children aged ≥ 3 months old - 14 years old with suspected PTB.

CONFLICT OF INTEREST

There is no conflict of interest of this study.

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Article Categories

C-reactive Protein and Hepcidin in Non-Dialysis Chronic Kidney Disease

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ABSTRACT

Complications such as anemia and its clinical consequences arise as chronic kidney diseases progress,. One renal anemia pathophysiology is a disruption of iron metabolism, regulated by the main iron exporter hormone, hepcidin. Chronic kidney disease patients were constantly in an inflammatory state, represented by an increased in C-reactive protein. This inflammatory state would facilitate the liver to secrete hepcidin, which would subsequently follow a decrease of iron circulation, thus resulting in functional iron deficiency. Both acute phase reactants which used thoroughly as markers in tropical and infectious diseases, had their own roles in chronic kidney disease. The correlation of c-reactive protein and hepcidin in chronic kidney disease patients was still controversial. To analyse the relationship between c-reactive protein and hepcidin in non-dialysis chronic kidney disease patients. We conducted an observational cross-sectional study with 40 non-dialysis chronic kidney disease patients who met the inclusion and exclusion criteria. Patients were enrolled with consecutive sampling and were examined for serum c-reactive protein and hepcidin levels.A total of forty subjects (67.5% male with mean age of 50.23 ± 1.04 years) were eligible for enrolment in this study. The most comorbid factor was hypertension (62.5%). The common stage for chronic kidney disease was stage 3 (40%). The mean hemoglobin value was 10.74 ± 0.36 g/dL, mean blood urea nitrogen was 39.98 ± 29.59 mg/dL, and serum creatinine of 4.12 ± 3.39 mg/dL. Mean serum c-reactive protein levels were 3.52 ± 5.13 mg/l. Mean hepcidin level were 94,03 ± 95,39 ng/ml. Serum C-reactive protein levels correlated positively (r=0.487) and significantly (p-value=0.001) with serum hepcidin value. C-reactive protein and hepcidin was significantly correlated in non-dialysis chronic kidney disease patients.

Keywords: *CRP; Hepcidin; CKD; non-dialysis; iron; liver*

ABSTRAK

Progresivitas penyakit ginjal kronis akan membawa komplikasi anemia dengan berbagai konsekuensi klinis. Salah satu patofisiologi anemia pada penyakit ginjal kronis dapat diakibatkan oleh gangguan metabolisme besi yang diatur oleh hormon eksporter utama besi yaitu hepsidin. Pasien penyakit ginjal kronis berada dalam kondisi inflamasi, yang diwakili dengan peningkatan c-reactive protein. Adanya inflamasi akan menyebabkan liver mensekresi hepsidin yang kemudian berdampak pada menurunnya kadar besi dalam sirkulasi yang dapat berdampak pada anemia defisiensi besi fungsional. Kedua reaktan fase akut yang biasa digunakan dalam penyakit tropik dan infeksi, ternyata juga memiliki peran dalam penyakit ginjal kronis. Hubungan antara c-reactive protein dengan hepsidin pada penderita penyakit ginjal kronis non-dialisis masih menjadi kontroversi. Menganalisis hubungan antara kadar c-reactive protein dengan hepsidin pada pasien penyakit ginjal kronis yang belum menjalani hemodialisis. Studi ini adalah studi analisis observasional cross sectional, diikuti 40 pasien penyakit ginjal kronis yang belum menjalani dialisis yang sesuai dengan kriteria inklusi dan eksklusi. Subjek penelitian di ambil secara konsekutif dan diperiksakan kadar c-reactive protein serum dan hepsidin serum. Empat puluh subjek penelitian ini, terdiri dari 27 subjek laki-laki dan 13 subjek perempuan dengan rerata usia 50,23 tahun. Penyakit komorbid terbanyak adalah hipertensi (62,5%). Stadium terbanyak adalah stadium 3. Rerata kadar hemoglobin pada penelitian ini sebesar 10,74 ± 0,36 g/dL, rerata blood urea nitrogen 39,98 ± 29,59 mg/dL, dan rerata serum kreatinin sebesar 4,12 ± 3,39 mg/dL. Rerata kadar c-reactive protein serum sebesar 3,52 ± 5,13 mg/l. Rerata kadar hepsidin serum sebesar 94,03 ± 95,39 ng/ml. Pada

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penelitian ini diperoleh hubungan positif (r=0,487) yang signifikan (p=0,001) antara c-reactive protein dan hepsidin. Didapatkan hubungan positif yang signifikan antara kadar C-reactive protein dan hepsidin pada pasien penyakit ginjal kronis non-dialisis.

Kata kunci: *CRP; hepsidin; PGK; non-dialisis; besi; liver*

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INTRODUCTION

Hepcidin and c-reactive protein (CRP) had their roles in infectious diseases for a period of time. Hepcidin lowered mammal's blood iron levels at the time the pathogen-infected the hosts. Low blood iron level hindered pathogen's growth so that infections might be stopped. C-reactive protein had its own roles in activating platelets, leukocytes, endothelial growth factors, complements and chemokines during infections to cease infection. However, the roles of these two markers in renal anemia in chronic kidney disease (CKD) have not been elucidated yet.

C-reactive protein has been one of the sensitive inflammation markers which correlate with hepcidin in CKD. There have been substantial studies to backed up and come against it. Chronic kidney disease, stated as a chronic state of low-grade inflammation, could initiate a chain of sequences that lead to secretion of CRP and hepcidin. However, hepcidin was first recognized by Ganz, et al. as liver expressed antimicrobial peptide-1 (LEAP-1) secreted during infection or high-grade inflammation, putting hepcidin into lower place in this chain of sequences than CRP.

C-reactive protein was proven to be inversely correlated with the estimated glomerular filtration rate (eGFR) and stage in CKD. CRP also correlated with other inflammation markers such as interleukin-6 (IL-6). Interleukin-6 was directly correlated with the secretion of CRP and hepcidin in the human liver.

Hepcidin is a major iron exporter hormone in mammals. It interacts with its receptor, ferroportin in gastrointestinal tracts and reticuloendothelial systems. Degradation and internalizing process of ferroportin inhibits daily iron intake entering circulation from duodenum and traps intracellular storage iron. These processes create a hypoferremia state which results in functional iron deficiency anemia. Anemia brings clinical consequences such as a decrease of quality of life, deterioration of eGFR, increased cardiovascular events, increased mortality rate, and even increased economical burden. High inflammation state and other confounding factors (anemia, duration of dialysis) was seen in CKD patients on dialysis which lead to sample selection of non-dialysis patients.

MATERIALS AND METHODS

Study design: This was an analytic observational study with cross-sectional design in CKD patients in Nephrology Outpatient Clinic at Dr. Soetomo General Hospital, Surabaya, Indonesia. This research was ethically approved by Health Research Ethics Committee of Dr. Soetomo Hospital. Written informed consent was obtained from all subjects. Chronic kidney disease, diagnosed using KDIGO criteria, are abnormalities in kidney function or structure that have occurred for more than 3 months. The stage was determined based on the decrease in eGFR with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[1,2] Inclusion criterias for the samples were non-dialysis stadium III-V chronic kidney disease patients. Patients with history of cancer, hepatitis B, hepatitis C, liver cirrhosis, diabetes mellitus, chronic inflammation (HIV-AIDS, obesity, rheumatic disease, geriatric patients), diagnosed with acute infection (urinary tract infection, respiratory infection, pneumonia, gastroenteritis), under oral and intravenous iron or erythropoietin

stimulating agents (ESA) therapy, hormonal therapy, history of blood transfusion, alcoholism, absolute iron deficiency, gastrointestinal bleeding were excluded.[1]

Data collection: Consecutive sampling was done to complete an amount of 40 samples. Direct interview was done by the author and blood samples were taken by professional healthcare and sent to Dr. Soetomo General Hospital laboratory to be examined. Inflammations in this research were represented by serum CRP. Serum level of CRP was measured using *extended range C-reactive Protein* method with reagent *Siemens Flex® Reagent Cartridge C-reactive Protein Extended Range CAT No. RCRP-3749*, an in vitro diagnostic test with a particle enhanced turbidimetric immunoassay (PETIA) technique, meant to quantitatively measure CRP level in human serum. Serum level of hepcidin was circulating level of hepcidin-25 in blood. Serum hepcidin level was measured using the Enzyme Linked Immunosorbent Assay (ELISA) method. Serum were stored in a deep freezer at a temperature of -80°C until the hepcidin measurement was performed. The reagent used was DRG Hepcidin-25 (bioactive) ELISA from *CAT No EIA-5782,* an enzyme immunoassay for in-vitro quantitative examination for hepcidin-25 peptide in serum and plasma.[3,4]

Statistical analysis: All data was analysed by Statistical Package for the Social Sciences (SPSS ver 23). Data was delivered in the form of analytic statistics. Data analysis was provided in mean ± standard error of mean (SE). Correlation of serum hepcidin with CKD stage was calculated by Pearson parametric test if it had normal distribution or Spearman parametric test if the data distribution was not normal. It was said to be significant if the p-value is <0.05.

RESULTS AND DISCUSSION

Patient Characteristics

A total of forty subjects (67.5% male with mean age of 50.23 ± 1.04 years) were eligible for enrollment in this study. This study was done in Nephrology Outpatient Clinic, Dr Soetomo General Hospital, Surabaya, Indonesia within the period of 1 June 2018 - 31 August 2018. The results of demographic and clinical characteristics of this study subjects were described in Table 1 and Table 2.

Table 1. Demographic Characteristics

Category	Result	Frequency
Sex		
Male		27 (67.5%)
Female		13 (32.5%)
Age (years)		
Mean ± SE	**50.23 ± 1.04**	
Range (Min - Max)	**27–58**	
BMI (kg/m^2)		
Mean ± SE	**20.54 ± 0.58**	
Range (Min - Max)	**14.57 – 24.38**	
CKD Stage		
Stage 3		16 (40%)
Stage 4		9 (22.5%)
Stage 5		15 (37.5%)

Twenty seven of 40 subjects were male (67.5%), the youngest is 27 years old and the oldest is 58 years old with mean age of 50.23 years old (SE 1.04), mean of body mass index (BMI) was 22.54 (SE 0.57). Based on CKD stage, 16 patients (40%) of the total sample had stage 3 CKD. (Table 1)

Table 2. Clinical characteristics

Clinical Data	Level	Frequency
Hemoglobin (g/dL)		
Mean ± SE	**10.74 ± 0.36**	
Range (Min - Max)	**7.30 – 15.70**	
BUN (mg/dL)		
Mean ± SE	**39.98 ± 4.68**	
Range (Min - Max)	**11 – 125**	
Creatinine serum (mg/dL)		
Mean ± SE	**4.12 ± 0.54**	
Range (Min - Max)	**1.22 – 16.53**	
Comorbid disease		
Hypertension (n)		25 (62.5%)
Urinary tract stone (n)		**7 (17.5%)**
Others		8 (20.0%)

Table 3. CRP level characteristics

Stage	CRP level (mg/L)			p
	Mean ± SE	Median	Range (Min-Max)	
Stage 3	1.66 ± 0.41	0.25	0.10 – 9.40	0.036
Stage 4	2.68 ± 0.49	1.10	0.10 – 8.90	
Stage 5	6,01 ± 1.11	2.50	0.30 – 21.10	
Total	3.52 ± 0.81	1.10	0.10 – 21.10	

Table 4. Hepcidin level characteristics

Stage	Hepcidin level (ng/ml)			p
	Mean ± SE	Median	Range (Min - Max)	
Stage 3	27.24 ± 3.24	23.18	0.12 – 70.14	0.000
Stage 4	84.69 ± 12.23	55.65	1.08 – 254.87	
Stage 5	170.88 ± 15.81	200.00	9.96 – 352.42	
Total	94.03 ± 15.08	53.98	0.12 – 352.42	

Clinical Characteristics

Mean hemoglobin level of 40 subjects was 10.74 g/dL with SE of 0.36. Mean of blood urea nitrogen (BUN) levels was 39.98 mg/dL with SE of 4.68. Mean level of serum creatinine was 4.12 with SE of 0.54. The most frequent comorbid factor was hypertension (62.5%) (Table 2).

Distribution of CRP levels by CKD stage results were mean CRP 166 mg/L (range 0.10 - 9.40), 2.68 mg/L (range 0.10 - 8.90), 6.01 mg/L (range of 0.30 - 21.10) in stage 3, 4, and 5 respectively. The overall mean of CRP levels in this study was 3.52 mg/L with a range 0.10 - 21.10. (Table 3)

Distribution of hepcidin value by CKD stage were mean hepcidin 27.24 ng/ml (range 0.12 - 70.14), 84.69 ng/ml (range 1.08 - 254.87), and 170.88 ng/ml (range 9.96 - 352.42) in stage 3, 4, and 5 respectively. Hepcidin level overall mean was 94.03 ng/ml (range 0.12 - 352.42). (Table 4)

Significance and Strength of CRP and Hepcidin Value Correlation in Non-Dialysis CKD Patients.

Distribution of CRP and hepcidin level data were used to analyze the correlation between CRP and hepcidin in CKD patients. *Kolmogorov-Smirnov* normality test showed that the distribution of CRP and hepcidin level data is abnormal (both p-value < 0.05). Spearman correlation test was used to further analyze the correlation between CRP and hepcidin levels. (Table 5)

Table 5. Result of Spearman correlation test

Variable 1	Variable 2	r_s	p
CRP	Hepcidin	0.487	0.001

Analysis of CRP and hepcidin value showed an association with a positive correlation coefficient of 0.487. The correlation of CRP and hepcidin value in this study was significant, indicated by the p-value = 0.001. The meaning of this positive correlation coefficient showed a unidirectional relation, if the CRP level increase, the hepcidin level would be increased consequently.

DISCUSSION

Chronic kidney diseases progressed alongside complications such as anemia and its clinical consequences. One of the renal anemia pathophysiologies was disruption of iron metabolism, regulated by main iron exporter hormone, hepcidin. Chronic kidney disease patients were constantly in an inflammatory state, represented by increased of CRP. This inflammatory state results in the liver secreting hepcidin, which subsequently followed a decrease in iron circulation, thus resulting in functional iron deficiency. Inclusion of stage 3 to 5 CKD patients was based on earlier studies that stated complications of CKD, particularly anemia, were more commonly seen in stage 3 to 5 CKD patients. Non-dialysis CKD patients were selected to reduce confounding factor such as duration of dialysis in CKD patients.[5,6]

Most of the study subjects were men with a percentage of 67.5%, similar to studies by Toima, et al., Mercadel, et al, Elmenyawi, et al.[5,6,7] Higher male prevalence than female could be influenced by numerous factors like hypertension, hyperglycemia, lifestyle, kidney structure and hormonal differences.[8]

The mean age in this study was 50.23 ± 1.04 years old, similar to studies by Mercadel, et al,

Toima, et al., 2010, Elmenyawi, et al.[5,6,7] Aging process influenced CKD progression and lesser function was expected from older nephrons.[9]

Mean hemoglobin result in this study was 10.74 g/dL with SE of 0.36. The results of this study were similar to other studies by Toima, et al., Peters, et al., and Goyal, et al. [5,10,11] Mean results of BUN value were 39.98 mg/dL with SE of 4.8. BUN and creatinine serum levels found in this study were similar to study by Toima, et al.[5]

The most frequent comorbid disease in this study was hypertension, at 62.5% of the total subjects. Study by Toima, et al., Peters, et al., and Goyal, et al., also mentioned hypertension as the most frequent comorbid disease found in CKD patient. [5,10,11] Hypertension was the highest prevalent chronic disease in Indonesia based on 2013 RISKESDAS study.[12] Hypertension risk factors were age, race, family history, obesity, high sodium intake, and smoking. [8]

In this study, higher mean CRP levels was seen in more advanced CKD stage. The mean total CRP level in this study was 3.52 mg/L with a SE of 0.81. This was similar to previous studies by Toima et al. who found CRP levels of 6.0 mg/L with a standard deviation of 0.9, Elmenyawi et al. who found mean CRP level was 4.28 mg/L with a standard deviation of 3.7, Rasheed et al. who found CRP mean levels were 7.59 mg/L in all CKD stages, and. [5,7,13] Fluctuations in CRP levels may also have been due to the highest staging differences in the population study. In a study by Elmenyawi et al the most frequent CKD stage in the population was stage 3 with mean CRP level at 3.52 and 4.28 mg/L.[7] In the study of Toima et al. and Rasheed et al. the highest staging in the population was stage 5 and mean CRP levels were, 6.0 and 7.59 mg/L.[5,13] This study found that CRP level increased along with a decrease in eGFR, which were consistent with other studies. [14,16]

Total mean hepcidin found in this study was 94.03 ng/ml with SE of 15.08**.** While Toima, et al. found mean hepcidin level of 84 ng/mL with a standard deviation of 18.6, Goyal, et al. and Uehata, et al. found mean hepcidin levels of 65.0 ng/mL and 15.4 ng/mL respectively. [5, 11,17]

Analysis of CRP and hepcidin levels in non-dialysis CKD patients revealed a moderate to significant relationship (correlation coefficient 0.487; p-value 0.001). This result indicated that an increase in CRP levels would lead to a directly proportional increase of hepcidin value. The results of this study were in accordance with studies by Toima et al., Peters et al., and Lee et al., who inferred a positive relationship between CRP and hepcidin levels. [5,10,15] Toima et al. organized a study in Egypt regarding the importance of hepcidin role as a novel biomarker which reflected iron status in CKD patients and its relationship with CRP levels. Thirty CKD patients and 10 healthy subjects, used as controls, were enrolled. The result showed a correlation in CRP and hepcidin value with R of 0.68 ($p = 0.001$).[5] Patients who had iron or erythropoietin therapy for the previous 21 days were excluded. Inclusion of diabetes mellitus patients might lead to a strong correlation found in this study. This study used the same method in CRP and hepcidin level measurement as Toima, et al.[5] Peters, et al. conducted an observational cross-sectional study of factors affecting hepcidin in 83 non-dialysis CKD patients and 48 dialysis CKD patients in the Netherlands. There was a weak positive relationship ($r=0.21$, $p <0.001$) between CRP and hepcidin levels which was probably related to inclusion of patients who were under erythropoietin therapy. The method used to measure CRP level was the same as in our study, but a different method (light chromatography mass spectrometry / LC-MS) was used in measuring hepcidin level.[10] Lee, et al. in Korea analysed whether hepcidin was a novel uremic toxin using multivariate analysis of various variables affecting hepcidin in 2090 non-dialysis CKD patients. They found a positive correlation between CRP and hepcidin with $r=0.23$ ($p <0.001$). Patients with intravenous iron, oral iron, and erythropoietin therapy were not excluded. These factors might have played a role as confounding factors to the weak correlation. This study used the same method in CRP and hepcidin level measurement as Lee, et al.[15]

The result of the Uehata et al. study result was different compared to this study. That study

included 505 samples of non-dialysis CKD patients and found no association between CRP and hepcidin levels ($r = 0.03$ and $p = 0.4$). Patients with liver cirrhosis were not excluded, while liver cirrhosis can induce negative feedback on hepcidin and CRP. The Level of CRP was measured using immunoagglutination detection method and hepcidin level was determined using LC-MS.[17] Another study by Goyal, et al. in India analyzed the relation between CRP and hepcidin levels in 100 non-dialysis CKD patients. They found similar results with this study, the correlation coefficient of 0.0001 and $p = 0.896$ between CRP and hepcidin levels. Patients having oral iron therapy were not excluded, while oral iron could induce positive feedback on hepcidin. C-reactive protein levels were measured using different EIA kits which could influence the absence of association of CRP and hepcidin.[11] Study by Wagner, et al., which analysed predictive factors of mortality in patients with non-dialysis CKD patients, showed contradiction to this study by stating that CRP level was not associated with hepcidin levels (correlation coefficient of 0.01 and $p < 0.001$). Their case control study stated that CRP and hepcidin (measured hepcidin using RIA method) were influenced by factors that change over time. The mean hemoglobin in their study was higher than this study (13.1 g/dL) while anemia could induce negative feedback on hepcidin.[18] These factors might have played a role in the absence of a correlation. Another study contrasting this study results was Macdougall et al. in the Netherlands who used a random sampling system and including patients with erythropoietin therapy which caused positive feedback on hepcidin. [19]

There were differences in the results of this study compared to previous studies. Different methods in measuring CRP and hepcidin levels could have contributed to this result. In earlier studies, the CRP level was measured using the immunoturbidimetric assay, immunoagglutination, or EIA method.[5,10,15] In previous studies, hepcidin level was measured by ELISA and LC-MS methods.[20] Studies conducted by Mercadel, et al., Macdougall, et al. used different methods to determine hepcidin levels.[6,21] Hepcidin could be measured using RIA, ELISA, and mass spectrometry-based methods.[22] Measurement using RIA detects hepcidin-25 greater than actual condition. Measuring hepcidin-25 using ELISA were accurate and cheap.[3,23] Mass spectrometry-based was indeed more accurate but not practical, requiring more instruments and too expensive. Besides differences in measurement methods, there were also differences in this study subjects' characteristics compared to previous studies.[24] History of erythropoietin therapy, a history of blood transfusion, iron therapy, diabetes mellitus, and other factors influencing CRP and hepcidin level were not excluded in previous studies, whereas it could have affected the results.[6,11,14,25]

This was a novel study of hepcidin and inflammation marker in CKD in Surabaya, although we were aware that the small number of subjects might interfere with the study results. Study with a larger, more homogenous sample, more markers of inflammation and iron might be needed in the future.

CONCLUSION

A significant positive correlation with $r_s = 0.487$, $p = 0.001$) was found between CRP and hepcidin levels in non-dialysis CKD patients. If there was an increase in serum CRP levels in non-dialysis CKD patients there was a tendency for an increase in serum hepcidin levels.

CONFLICT OF INTEREST

There is no conflict of interest of this paper.

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Case Report

Gastric Perforation Associated with Candidiasis and NSAIDS

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ABSTRACT

Invasive candidiasis is an important health-care-associated fungal infection. Candida is often described as an opportunistic pathogen. It is commensal flora in the gastrointestinal tract. Invasive candidiasis can happen usually because of a consequence of increased or abnormal colonization together with a local or generalized defect in host defenses. Candidiasis can occur in patients with HIV, therapy with a broad-spectrum antibiotic, transplant organ, and immunocompromised. Most cases of gastric perforation occur as complications of Peptic Ulcer Disease (PUD), Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) and gastric neoplasms, but candidiasis as a cause of gastric perforation is very rare. This study aims to reveal the correlation between gastric perforation with candidiasis and NSAIDs. It was reported that a 57-year-old East Java Indonesian female presented with severe epigastric pain, generalized peritonitis, fever, nausea also vomiting and had a history of NSAIDs used for five years. The patient was taken to the general surgery of Dr. Sutomo Surabaya Hospital and performed exploratory laparotomy. A gastric perforation was discovered in the antrum. Microbiology culture examination from biopsy gastric tissue revealed an intense fungal growth from sabouraudagar medium and there is no other microorganism that grew in aerobic culture. Candida albicans was identified by VITEK® 2 COMPACT. Histopathological examination from biopsy gastric tissue was performed by Olympus CX-21 microscope, showed invasive Candida albicans consisting of numerous fungal yeasts and pseudohyphae invading and destroying the gastric wall. The patient was subsequently treated with fluconazole anti-fungal and discharge home after nine days postoperative period in good condition. From this result, we suggest using an antifungal treatment for patients who use NSAIDs for long periods to prevent candidiasis.

Keywords: *Candida albicans, fluconazole, gastric perforation, histopathological, NSAIDs, peritonitis*

ABSTRAK

Kandidiasis invasif adalah infeksi jamur penting yang berhubungan dengan perawatan kesehatan. Candida sering digambarkan sebagai patogen oportunistik. Candida merupakan florakomensal dalam saluran pencernaan. Kandidiasis invasif dapat terjadi sebagai konsekuensi dari peningkatan atau kolonisasi abnormal Candida bersama dengan kurangnya sistem imun lokal atau umum dalam tubuh host. Kandidiasis dapat terjadi pada pasien dengan HIV, terapi dengan antibiotik spektrum-luas, transplantasi organ, dan immunocompromised. Sebagian besar kasus perforasi gastric terjadi sebagai komplikasi dari Penyakit Ulkus Peptikum (PUD), Obat Antiinflamasi Nonsteroid (NSAID) dan neoplasma lambung, tetapi kandidiasis sebagai penyebab perforasi gastric sangat jarang terjadi. Tujuan kami dalam laporan kasus ini adalah mengungkapkan korelasi antara perforasi gastric dengan kandidiasis dan pemakaian NSAIDs. Kami melaporkan seorang wanita Indonesia Jawa Timur berusia 57 tahun yang mengalami nyeri epigastrium parah, peritonitis menyeluruh, demam, mual juga muntah dan memiliki riwayat penggunaan NSAID selama lima tahun. Pasien dirawat di bagian bedah umum Rumah Sakit Dr. Sutomo Surabaya dan dilakukan laparatomi eksplorasi. Perforasi gastric ditemukan di bagian antrum. Pemeriksaan kultur mikrobiologi dari jaringan biopsi menunjukkan pertumbuhan jamur yang banyak pada media sabouraud dextrose agar dan tidak ada mikroorganisme lain yang tumbuh di kultur aerob. Identifikasi di lanjutkan

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dengan menggunakan VITEK® 2 COMPACT hasilnya adalah Candida albicans. Pemeriksaan histopatologis dari jaringan biopsi dilakukan dengan mikroskop Olympus CX-21, menunjukkan Candida albicans invasif yang terdiri dari sejumlah yeast dan pseudohypae yang menyerang dan menghancurkan dinding gastric. Pasien kemudian diobati dengan flukonazol anti jamur dan pulang ke rumah setelah sembilan hari periode pasca operasi dalam kondisi baik. Dari hasil case report ini, kami menyarankan penggunaan pengobatan antijamur untuk pasien yang menggunakan NSAIDs dalam jangka waktu lama untuk mencegah kandidiasis.

Kata kunci: *Candida albicans, fluconazole, gastric perforasi, histopatological, NSAIDs, peritonitis*

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INTRODUCTION

Invasive candidiasis is an important health-care-associated fungal infection that can be caused by several *Candida* spp.; the most common species is *Candida albicans*. The spectrum of disease of invasive candidiasis ranges from minimally symptomatic an candidiasis to fulminant sepsis with associated mortality exceeding 70%.[1] *Candida albicans* are common commensal organisms in the skin and gut microbiota, and disruptions in the cutaneous and gastrointestinal barriers (for example, owing to gastric perforation) that promote invasive disease.1 The common causations of gastric perforation are Peptic Ulcer Disease (PUD), Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), gastric neoplasms and strong antacid consumption, but it is very rare due to candidiasis.1 Candidiasis can occur in patients with HIV, transplant organs, therapy with broad-spectrum antibiotics and immunocompromised.[2–4] This case is reportedly rare because in literature there were only two cases that have been published.[5, 6] This patient is immunocompetent without other histories of chronic illness, except gout arthritis. The patient had been using NSAIDs to relieve gout pain for almost 5 years. Here, we report the first history associated with invasive candidiasis and review the relevant literature.

CASE REPORT

A 57-years-old woman came to a emergency unit of RSUD Dr Sutomo hospital with a history of abdominal pain since three days earlier.

The pain was epigastric; severe, deep-seated, progressive, non-radiating, not relieved by food intake and vomiting and not associated with diarrhea. Her condition worsened 1 day to presentation with generalized abdominal pain and abdominal wall rigidity. There was abdominal distension, no change in bowel habit, no blood in the stool, no associated fever, weight loss, alcohol binge or trauma. There was no history of changes in pattern of bowel movements, diabetes mellitus, hypertension or Peptic Ulcer Disease (PUD), except the history of using NSAIDs for almost 5 years due to gout arthritis. On clinical examination, an elderly woman was conscious and alert but in obvious painful distress. She was a febrile, not pale, anicteric, not cyanosed, no pedal edema and no dehydrated. On physical examination, blood pressure was 115/80 mmhg, pulse rate 100x beats/minute, respiratory rate 22 x breath/minute, temperature 37.6ºC. The inspection abdomen showed distended, tense, bowel sound decreased, and defense muscular on all abdominal regions. The examination of the liver, spleen, kidneys, and rectum were normal. Diagnosed as peritonitis was made. For initial treatment in the emergency unit, she was given metronidazole, dexamethasone, metamizole, ranitidine and tutofusin as fluid hydration. The preoperative laboratory examinations showed hemoglobin concentration 6.3 g/dl, leucocyte 10.310/ul, hematocrits 23.8%, thrombocyte 457.000/ul, creatinine 1,33 mg/dl, SGOT 30 U/L, SGPT 17 U/L, blood sugar 109 mg/dl, albumin 2.7 g/dl, serum electrolyte concentration sodium (Na+) 137 mmol/L, potassium (K+) 3.4 mmol/L, chloride (Cl−) 101 mmol/L, and non-reactive

Hbsag examination. Thorax photo showed lung inflammation with minimal right pleural effusion. BOF (Bluch Over Sich) examination showed air-fluid level. The patient was measured 1.57 m tall, weighed 90 kg, and had a body mass index of 36.5 kg/m2. An emergency exploratory laparotomy was performed and found a hole size 2 x 1 cm by 2 x 3 cm of gastric perforation covered with fibrinous exudate within the peritoneum. Gross macroscopy gastric tissue was irregular, weight < 1 gram, greyish-white color, and solid consistency. Microscopic histopathological examination showed pieces of tissue with erosive and ulcerative surfaces coated in necrotic areas and inflammation of neutrophils, lymphocytes and histiocytes. In the muscular layer until serious, fat was obtained in lymphocyte inflammation cells, histiocytes and plasma cells. Gram (Figure 1) and KOH staining from gastric tissue showed yeast and fungal hyphae. There were no *Helicobacter pylori*-like microorganisms. Microbiology culture used gastric tissue and gastric liquid 3cc specimens. The liquid looked red. Both specimens examined aerobic, anaerobic and Sabouraud Dextrose Agar/fungal culture. Aerobic culture did on Blood Agar Plate, Chocolate and Mac Conkey Agar. They were incubated in 37ºC for 18-24 hours. Anaerobic culture did on Brucella Agar and Cooked Meat Medium, incubated in an anaerobic jar for 48 hours. Fungal culture on Sauboroud Dextrose Agar was incubated in two temperatures 25ºC and 37ºC to differentiate from mold, grew creamy colonies. Lactophenol Cotton Blue staining was also performed to identify yeast from the colony (Figure 2). Three cultures (aerobic, anaerobic and fungal) revealed *Candida* colonies. The result identification using VITEK® 2 COMPACT was *Candida albicans*. The histopathological examination was performed using Olympus CX- 21 microscope. It showed numerous yeast and hyphae which invaded tissue gastric.

RESULTS

Gastric tissue was put into formalin and then it was compacted with wax called *paraffin*

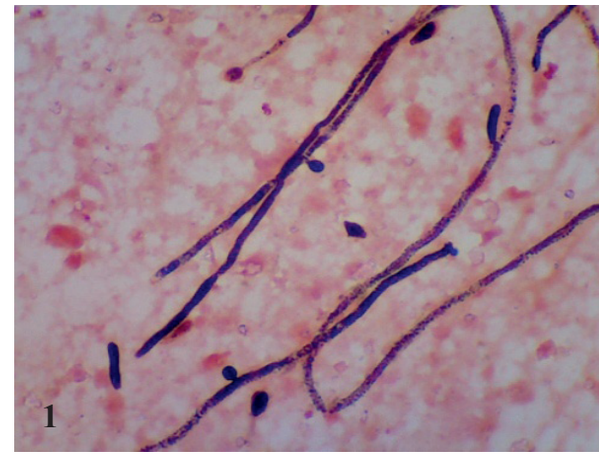

Figure 1. Gram staining from direct gastric tissue specimen, showed yeast and pseudohyphae.

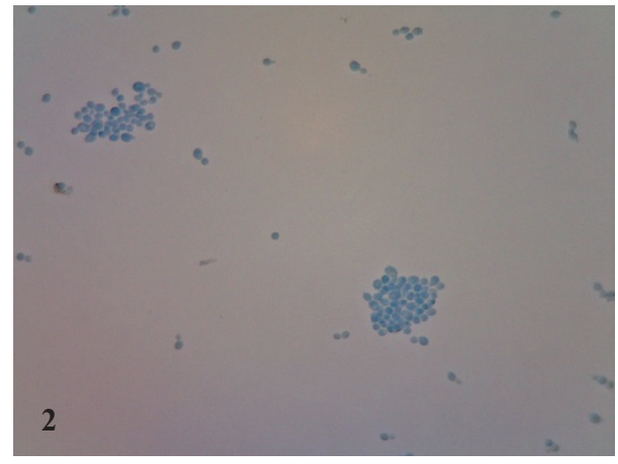

Figure 2. Lactophenol Cotton Blue staining from colony, showed fungal yeast

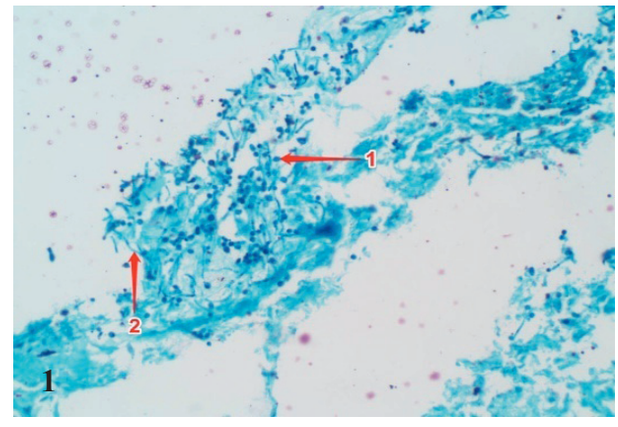

Figure 1. Gram Twort Staining 400x arrow (1) Yeast (2) Hyphae

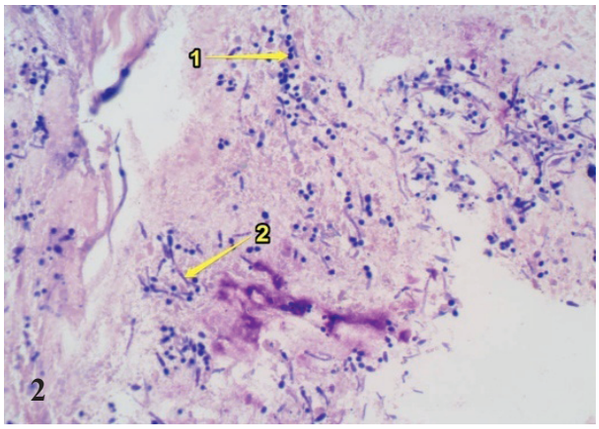

Figure 2. Giemsa staining 400x arrow (1) Yeast (2) Hyphae

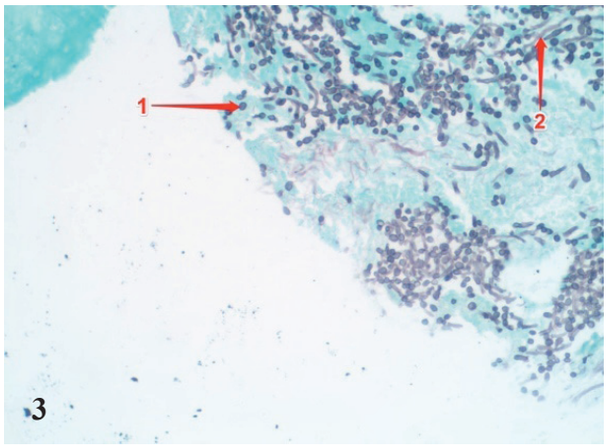

Figure 3. GMS (Gomori Methenamine Silver) staining 400x arrow (1) Yeast (2) Hyphae

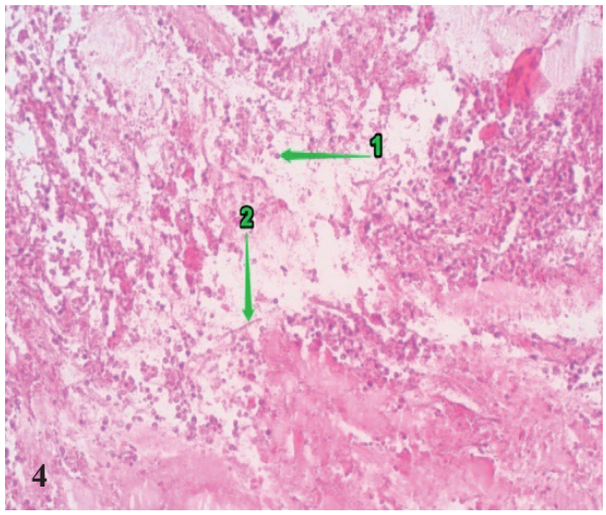

Figure 4. HE(Hematoxyline Eosin) staining 400x arrow (1) Yeast (2) Hyphae

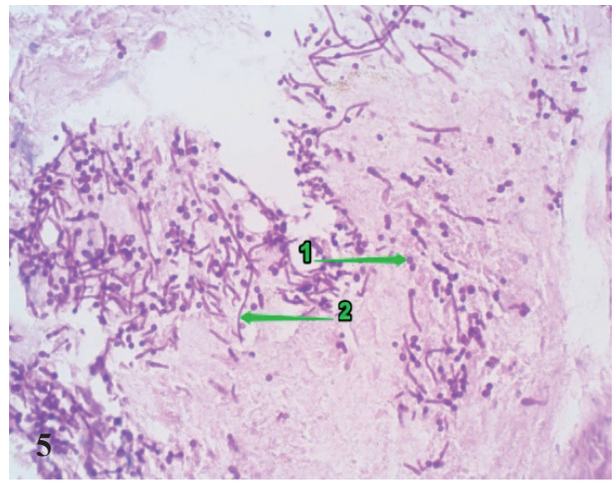

Figure 5. PAS (Periodic Acid Schiff) staining 400x arrow (1) Yeast (2) Hyphae

Block. After that, we used microtome to slice and staining it with Gram Twort (Figure 1), Giemsa (figure 2), GMS (Gomori Methenamine Silver) (Figure 3), HE (Hematoxyline Eosin) (Figure 4) and PAS (Periodic Acid Schiff) (Figure 5).

Therapies treated for this patient were fluconazole anti-fungal, metronidazole, levofloxacin, ranitidine, and metamizole. Patient was hospitalized for nine days and discharged in a good condition.

DISCUSSION

Invasive candidiasis are frequently associated with high morbidity rates.[5] *C. albicans* is a fungus that can exist in three morphotypes: budding yeast, pseudohyphae and true hyphae.[2–4, 7] Fungal yeast-mycelium dimorphism is of interest because of the economic and medical importance of dimorphic fungi and because these organisms may serve as a model for studying differentiation.[2–4, 7] These yeasts often cause opportunistic fungal infection in human patients who have become immune-compromised by anticancer therapy, HIV infection, organ transplantation or therapy with broad-spectrum antibiotics, leading to severe fungal infection.[1, 7–9] Candidiasis is a rare cause of gastric perforation. Using Nsaids for long period can make *Candida* colonize and invade gastric tissue causing perforation.[10–13]

Recent studies suggest that *Candida albicans* colonization is associated with several gastrointestinal inflammatory disorders and is also responsible for the delay in ulcer healing.[11, 12, 14] In animal models such as mice, *C. albicans* colonization of the stomach results in the expansion of regulatory T cell populations[15] and regulatory T cells are associated with immunosuppressive effects on the host.[12] In the setting of inflammation, *Candida* colonization appears to exacerbate inflammation.[1] NSAIDs toxicity on the gastroduodenal mucosa is well known, as non-invasive procedures capsule endoscopy for diagnosis of NSAIDs enteropathy syndrome is extensively used.[23] The toxicity of NSAIDs on gastrointestinal mucosa is due to cyclooxygenase (COX) inhibition. COX is an enzyme responsible for the formation of prostanoids, including thromboxane and prostaglandins. The COX inhibition can provide relief from the symptoms of inflammation and pain, but it also reduces prostaglandin 2 (PGE2) synthesis.[16–19, 21] The PGE2 reduction can make the mucosal hypovascularization, hypomucus formation and unbalances the immune tolerance state, as PGE2 produced by resident stromal cells in the small intestine. Lamina propria is in part responsible for the immune tolerance for bacteria and other microorganisms that colonize the digestive tract such as *C. albicans*.[16, 18] This colonization could be modulated by NSAIDs and the viability of yeast cells in the digestive tract as nitric oxide produced after 18–24 h administrated with NSAIDs.[24] Other mechanisms show that NSAIDs inhibit mitochondrial activity b-oxidation and consequently growth of yeast cells.[21].

Documented studies explain that enterobacterial translocations are considered as the first step for necroinflammatory lesions detected in the pathogenesis of indomethacin-induced intestinal on the digestive mucosa lesions.[4,9,10] In immunocompromised patients, colonization with *C. albicans* is associated with increased levels of proinflammatory cytokines (IL 17).[22] It may be assumed that colonization with *C. albicans* may enhance the necroinflammatory lesions produced by NSAIDs on digestive tract mucosa.[23–25] Some studies have found that *C. albicans* aggravates duodenal ulcer perforation in the experimental model of cysteamine-induced duodenal ulcer perforation, granulocytic infiltration and number of eosinophils infiltrating the ulcer base being significantly greater.[25] Other researchers specify that *C. albicans* colonization is mediated through prostaglandins which this fungus can synthesize these molecules.[18, 19]

CONCLUSION

The interaction between NSAIDs and *Candida albicans* colonization in the digestive tract needs some consideration because it has potential damage to the gastrointestinal.[11] Their interaction on the same organism may have clinical relevance since the use of these anti-inflammatory drugs is increased and associated with both gastric and enteral severe toxicity, in part by decreasing the immune gut tolerance.[1, 11, 12] Subsequently, *C. albicans* may become pathogenic in these conditions. It may be assumed that colonization with *C. albicans* may enhance the necroinflammatory lesions produced by NSAIDs on digestive tract mucosa.[9,22,23] It is suggested that for patients who use NSAIDs for long periods should be given antifungal treatment to prevent candidiasis.

CONFLICT INTEREST

There is no conflict interest of this paper.

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Case Report

Disseminated Tuberculosis Mimicking Lung Cancer with Multiple Bone Metastasis: A Case Report

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ABSTRACT

Tuberculosis (TB) is a contagious infectious disease caused by Mycobacterium tuberculosis (Mtb) of which attacking various organs particularly the lungs. Tuberculosis can occur together with malignancy or manifest as malignancy. Lung tuberculosis may appear in a variety of clinical and radiological manifestations caused by other diseases including tumors. These tumors are called pseudo-tumors. TB pseudo-tumor is a rare manifestation that can occur in immunocompetent patients in both primary and post-primary TB. The clinical presentation of TB pseudo-tumor is nonspecific and the clinical suspicion must be increased to diagnose related diseases. Radiological features can also be challenging to be distinguished from actual tumors. The classic manifestations of pulmonary TB are generally easy to diagnose due to the distinctive clinical and radiological characteristics nonetheless some pulmonary TB symptoms are also often found in patients with lung cancer. Infection patients resemble malignancies most were asymptomatic (> 27%) and the remaining 27% showed symptoms that varied with the average symptoms experienced about 1 month earlier. Clinical presentations that require a lot of misdiagnosis result in delayed treatment and unnecessary procedures. Establishing a diagnosis in cases of tuberculosis that causes malignancy is very important since the management and outcomes of the infection and malignancy process are quite different. Consequently we report a 24-year-old man with tuberculosis possible lung cancer with multiple bone metastase. Extrapulmonary tuberculosis which attacks bones and joints constitutes 10% to 20% of all TB cases. The location of bone and joint TB generally develops in the lumbar or thoracic vertebrae.

Keywords: *tuberculosis, pseudo-tumour, lung cancer, bone metastasis, extrapulmonary tuberculosis.*

ABSTRAK

Tuberkulosis (TB) merupakan penyakit infeksi menular yang disebabkan oleh Mycobacterium tuberculosis (Mtb) dan dapat menyerang berbagai organ terutama paru.Tuberkulosis dapat terjadi bersamaan dengan keganasan ataupun bermanifestasi menyerupai keganasan. Tuberkulosis paru dapat muncul dalam berbagai manifestasi klinis dan radiologis yang menyerupai penyakit lain termasuk tumor. Lesi berbentuk massa dan menyerupai tumor ini disebut sebagai pseudotumor. Pseudotumor TB merupakan manifestasi langka yang dapat terjadi pada pasien imunokompeten baik pada TB primer maupun post primer. Presentasi klinis dari pseudotumor TB tidak spesifik dan kecurigaan klinis harus ditingkatkan untuk mendiagnosis adanya infeksi tersebut. Gambaran radiologis juga sulit dibedakan dengan tumor sebenarnya. Manifestasi klasik TB paru secara umum mudah didiagnosis karena menunjukkan karakteristik klinis dan radiologis yang khas namun beberapa gejala TB paru juga sering ditemukan pada penderita kanker paru. Pasien infeksi menyerupai keganasan sebagian besar asimptomatis (>27%) dan 27% sisanya menunjukkan gejala yang bervariasidengan rata-rata gejala yang dialami muncul sekitar 1 bulan sebelumnya. Presentasi klinis yang bervariasi mengakibatkan misdiagnosis yang berakibat pada keterlambatan pengobatan dan prosedur diagnostik yang tidak perlu. Penegakan diagnosis pada kasus tuberkulosis yang menyerupai keganasan merupakan hal yang sangat penting karena manajemen dan luaran dari proses infeksi dan keganasan sangat berbeda. Berikut kami laporkan

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seorang laki-laki berusia 24 tahun dengan tuberkulosis yang menyerupai kanker paru dengan metastasis tulang multipel. Tuberkulosis ekstraparu yang menyerang tulang dan sendi merupakan 10% hingga 20% dari semua kasus TB. Lokasi TB tulang dan sendi umumnya berkembang pada vertebra regio lumbal atau toraks.

***Kata kunci**: tuberkulosis, pseudotumor, kanker paru, metastasis tulang, tuberkulosis ekstraparu.*

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INTRODUCTION

Tuberculosis (TB) is a contagious infectious disease caused by Mycobacterium tuberculosis (Mtb), which attacking various organs affected by the lungs.[1] This disease causes 1 in 10 patients in the world and is a cause of disease for patients with Human Immunodeficiency Virus / Acquired Immune Deficiency Syndrome (HIV/AIDS).[2] The highest TB morbidity and mortality are suspended within middle and low revenue countries due to poverty, malnutrition, and low endurance.[3] Indonesia with a population of 264 million people is of the opinion that the number of funds with the highest TB in the world. The World Health Organization (WHO) reports as many as 842,000 people suffering from TB in Indonesia and 116,000 who have died caused by TB.[2]

Lung tuberculosis may appear in a variety of clinical and radiological manifestations caused by other diseases including tumors.[4] These tumors are called pseudo-tumors. TB pseudo-tumor is a rare manifestation that can occur in immunocompetent patients in both primary and post-primary TB. Lesions removed from the parenchyma were higher than those in enlarged lymph nodes.[5] The clinical presentation of TB pseudo-tumor is nonspecific and the clinical suspicion must be increased to diagnose related diseases. Radiological features can also be challenging to be distinguished from actual tumors. This may cause late in diagnosis and carried out morbidity among patients.

CASE REPORT

The patient's general condition is quite frail, with blood pressure of 80/50mmHg, tachycardia, febrile, and pain scale of Wong Baker is 4, anemic conjunctiva and has a mass with Ø ± 4cm in the parietal region of the region. Reddish wound on the sternum region measuring 2x5cm in size. Lung examination revealed decreased fremitus palpation and faint percussion in 1/3 of the right hemi thorax accompanied by decreased breath sounds. There are 8x9cm pressure sores in the sacrum region, 2x3cm and 3x4cm sizes in the calcaneus regions dextra and sinistra with slough and necrotic tissue. There is inferior paraplegic, hypesthesia as high as the ThX segment, as well as urinary retention.

Laboratory results showed leukocytosis (WBC 19,130), granulocytosis (91.8% neutrophils), increased LDH (253), and CEA 2.93ng/ml (cut off 5ng/ml). The chest radiograph shows an inhomogeneous opacity at 1/3 of the extra hemi thorax (Figure 1). A thoracolumbar MRI (Fig. 7A) shows destruction of the lamina, spinous process, VC pedicle 6.7 accompanied by soft tissue mass bulging and surrounding necrotic areas. Soft tissue mass bulging was also seen in anterior Vth 1-3, destruction of the VTh12 corpus with

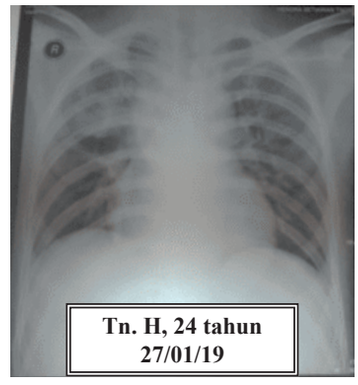

Figure 1. Result of CT scan thorax during in-patient

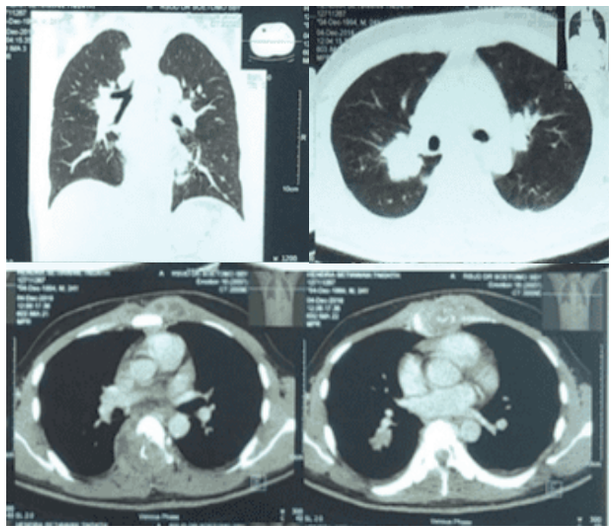

Figure 2. Right lung central mass findings that show contrast enhancement on CT scan thorax result

anterior to left lateral paravertebral soft tissue, and destruction of the corpus sternum and spiculated mass of ± 4.9 x 3.5 cm in the middle of the right lung. These findings direct the suspicion of a right lung central mass and the process of metastasis.

The CT scan thorax result with contrast supports the image of malignancy by finding (Figure 2) solid lesions (36 HU), indistinct borders, spiculated edges, size ± 3.5 x 2.6 x 2.8 cm in the center of the right lung with contrast enhancement (77 HU), visible Abutting lesions of the superior and inferior branches of the right bronchus.

Result of MSCT head showed slight hyper dense (47HU) lesions in the left parietal subdural region with a thickness of ± 1.6cm, 4.7x4.4cm wide with a contrast enhancement rim (72HU)

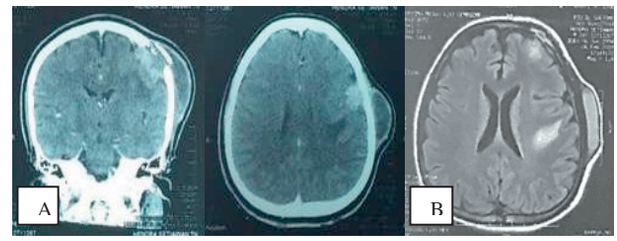

Figure 3. A) Result of head MSCT, B) Head MRI, both showing solid lesions in the left periental subdural region with *rim contrast enhancement*

which decomposed the left parietal calvarias and formed soft tissue bulging into the extra cranial. Head contrast MRI shows solid, extra axial, firm boundary, irregular margins in the left parietal lobe convex of 4.4x5.1x6.4cm with contrast enhancement rim and perifocal edema around it. MR spectroscopy showed an increase in intralesional Ch/Cr ratio, increased lipid-lactate perillion. Perfusion MR showed an increase in rCBV that did not return to baseline. This feature can be a metastatic process (when compared with previous CT head scans of enlarged lesions and perifocal edema extending), retention cysts in the left frontal sinus (Figure 3).

Furthermore, the patient underwent fiber optic bronchoscopy (FOB) as a diagnostic step. The FOB results showed intraluminal mass in the intermediate trunk wall and infiltrative mass in the superior lobe bronchial lumen with BAL cytology results in the right and sinistra, brushing, aspiration biopsy, and forceps biopsy showed no signs of malignancy. Then followed by CT-guiding FNAB examination on the right lung

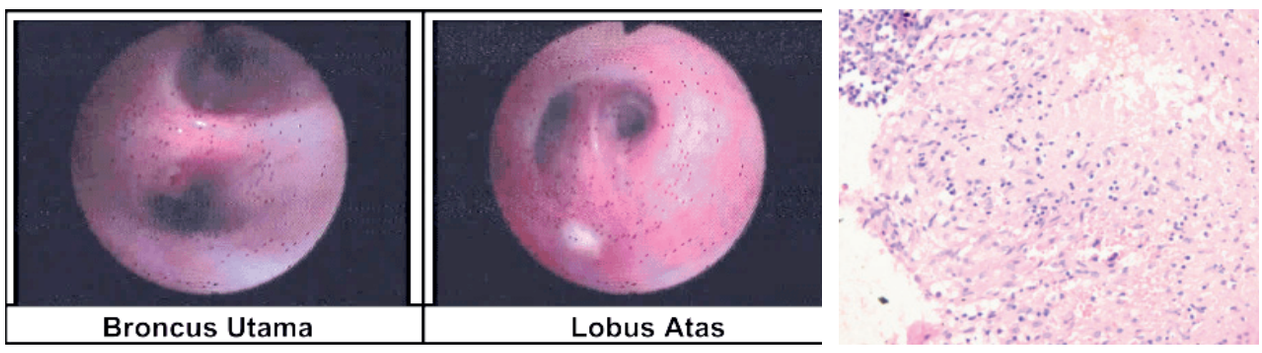

Figure 4. FOB of Mr. H (right) appears intralumen mass of the right superior lobe bronchus; CT-guiding FNAB results (left) show granulomatic inflammation

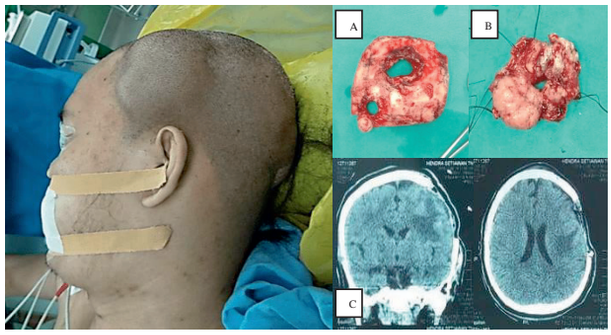

Figure 5. Clinical conditions of the left pariental region of the tumor on the patient's head. A) Post resection calvaria tissue, B) Resected tumor tissue, C) CT scan of the head post-operative evaluation

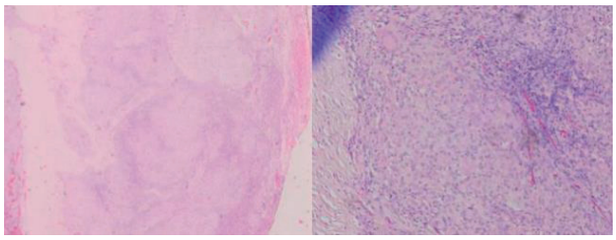

Figure 6. Histopathological examination of the head tumor showed granulomatic inflammation

obtained granulomatic inflammation according to tuberculosis (Figure 4). Patients were given Anti Tuberculosis (OAT) drugs exjuvantibus category I and then underwent open biopsy of the sternum lesion. Exploration of the operative field revealed a superficial abscess and the drainage of the abscess was carried out with the results of watercolor and culture of the pus sternum without the presence of acid fast bacili (AFB) germs and aerobic germ growth. Pus cytology was not found malignant cells in the same specimen.

Surgical excision of head tumors in patients is performed as a diagnostic and therapeutic step because the results of previous examinations are not conclusive. Exploration of the field of surgery in the left parietal region obtained sub cutis capsules filled with liquid likely cheese porridge under duramater, continue with tumor resection, duraplasty and drainage. Bone is not returned for the purpose of decompression. After the patient's surgery a CT scan of the head is evaluated (Figure 5).

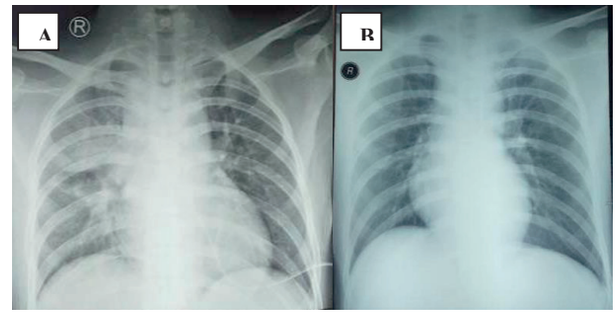

Figure 7. Result of thorax images. A) Before diagnosed with TB, B) 5 months after TB treatment.

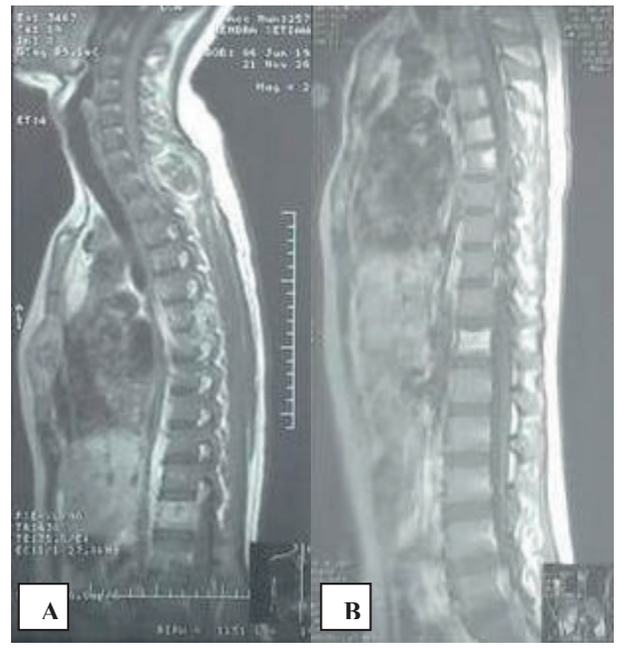

Figure 8. Thoracolumber MRI: A) Before TB diagnosis, B) 5 months after TB treatment

Tumor resection tissue was examined by rapid molecular tests, AFB smear, culture and Mtb sensitivity test, and histopathology. Molecular rapid test results of pus os parietal obtained low detected Mtb, rifampicin resistant not detected; AFB smear scanty positive; culture and sensitivity test of Mtb negative. Histopathological examination results of calvarias and duramater bone tissue surgery showed granulomatous inflammation and AFB bacteria according to tuberculosis (Figure 6).

Patients also underwent 3 methods of HIV testing with a negative result and a T helper lymphocyte count (absolute CD4) with a result

of 503 cells/μL (normal range 410-1590 cells/μL). The patient was formerly diagnosed with TB and given a Category I OAT plus streptomycin for 2 months then continued with a 10-month follow-up phase.

Evaluation of treatment showed clinical improvements such as weight gain, loss of cough as well as chest and back pain. The postoperative wound dried up and there was sensory and motor repair of the inferior limb. Chest radiographs in the fifth month of treatment showed a picture of inhomogeneous opacity disappearing (Figure 7). Results of thoracolumbar MRI appear soft cervical region soft tissue bulging disappears even though there is still a picture of corpus VTh5, 6, 7 destruction, right-sided transverse process, thoracic cord compression level VTh5-7 accompanied by cord edema and skip lesion in VTh12, compared to previous MRI improvement (Figure 8).

DISCUSSION

Tuberculosis (TB) is a contagious infectious disease caused by Mtb and can attack various organs especially the lungs.[1] As many as ⅓ of the world's population is infected with Mtb, however only 5% to 10% of the population are at risk of developing into TB within 1 or 2 years after infection (primary TB) or after (post-primary TB).[6] TB diagnosis is determined based on objections, history taking, clinical examination, laboratory, and other support. Bacteriological laboratory examination (direct microscopic sputum examination, rapid molecular examination, and culture), radiological examination (x-ray, CT scan, and MRI), histopathological examination, drug sensitivity test, and serological tests also play an important role in the diagnosis of TB.[7]

Tuberculosis manifestations are vary which can sometimes be a challenge for medical doctors to identify this disease.[8] The classic manifestations of pulmonary TB are generally easy to diagnose due to the distinctive clinical and radiological characteristics nonetheless some pulmonary TB symptoms are also often found in patients with lung cancer.[9] Infection patients resemble malignancies most were asymptomatic (> 27%) and the remaining 27% showed symptoms that varied with the average symptoms experienced about 1 month earlier.[4]

The most common symptoms are coughing, chest pain, and fever. Other symptoms that are less common include weight loss, dyspnea, fatigue, malaise, hemoptysis, and night sweats.[9,10] In this case, the patient presents in a seizure condition, history taking does not show any typical clinical symptoms of pulmonary TB. Initial complaints were severe chest pain when coughing and weakness of both limbs to paralysis so they could not be distinguished from malignancy.

Some patients with pseudo tumor are also accompanied by comorbidities such as old age, diabetes mellitus, alcoholics, and chronic obstructive pulmonary disease, or patients with immunocompromised conditions such as in people with HIV / AIDS or those who undergo such organ transplants.[9,11] These immunocompromised conditions are not found in patient. The incidence of idiopathic lymphocytopenia (low CD4 + T cell levels) is also not found in patients although some studies have shown that low levels can be associated with severe TB infection that is spread.[11]

Data on extrapulmonary TB cases is limited, although the prevalence of extrapulmonary TB cases is reported as 4% to 48% in various countries.[12] Extrapulmonary TB symptoms were according to the location of the organ being attacked. Nonspecific symptoms such as anorexia, fatigue, myalgia, headaches, and neck stiffness, disturbance of consciousness, and behavior changes for about 2-8 weeks can occur in meningitis TB.[7] Patients in the emergency room with complaints of seizures that can also occur in patients with tumor metastases to the brain. The process of metastasis causes disruption of the blood-brain barrier that leads to cerebral edema and increased intracranial pressure.[10]

Tumor markers are one of the diagnostic modalities that are widely used to get rid of cancer from other benign diseases. High levels of tumor markers indicate the presence of cancer. Carcinoembryonic antigen (CEA), cancer antigen (CA) 125, CYFRA 21-1, and SCCAg are tumor

markers that can be used in the diagnosis of non-small cell carcinoma lung cancer (NSCLC), while pro-gastrin-releasing peptide and neuron-specific enolase (NSE) is commonly used in the diagnosis of small cell carcinoma lung cancer (SCLC). However, there are reports that abnormal tumor marker results can also occur in benign diseases including pulmonary TB.[9] The results of CEA in patients are within normal limits so it does not support the direction of malignancy, especially NSCLC.

Radiological features of the chest X-ray of most TB patients resembling malignancy are similar to cancer even though about 20% of cases still allow an infection to be described. The most common radiological features encountered are solitary round nodules (46%), cavities, and lobulated masses. The location of the lesion varies and there is no specific dominant location.[9] Chest X-ray of the patient shows inhomogeneous opacity with irregular edges. This picture does not show the typical characteristics of pulmonary TB so it leads to misdiagnosis.

Malignancy images on CT scans show a variety of morphologies including speculated, lobulated edges, blood vessel convergence signs, pleural indentation, ground-glass opacity, and thick-walled and irregular cavities. In contrast, nodules with smooth edges, bronchus signs, and round shapes are considered benign lesions. However, research shows that pulmonary TB can show one or more features of malignancy. Asymptomatic pulmonary TB patients have a higher frequency of spiculated margins rather than lung cancer.[9] As a result, there is an overlap between pulmonary TB and lung cancer on chest CT images as in the case. The thoracic CT scan of the patient shows solid lesions with indistinct borders, spiculated edges, and contrast enhancement. The lesion also appears to titrate the right bronchus.

Extrapulmonary TB which attacks bones and joints constitutes 10% to 20% of all TB cases.[13] Bone and joint tuberculosis results from hematogenous or primary focal lymphogeneous spread or reactive infection focus. Concurrent active pulmonary TB occurs in only 30% of cases of bone and joint TB.[14,15] The location of bone and joint TB generally develops in the lumbar or thoracic vertebrae.[14] In this case, in addition to finding a pulmonary pseudotumor, large bone TB is also found in several organs such as the cervical vertebrae, sternum, and calvarias. Diagnosis is difficult because the initial manifestations that are not typical and in advanced conditions can resemble malignancy.[16]

Contrasting thoracolumbar MRI examination in this case shows multiple bone destruction in the vertebrae and sternum and abscess formation in the posterior cervical vertebrae which is rarely found in cases of spondylitisTB. CT scan on spondylitis TB can be found disco-vertebral lesions and paravertebral abscesses and the spread of disease to the soft tissue that is more clearly seen on MRI.[17] Spine MRI for spondylitis TB is preferred because it can detect early marrow and paraspinal soft tissue changes.[18] Typical radiological findings include vertebral end plates thinning, loss of disc height, bone destruction, new bone formation and soft tissue abscesses often involving multiple vertebral involvements, fusion and collapse.[17]

Destruction of the sternum by the formation of abscesses found in patients can be caused by four mechanisms namely thickening of the pleura that causes the spread of TB germs in lymphogen, localized empyema of tuberculosis pleurisy, and spread of TB germs from the thoracic cavity due to medical measures, and hematogenous infiltration due to milier TB. However, the results of AFB smear pus regional sternum in patients gave negative results. Mtb culture two months later also gave negative results. Nonaka et al. reported that the rate of positive AFB in sternal abscess was 35% and the positive culture rate was 60%.[19]

Calvaria involvement within this case is also infrequent. Calvarial TB generally occurs at the age of 11 to 20 years with a ratio of men and women 2:1.[20] Radiological features found in calvarial TB in the form of lesions with clearly defined sclerotic type and lytic type which diffuse into the cranium. Calvarial TB often appears as painful scalp swelling, subgaleal abscess (Pott's puffy tumor), sinus discharging, and extradural granulation tissue. The involvement of isolated skull bones is very rare.[21] As many

as 42% of cases with a history of trauma or previous surgery, 52% with painless swelling accompanied by discharging sinus, and seizures in a small proportion of patients. The presence of granulation tissue in the extradural space can cause focal neurological deficits.[20]

Seizures that occur in patients initially allegedly due to the process of metastasis in the brain due to the effect of mass pressure that results in increased intracranial and meninges involvement. However, postoperative calvaria tissue examination showed rapid molecular test results Mtb detected low, rifampicin resistant not detected; AFB positive scanty smear, and histopathological examination found granulomatous inflammation and smear germ according to tuberculosis. This is the basis for the diagnosis of TB in patients.

Provision of ATD is the main therapy in this case. Some experts recommend giving ATD 9-12 months if there are multiple with vertebral lesions, cervical levels, and neurological deficits that cannot yet be evaluated.[21,22] Patients with calvarial TB are advised to administer ATD 18-24 months and the therapeutic response must be monitored by physical examination, laboratory and radiological.[23] In this case, the patient was given regiment ATD first category plus streptomycin for 2 months of intensive phase and continued with 10 months of advanced phase after seeing the results of evaluation of therapy. This is ended by considering multiple lesions and involvement of the central nervous system. The results of the treatment evaluation showed clinical improvement with weight gain, increased right and left inferior limb muscle strength, sensory improvement, and loss of lesions on chest X-ray and vertebral abscess on thoracolumbar MRI with contrast.

Some studies show better results if ATD is combined with surgery because of the large area of a bone that can be the focus of TB infection. Surgery for calvarial TB is aimed at establishing the diagnosis, removing thick extradural granulation tissue and necrotic bone, and eliminating the effects of mass urgency.[23] Surgery is only performed on large extradural abscesses which result in focal neurological deficits or scalp swelling with sinus formation and fulminant secondary infection. In some cases, complete excision and extirpation of the sinus tract are recommended.[24] Surgery has also been developed to prevent paralysis due to spondylitis TB. Posterior spinal arthrodesis is currently used to control mechanical instability, prevent kyphosis progression, and correct vertebral deformity in pan vertebra surgery. Ancient conservative therapy with immobilization using corsets and sleeping on hard beds in spondylitis TB is beginning to be abandoned from the time when it gives unsatisfactory results.[25]

CONCLUSION

TB diagnosis is determined based on objections, history taking, clinical examination, laboratory, and other support. Bacteriological laboratory examination (direct microscopic sputum examination, rapid molecular examination, and culture), radiological examination (X-ray, CT scan, and MRI), histopathological examination, drug sensitivity test, and serological tests also play an important role in the diagnosis of TB.

Tumor markers are one of the diagnostic modalities that are widely used to get rid of cancer from other benign diseases. The results of CEA in patients are within normal limits so it does not support the direction of malignancy, especially NSCLC.

The Chest X-ray of the patient shows inhomogeneous opacity with irregular edges. This picture does not show the typical characteristics of pulmonary TB so it leads to misdiagnosis. Malignancy images on CT scans show a variety of morphologies including speculated, lobulated edges, blood vessel convergence signs, pleural indentation, ground-glass opacity, and thick-walled and irregular cavities. Asymptomatic pulmonary TB patients have a higher frequency of spiculated margins rather than lung cancer. As a result, there is an overlap between pulmonary TB and lung cancer on chest CT images as in the case.

In this case, in addition to finding a pulmonary pseudo tumor, large bone TB is also found in several organs such as the cervical vertebrae,

sternum, and calvarias. Contrasting thoracolumbar MRI examination in this case shows multiple bone destruction in the vertebrae and sternum and abscess formation in the posterior cervical vertebrae which is rarely found in cases of spondylitis TB. The results of AFB smear pus regional sternum in patients gave negative results.

Postoperative calvarial tissue examination showed rapid molecular test results Mtb detected low, rifampicin-resistant not detected; AFB positive scanty smear, and a histopathological examination found granulomatous inflammation and smear germ according to tuberculosis. This is the basis for the diagnosis of tuberculosis in patients.

In this case, the patient was given regiment ATD first category plus streptomycin for 2 months of intensive phase and continued with 10 months of advanced phase after seeing the results of evaluation of therapy. The results of the treatment evaluation showed clinical improvement with weight gain, increased right and left inferior limb muscle strength, sensory improvement, and loss of lesions on chest X-ray and vertebral abscess on thoracolumbar MRI with contrast.

CONFLICT OF INTEREST

There is no conflict of interest of this study.

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Case Report

Effect of Zinc(II)-2,4,5-triphenyl-1*H*-imidazole Complex Against Replication DENV-2 in Vero Cell

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ABSTRACT

Dengue virus (DENV) is a significant pathogen emerging worldwide as a cause of infectious disease. DENVs are transmitted to humans through female mosquitoes from Aedes aegypti and Aedes albopictus species. Indonesia is one of the largest countries in the world in dengue endemic regions worldwide. Dengue fever was occurred for the first time as an outbreak in Surabaya and Jakarta in 1968. Many efforts have been made to prevent and treat DENV infections, and clinical trials of a number of vaccines are currently underway. Antiviral testing of DENV is an important alternative for drug characterization and development. Complex compounds are formed as a result of metal and organic complex reactions. Complex compounds can be used as an anti-inflammatory, antimicrobial antifungal, antibacterial, antivirus. The Zn^{2+} ion can be used as an antiviral candidate. The purpose of this project was investigated Zinc(II)-2,4,5-triphenyl-1H-imidazole antiviral compound to be further tested for inhibitory effect on the replication of DENV-2 in cell culture. DENV replication was measured by antiviral activity assay and cytotoxicity assay. The inhibitory activity of Zinc(II)-2,4,5-triphenyl-1H-imidazole complex compound was determined by Viral ToxGloTM Assay. The cytotoxicity of Zinc(II)-2,4,5-triphenyl-1H-imidazole complex compound was determined by CellTiter96® AQ_{uoeus} assay. The inhibitory concentration (IC_{50}) of Zinc(II)-2,4,5-triphenyl-1H-imidazole against dengue virus type-2 was 34.42 µg/ml. The cytotoxic concentration (CC_{50}) of compound against Vero cell was <100 µg/ml. The results of this study demonstrate the antidengue serotype 2 inhibitory activity of investigated Zinc(II)-2,4,5-triphenyl-1H-imidazole complex and its high toxicity in Vero cells. Further studies are not required before investigated Zinc(II)-2,4,5-triphenylimidazole can be applied in the treatment of DENV-2 infections.

Keywords: *Zinc (II), complex compound, cytotoxicity, inhibitory activity, DENV-2*

ABSTRAK

Virus Dengue (DENV) adalah patogen signifikan yang muncul di seluruh dunia sebagai penyebab penyakit menular. DENV ditransmisikan ke manusia melalui nyamuk betina dari spesies Aedes aegypti dan Aedes albopictus. Indonesia adalah salah satu negara terbesar di dunia di daerah endemik demam berdarah di seluruh dunia. Demam berdarah terjadi untuk pertama kalinya sebagai wabah di Surabaya dan Jakarta pada tahun 1968. Banyak upaya telah dilakukan untuk mencegah dan mengobati infeksi DENV, dan uji klinis sejumlah vaksin saat ini sedang berlangsung. Pengujian antivirus DENV adalah alternatif penting untuk karakterisasi dan pengembangan obat. Senyawa kompleks terbentuk sebagai hasil dari reaksi kompleks logam dan organik. Senyawa kompleks dapat digunakan sebagai anti-inflamasi, antimikroba antijamur, antibakteri, antivirus. Ion Zn^{2+} dapat digunakan sebagai kandidat antivirus. Tujuan dalam proyek ini adalah menyelidiki senyawa antivirus Zink(II)-2,4,5-trifenil-1H-imidazol yang diuji lebih lanjut untuk efek penghambatan pada replikasi DENV-2 dalam kultur sel. Replikasi DENV diukur dengan uji aktivitas antivirus dan uji sitotoksisitas. Aktivitas penghambatan senyawa kompleks Zinc(II)-2,4,5-triphenyl-1H-imidazol ditentukan dengan Viral ToxGloTM Assay. Sitotoksisitas senyawa kompleks Zinc(II)-2,4,5-triphenyl-

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1H-imidazol ditentukan dengan uji CellTiter96® AQuoeus. Konsentrasi penghambatan (IC_{50}) Zinc(II)-2,4,5-trifenil-1H-imidazol terhadap virus dengue tipe-2 adalah 34,42 µg/ml. Konsentrasi sitotoksik (CC_{50}) senyawa terhadap sel Vero adalah <100 µg/ml. Hasil penelitian ini menunjukkan aktivitas penghambatan serotipe 2 antidengue dari Zinc(II)-2,4,5-trifenil-1H-imidazol yang diteliti dan toksisitasnya yang tinggi dalam sel Vero. Studi lebih lanjut tidak diperlukan sebelum investigasi Zinc(II)-2,4,5-trifenil-1H-imidazol dapat diterapkan dalam pengobatan infeksi DENV-2.

Kata kunci: *Seng (II), senyawa kompleks, sitotoksisitas, aktivitas penghambatan, DENV-2*

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INTRODUCTION

Dengue virus (DENV) is a virus carried by the *flavivirus* genus of the family Flaviviridae. Dengue virus (DENV) consists of four serotypes which is dengue virus type 1, dengue virus type 2, dengue virus type 3, and dengue virus type 4. Dengue virus is transmitted to humans through female mosquitoes from *Aedes aegypti* and *Aedes albopictus* species. World Health Organization (WHO) reported 390 million dengue infections per year.[1] Indonesia is one of the largest countries in the world with dengue endemic areas. Surabaya and Jakarta were the cities where dengue disease was first reported in Indonesia in 1968.[2] Many studies have been conducted to overcome the threat of dengue virus infections, and clinical trials of a number of vaccines are currently on the way.[3] Antiviral testing on DENV is a very important method in the development and characterization of drugs. Supplementary to vaccines, inhibitors in each natural cycle of viral replication have the potential to cure dengue virus infection and indeed compounds such as RNA replication inhibitors have been tested as such.[4] However, there is no commercially available drug with antiviral activity for DENV.[5]

Ligand 2,3,5-triphenyl-1*H*-imidazole compound is a derivate of imidazole. Imidazole-containing drugs that have strong therapeutic properties have encouraged scientists to synthesize many novel chemotherapeutic agents consisting of these entities. N^5-(4-fluorophenyl)-N^4-(2-(pyridin-4-yl)benzyl)-1*H*-imidazole-4,5-dicarboxami-de, a derivate of imidazole, was reported anti-DENV activity.[6] 4-carbamoyl-5-(4,6-diamino-2,5-dihydro-1,3,5-triazin-2-yl) imidazole-1-β-D-ribofuranoside is examined for four different types of viruses from the flaviridae family *in vitro*, including hepatitis C virus (HCV), Japanese viral encephalitis (JEV), West Nile virus (WNV), and dengue virus (DENV) *in vitro* against NTPases/helicases. The compound showed activity highly active against WNV with IC_{50} was 23 µM.[7]

Complex compounds are formed as a result of metal and organic compound reactions. Complex compounds can be used as an anti-inflammatory[8], antimicrobial[9], antifungal, antibacterial[10], and antivirus[11]. Based on previous research, Copper(II)-imidazole derivatives can be used as antiDENV-2, can be used as low toxicity and potential as drug candidates. The compound exhibited adsorption inhibitory activity against DENV-2 at IC_{50} = 2.3 µg/ml.[12]

The Zn^{2+} ion can be used as an antiviral candidate.[13] Zn^{2+} ions can change the activity of various transcription factors and thus, patterns of cellular and viral gene expression.[14] Thus, the antiviral test of the compound Zinc(II)-2,4,5-triphenyl-1*H*-imidazole was investigated.

MATERIALS AND METHODS

Chemicals and Media

Chemical reagents used in this research were Zinc(II)-2,4,5-triphenyl-1*H*-imidazole complex compound, Minimum Essential Eagle Medium (Sigma-Aldrich, Germany), dengue virus serotype 2 Surabaya isolate (KT012509), Vero cells (African Green Monkey Kidney), Viral ToxGlo™ assay (Promega, USA), CellTiter96®

AQ_{uoeus} One Solution Cell Proliferation Assay (Promega, USA).

Antiviral Activity Assay

Confluent monolayers of Vero cells were prepared on a 96-well plate (1×10^6 cells/10 ml) and counted using a hemocytometer, and the titer of DENV-2 (2×10^4 FFU/well) was expressed in Foci-Forming Units (FFU) after incubating at 37°C for 2 days. The concentrations of Zinc(II)-2,4,5-triphenyl-1*H*-imidazole were 50 µg/mL; 25 µg/mL; 12.5 µg/mL; 6.25 µg/mL; 3.13 µg/mL; 1.57 µg/mL; 0.78 µg/mL; and 0.39 µg/mL with addition 100 µL Viral ToxGlo™ Assay per well. The 50% inhibitory concentration (IC_{50}) of DENV-2 replication by each compound was further investigated by using GloMax® Discover System.

Cytotoxicity Assay

A cytotoxicity assay was performed using CellTiter96® AQ_{uoeus} One Solution Cell Proliferation reagent. The CellTiter96® Assay is a modification of the MTT assay method portrayed by Akter.[15] The concentrations of Zinc(II)-2,4,5-triphenyl-1*H*-imidazole were 100 µg/mL; 200 µg/mL; 400 µg/mL; 600 µg/mL; 800 µg/mL; and 1000 µg/mL. The medium was allowed to equilibrate for 1 hour; then 20µl/well of CellTiter 96® AQ_{ueous} One Solution Reagent was added. After 1 hour at 37°C in a humidified, 5% CO_2 atmosphere, the absorbance at 490nm was recorded using GloMax® Discover System.

Viral Detection by Reverse Transcriptase-Polymerase Chain Reaction

RNA replication was estimated using the Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). The purpose of this assay was to known RNA replication after treatment. Briefly, DENV-2 RNA was extracted from the DENV-2 infected cells and cell culture supernatant using RNA extraction kit by Qiagen, Germany. The two-step kit (Toyobo, Japan) was used for cDNA synthesis and Polymerase Chain Reaction (PCR) following the manufacturer's instructions. Primer oligonucleotide sequences were as follows by Bhatnagar et. al. 2012.[16] Amplification condition was 54 °C for one minute (annealing temperature) and the amplified product was the analyzed on 1.5% agarose gel.

RESULTS AND DISCUSSION

The cytotoxicity of Zinc(II)-2,4,5-triphenyl-1*H*-imidazole complex compound was determined by CellTiter96® AQ_{uoeus} assay and the recorded CC_{50} value is <100 µg/ml to Vero cells. When compared with a previous study, Copper(II) was found to be nontoxic to human erythrocyte cells to concentrations of 500 µg/ml.[17] CC_{50} is the cytotoxicity level of $[Cu(2,4,5\text{-triphenyl-1}H\text{-imidazole})_2]_n$ (compound) to cause death to 50% of Vero cells.[12] The toxicity value of Cobalt(II) complex with 2,4,5-triphenyl-1*H*-imidazole ligand was 362.24 mg/L, which was not toxic.[18] The toxicity value of 2-methyl-4,5-diphenyl-1*H*-Immidazole ligand compound was 192,3 µg/ml.[19] The toxicity of $[Mn(2\text{-}(4\text{-chlorophenyl})\text{-}4,5\text{-diphenyl-1}H\text{-imidazole})_2(H_2O)_2]\cdot 2H_2O$ was >200 µg/ml which had less toxicity.[20] Zinc(II)–2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxycromen-4-one complex compound defined cytotoxicity with CC_{50} at 3.59 µg/ml.[21] But, the metal-free imidazole more toxic for Vero cells (CC_{50} = 5.03 µg/ml).[22] Activity against HIV-1 strain IIIB and HIV-2 strain ROD in MT-4 cells (CC_{50}) by zinc(II) complexes with hexyl-Me_2-cyclam (HMC; 3,14-dimethyl-2,6,13,17-tetraazatricyclo(16.4.0.$0^{7,12}$)-docosane) were >372 µM and >372 µM with selectivity index >35 and >3. Activity against HIV-1 strain IIIB and HIV-2 strain ROD in MT-4 cells (CC_{50}) by Zn(II)–HMC diacetate were 110.67 ± 12.67 µM and 110.67 ± 12.67 µM with selectivity index 32 and <1.[23]

The complex stability is highly dependent on both the metallic ion and the ligands. As for the central ion (M^{2+}), Zn(II) more unstable than Cu(II), Mn(II), and Co(II). The Zn(II) complex has grater polarizability that that Cu(II), Mn(II), and Co(II) because it contains more *d*-electrons, and the Zn(II) complex produced more product ions soluble in water.[24] This effect causes Zn(II) to be more toxic, because Zn^{2+} in the medium are

more numerous, so it damages the cell wall faster than complex compound that have high stability such as Cu(II), Mn(II), and Co(II).

The percentage inhibition of the development of dengue virus type-2 by the test sample of Zinc(II)-2,4,5-triphenyl-1*H*-imidazole complex compound was shown on figure 1. The IC_{50} value was determined from the concentration–response curve (Figure 1); the IC_{50} value was 34.42 µg/ml, R^2 was 0.9196. Based on the value of the IC_{50} Zinc(II)-2,4,5-triphenyl-1*H*-imidazole complex compound was a medium toxic compound.

Antiviral activity was also shown in Figure 2, these findings were corroborated by results obtained from RT-PCR which indicated significant reduction in the amount of DENV-2 genomic RNA levels. The highest percentage of viral inhibition was observed after treating the infected cells with 50 µg/ml.

Based on the previous study, $[Cu(2,4,5\text{-triphenyl-1}H\text{-imidazole})_2]_n$ complex compound exhibited adsorption inhibitory activity against DENV-2 at IC_{50} = 2.3 µg/ml. The inhibition at IC_{50} was not significantly high ($p<0.005$) compared to that of the metal-free imidazole (IC_{50} = 0.13 µg/ml).[12] The maximal inhibitory concentration (IC_{50}) of Copper(II)chloride Dihydrate against DENV-2 was 0.13 µg/ml.[22]

Activity against HIV-1 strain IIIB and HIV-2 strain ROD in MT-4 cells (IC_{50}) by zinc(II) complexes with hexyl-Me_2-cyclam (HMC; 3,14-dimethyl-2,6,13,17-tetraazatricyclo(16.4.0.$0^{7,12}$) docosane) were 10.51 ± 0.23 µM and 133.78 ± 14.10 µM. Activity against HIV-1 strain IIIB and HIV-2 strain ROD in MT-4 cells (IC_{50}) by Zn(II)–HMC diacetate were 3.50 ± 0.33 µM and >110.67 µM.[23] Anti-HIV-1 activity (IC_{50}) in C8166/IIIB, MT-4/GUN1 and PBLs/IIIB were 8.0 µg/ml, 3.5 µg/ml, and 9.3 µg/ml, respectively. The IC_{50} value of the Cobalt(II)–Morin complex for DENV-2 was 3.08 µg/ml.[25] MB21, a benzimidazole derivative, was found to be the most potential inhibitor of cloned proteases (IC_{50} = 5.95 µM).[26]

This study suggest that of Zinc(II)-2,4,5-triphenyl-1*H*-imidazole complex compound can't be an attractive antiviral option. It would be interesting to further investigate whether 2,4,5-triphenyl-1*H*-imidazole complex with other metal. The result of this study, Zinc(II)-2,4,5-triphenyl-1*H*-imidazole complex compound more toxic than Cu(II)-2,4,5-triphenyl-1*H*-imidazole, this is caused by the Zn (II) complex being unstable compared to the Cu (II) complex.

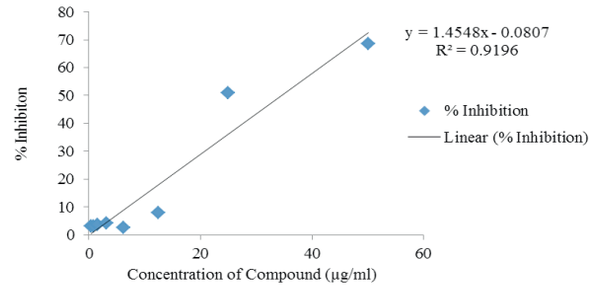

Figure 1. Inhibition of DENV-2, at variation concentrations of Zinc(II)-2,4,5-triphenyl-1*H*-imidazole complex compound

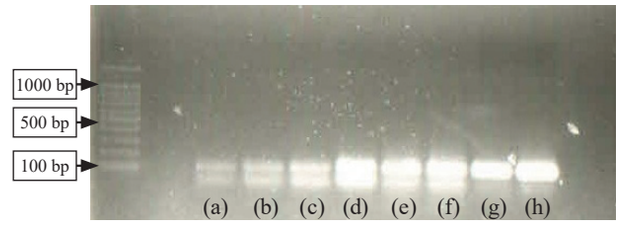

Figure 2. Electrophoresis on 1.5% agarose of RT-PCR after treatment, molecular weight marker (100 bp), (a) treatment with 50 µg/ml compound, (b) 25 µg/ml, (c) 12.5 µg/ml, (d) 6.25 µg/ml, (e) 3.13 µg/ml, (f) 1.57 µg/mL, (g) 0.78 µg/mL, and (h) 0.39 µg/mL

CONCLUSION

Further studies are not required before Zinc(II)-2,4,5-triphenyl-1*H*-imidazole can be applied in the medication of DENV-2 infections. This study did not show the potential of the Zinc(II)-2,4,5-triphenyl-1*H*-imidazole complex as a candidate for antiviral agents against DENV-2 because it was shown to be toxic to Vero cells.

CONFLICT OF INTEREST

There is no conflict of interest of this paper.

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Original Article

Prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) Carrier in Hemodialysis Patients at Dr. Soetomo Academic General Hospital

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ABSTRACT

Chronic kidney disease (CKD) is now a global epidemic, and the prevalence is increasing worldwide. Hemodialysis is one of the ways to treat by kidney function replacement. Infection is the number two cause of death in patients with hemodialysis (HD). Methicillin-resistant Staphylococcus aureus (MRSA) is a common cause of bacteriemia in patients with dialysis. The epidemiological data of MRSA carriers in CKD in Indonesia are still scarce. This study was to determine the prevalence of MRSA carriers in patients at The Kidney and Hypertension Outpatient-clinic and Hemodialysis Installation at Dr. Soetomo Academic General Hospital, Surabaya Indonesia. The study design was descriptive-analytic with a cross-sectional study design. Sampling was collected consecutively. Data on the general characteristics of the research subjects will be analyzed using a Chi-Squared test. There were 150 CKD stage five patients included in this study, the number of patients has MRSA carrier were 6 (4%), among them, subjects underwent HD MRSA carrier were 2 subjects(2.7%), while for non-HD patients with MRSA were 4 subjects (5.3 %). There were no significant differences in MRSA carriers between HD and non HD groups (p=0.404). Comorbid factors that accompany MRSA carriers are diabetes mellitus, hypertension, kidney stones, gout, and systemic lupus erythematosus (SLE). ***Conclusions****: This study found, there were no significant differences in the incidence of MRSA carriers in stage five CKD non HD or HD groups. MRSA colonization exists in stage five CKD sufferers, so awareness of MRSA colonization.*

Keywords: *Chronic Kidney Disease, Hemodialysis, MRSA, Diabetes Mellitus, Hypertension, Indonesia.*

ABSTRAK

Penyakit ginjal kronis (CKD) saat in menjadi epidemi global, dan prevalensi meningkat di seluruh dunia. Hemodialisis adalah salah satu cara untuk terapi penggai ginjal. Infeksi merupakan penyebab kematian nomor dua pada pasien dengan hemodialisis (HD). Staphylococcus aureus yang resisten terhadap metisilin MRSA) adalah penyebab tersering bakteriemia pada pasien dengan dialisis. Saat ini data epidemiologis pembawa MRSA pada penderita CKD di Indonesia belum lengkap. Penelitian ini untuk mengetahui prevalensi pembawa MRSA pada pasien-pasien di Klinik Rawat Jalan Ginjal dan Hipertensi dan Instalasi Hemodialisis di Rumah Sakit Umum Dr. Soetomo, Surabaya Indonesia. Desain penelitian adalah deskriptif-analitik dengan desain penelitian cross-sectional. Pengambilan sampel dikumpulkan secara berurutan. Data karakteristik umum dari subjek penelitian akan dianalisis menggunakan uji Chi-Squared. Terdapat 150 pasien CKD stadium lima yang masuk didalam penelitian ini, jumlah pasien yang menjadi pembawa MRSA ada 6 subjek (4%), di antara mereka, subjek yang menjalani HD sebagai pembawa MRSA ada 2 subjek (2,7%), sedangkan untuk pasien non-HD dengan pembawa MRSA ada 4 subyek (5,3%). Tidak ada perbedaan yang signifikan antara pembawa MRSA antara kelompok HD dan non HD (p = 0,404). Faktor komorbid yang menyertai pembawa MRSA adalah diabetes mellitus, hipertensi, batu ginjal, asam urat, dan systemic lupus erythematosus (SLE). Penelitian ini mendapatkan, tidak ada perbedaan

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yang signifikan pada kejadian pembawa MRSA pada stadium lima CKD non HD atau kelompok HD. Kolonisasi MRSA ditemukan pada penderita CKD stadium lima, sehingga kesadaran pada kolonisasi MRSA.

Kata kunci: *Penyakit Ginjal Kronis, Hemodialisis, MRSA, Diabetes Mellitus, Hipertensi, Indonesia.*

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INTRODUCTION

Chronic kidney disease is a serious public health problem because there has been an increase in the number of patients, morbidity, and mortality.[1] MRSA can be spreading from hospital to community, in addition to hospital-hospital transfers.[2] In CKD patients with a history of hemodialysis with the use of vascular access, they should always be aware of the possibility of bacterial infections, it causes of death number 2 in patients with hemodialysis.[3,4,5] Vascular access is needed to obtain a large, enough blood flow. This access can be in the form of a fistula (artery-vein), graft, or intravenous catheter, which functions to drain blood during HD. Vascular access is one of the main risk factors for bacteremia (15.16%) and infections associated with frequent hospitalization and death (about 17.18%) in patients with hemodialysis.[6] The percentage of fatalities in CKD stage 5 patients due to infection is quite large. Patients who undergo hemodialysis are very susceptible to infection, especially Methicillin-resistant Staphylococcus aureus (MRSA). There was a meta-analysis study that estimates the prevalence of MRSA colonization in dialysis patients, the time and long-term risk of MRSA infection. From the data of 5596 dialysis patients, the prevalence of MRSA colonization was 6.2% (95% confidence interval, 4.2% to 8.5%). Prevalence increased over time but remained stable after 2000. Over a long period (6-20 months), the likelihood of developing MRSA is around 19% in patients who have hemodialysis compared with patients with hemodialysis without MRSA colonization of about 2%.[7] The infection will also worsen kidney function or will add a burden to the already poor kidney function, which will contribute to the increase in morbidity, mortality, and costs, therefore infection problems in stage five CKD patients are critical.[8]

Chronic kidney disease (CKD) is often associated with several immunological abnormalities, both congenital immune system disorders, and adaptive immune systems and, therefore, can increase susceptibility to infection.[9] Infection enhanced with the use of a central catheter compared to AV fistula. Infections originating from the use of vascular access are often associated with microorganisms *Staphylococcus aureus* and *Staphylococcus epidermidis*.[10] MRSA is a major nosocomial pathogen that affects inpatients. Endemic MRSA strains originate from the hospital. Most of the HD units have had patients with MRSA colonization of bacteria.[5] Bacteriemia or infection through the blood caused by S. aureus is a significant cause of high morbidity and mortality.[5,11]

Based on the above considerations, the researcher wants to examine the prevalence of MRSA carriers in CKD stage five patients in the Outpatient Clinic and Hemodialysis Installation, as an essential data on the incidence of MRSA carriers.

METHODS

The study was a descriptive-analytic study with a cross-sectional design. The research subjects were obtained by consecutive sampling. The study was conducted in the Outpatient Clinic and Hemodialysis Installation of Dr. Soetomo general hospital, Surabaya Indonesia in July - August 2018. There were two research groups, namely Stage five non-HD CKD and the Stage five CKD who had undergone HD treatment.

The study sample was selected through the inclusion and exclusion criteria of the population of Stage five CKD patients in the Hemodialysis Unit and Kidney and Hypertension Outpatient clinic. The requirements of participants: over 18 years old, willing to take part in this study, and signed informed consent.

Participants who were not included in the study, if one or more of the following criteria found: Subjects with decreased consciousness or sepsis.

Specimens were collected by using sterile dry cotton swabs, and instructions were given on how to take swab samples from anterior nares and throat. One swab was used for both nostrils. All swabs were transported to the Laboratory and directly inoculated into 5 ml of Phenyl mannitol salt broth (Difco), incubated overnight at 37^{o}C and then subcultured onto MRSA-Chromagar, and further identification using Vitex 2, as standard microbiological procedure in the Microbiology Laboratory at Dr. Soetomo General Hospital.[12,13,14]

Table 1. General characteristics

Characteristics Subject	N (%)
Age (Years)	
- Average ± SD	52.1±11.8
- age range	19-78
Co-morbid Factors	
- Diabetes mellitus	69 (46%)
- hypertension	129 (86%)
- Kidney stones	29 (19%)
- Gout	30 (20%)
- Hepatitis B	2(1.3%)
- SLE	1(0,66%)
- Cervical cancer	6(4%)
- HIV	1(0.66%)
- Others (Hepatitis C, ovarian cyst, UTI)	0 (0%)
Types of Vascular Access	
- AV fistula	74(49%)
- CVC	1(0.66%)
HD Frequency	
- Never been HD	75(50%)
- HD 1x / week	1(0,66%)
- HD 2x / week	74(49%)
Long HD	
<4 years	51(34%)
4-6 years	14(9.3%)
> 6 years	10(6.660%)

RESULTS

There were 75 subjects in the HD group, and 75 subjects in the non HD group, prevalence MRSA carriers were found in 6/150(4%) of total samples. Data on the general characteristics of research subjects in Table 1. In the non HD group, there were 45 male subjects (60%), and 30 female subjects (40%). In the HD group, there were 36 male subjects (48%), and 39 female subjects (52%).

In this study, the number of subjects with MRSA (+) carriers in patients who received hemodialysis was two (2.7%), and the number of MRSA carriers (+) in patients who have not undergone hemodialysis as many as four patients (5.3%). See Table 2.

Carrier prevalence of MRSA with comorbid DM, there were 69 (46%) patients suffering from DM, and 4 of them became MRSA carriers.

In this study in the non HD group who suffered HT as many as 65 (86.7%) with MRSA carrier incidence rates of 4 patients. Whereas in the HD group, who suffered HT as much as 64 (85.3%) with MRSA carrier incidence rate of 1 patient.

In the non-HD group who suffered kidney stones as many as 20 (26.7%), one patient with an MRSA carrier.

In the HD group suffering from kidney stones as many as 9 (12%), none of them with MRSA

In this study in the non-HD group who suffered from hepatitis B was 2 (2.7%). In the HD group subject suffering from hepatitis B were 9 (12%), but from both groups, there were no carriers of MRSA

In this study, the hepatitis C comorbid factor was not found, because the HD group with the hepatitis C comorbid factor was not willing to participate in this study.

In this study, there were no MRSA carriers with comorbid factors in urinary tract infections in either group HD and non HD subjects.

This study found only one patient with SLE in the HD group, and also as a carrier of MRSA.

Table 2. Prevalence of MRSA carriers

Characteristics Subject	MRSA Carriers (N)
Co-morbid factors	
- Diabetes mellitus	4(5.8%)
- hypertension	5(3.9%)
- Kidney stones	1(3.4%)
- Gout	2(6.67%)
- Hepatitis B	0(0%)
- SLE	1(100%)
- Cervical cancer	0(0%)
- HIV	0(0%)
- Others (Hepatitis C, ovarian cyst, UTI)	0 (0%)
Types of vascular access	
- AV fistula	2(2.7%)
- CVC	0(0%)
HD frequency	
- Never been HD	4(5.3%)
- HD 1x / week	0(0%)
- HD 2x / week	2(2.7%)
Long HD	
<4 years	2(3.9%)
4-6 years	0(%)
> 6 years	0(%)

For other comorbid factors such as cervical cancer, ovarian cancer, ovarian cyst, and HIV, there were no MRSA carriers.

The HD group that used double-lumen vascular access was one (1.33%) with an MRSA carrier occurrence rate of zero, while in the HD group who used AV Shunt vascular access as many as 73 (97.3%) with an MRSA carrier of two patients.

The HD group who had done two times/week was 74 (97.3%) patients, with two MRSA carriers. There was no MRSA carriers found in HD group who had done one time/week.

In the HD group with a length of time of HD less than 4 years as many as 51(68%) patients, with MRSA carriers of two (3.9%) patients, while the period of time undergoing HD 4-6 years was 14 (18.6%) patients, with MRSA carrier event of zero, while the length of time to experience HD more than 6 years was 10 (13.3%) patients, with MRSA carrier was zero.

DISCUSSION

Patients undergoing dialysis are vulnerable to get MRSA infections, especially they have carries or colonization of MRSA in their nose or throat.

This study found 150 patients, consist of 81 (54%) male and 69 women (46%), total MRSA carriers there were 6 (4%), in women as many as 3 (4.3%) patients, and 3 (3.7%) patients in male, where statistically was no significant difference ($p = 0.84$). This result of MRSA carriers were lower than the result from a previous study in the same hospital in surgical and medical wards in the year 2016, there were 52 (8.1%) of 643 patients on admission were colonized with MRSA, this result was higher than our study, This is possible because most of the patients were referred from other hospitals.[15]

In this study, the prevalence of MRSA carriers who had not received HD treatment 4(5.3%) patients, compared to those who had received HD treatment 2(2.7%) patients, but the statistical difference was not significant ($p = 0.45$). In the research of Wang et al. found a little higher prevalence of MRSA carriers in patients with HD, the prevalence of S. aureus colonization in hemodialysis patients around 22.4% consisting of 16.5% MSSA and 5.9% MRSA.[16]

This study had a mean age of 54 research subjects with the youngest age range of 19 years and the oldest 78 years in the non-HD group, with MRSA carrier incidence rates of four patients (50, 52, 61, and 76 years). As for the HD group, the average age of the study subjects was 49 years, with the youngest age range being 29 years and the oldest being 75 years with MRSA carrier incidence rates of two patients (ages 44 and 74 years). This research similar to the following study conducted by Celik et al., 2011 which reported that patients with hemodialysis found higher MRSA carriers incidence rates in the age group between 55-64 years (30.55%) and MRSA carrier incidence rates that were the youngest with a range between 25-34 years.[17]

Non-HD group research subjects having comorbid diabetes mellitus as many as 45 people (60%) with an MRSA carrier occurrence rate of four patients. Whereas for the HD group, who had DM comorbid factors as many as 24 patients (32%) with an MRSA carrier incidence rate of zero. Other studies also reported similar data based on data from Kang et al., 2012 found that out of a total of 296 research subjects. HD group with DM comorbid factors were 125 people.[18] MRSA colonization was 11 patients (57.9%), while MRSA colonization was not found 114 (41.2%). Research conducted by Lai et al., 2011 found that of 306 research subjects with DM comorbid factors in HD patients with MRSA carriers as many as 11 people (37.93%), while subjects without MRSA carriers were 147 people (53.07%).[19] Another study Yeoh et al., 2014, found that DM increases the high risk for MRSA colonization infection (odds ratio 4.2).[20] Research conducted by Saxena et al., 2009 found that the prevalence of type 2 DM increases three times higher risk factors for MRSA nasal carriers compared to non-DMs (72.4% vs. 24.6%) in patients with hemodialysis (RR. 2.97, $p < 0.0001$). In nasal carriers, about 72.4% in dialysis patients with type 2 diabetes, 29% higher than in non-DM HD patients.[21]

This study found hypertension in the non-HD group was sixty-five patients (86.7%) with an MRSA carrier occurrence rate of four patients. Whereas for the HD group, who had hypertension comorbid factors as many as sixty-four patients (85.3%) with an MRSA carrier occurrence rate of one patients. A study conducted by Kang et al., 2012, found that MRSA carriers in HD patients with hypertension as comorbid as many as 84.2%.[18]

This study found that hepatitis B comorbid factors in the non-HD group were two patients (2.7%) with an incidence of MRSA carrier of zero (0%). Whereas for the HD group who had hepatitis B comorbid factors as many as nine patients (12%) with an MRSA carrier occurrence rate of zero (0%). This result concordance with research conducted by Kang et al., 2012 found that of the subjects as many as 296 patients who had hepatitis B comorbid factors as many as 32 patients (10.8%) with MRSA carrier events as much as zero (0%).[18]

CONCLUSION

In this study, the prevalence of MRSA in subjects with stage five CKD were 6/150 (4%) there were no significant differences in the incidence of MRSA carriers in stage five CKD non HD or HD groups. This study shows that MRSA colonization exists in stage five CKD sufferers who have or who have not received HD therapy.

CONFLICT OF INTEREST

There is no conflict of interest of this paper.

ACKNOWLEDGEMENT

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5. Canadian Dental Hygienists Association. Dental hygiene: definition and scope. Ottawa: Canadian Dental Hygienists Association; 1995.

E-book

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