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CONTENTS

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Original Article

Risk Factors of Covid-19 Confirmed Died Patients in Dr. Kariadi Hospital: A Retrospective Study

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ABSTRACT

Covid-19 is a communicable disease causing global pandemic. Some factors inflict worse infection. This study aims to investigate risk factors of Covid-19 confirmed died patients at Dr. Kariadi Hospital Semarang. It is a retrospective study with a total sample of all Covid-19 confirmed patients involving died and healed patients from March to June 2020. Data was gathered from screening forms and analysed with Chi Square (confidence interval of 95%). This study found sixteen risk factors of Covid-19 confirmed died patients involving age (p= 0.000; OR= 8.803; 95% CI 3.982-19.462), entrepreneur (p= 0.041; OR= 14.894; 95% CI 1.12-198.65), farmer/trader (p= 0.029; OR= 25.625; 95% CI 1.40-469.25), contact history (p= 0.000; OR= 12.923; 95% CI 6.163-27.097), fever (p= 0.000; OR= 4.877; 95% CI 2.647-8.984), dyspnea (p=0.000; OR= 17.018; 95% CI 8.523-33.977), cough (p= 0.009; OR= 2.178; 95% CI 1.205-3.935), lethargic (p=0.010; OR= 2.282; 95% CI 1.205-4.323), cold (p= 0.002; OR= 0.180; 95% CI 0.054-0.600), diabetes (p=0.000; OR= 9.767; 95% CI 3.932-24.263), COPD (p= 0.001; OR= 6.360; 95% CI 2.164-18.690), hypertension (p= 0.043; OR= 2.436; 95% CI 1.008-5.887), cancer (p=0.001; OR= 9.647; 95% CI 2.413-38.579), heart disease (p= 0.000; OR= 12.226; 95% CI 2.4-62.294), neurological disorders (p=0.008; OR= 6.057; 95% CI 1.650-22.232), and immune disorders (p=0.031; OR= 1.625; 95% CI 1.186-113.899). Adequate handling is needed to prevent death. in patients with confirmed Covid-19 who have risk factors.

Keywords: *Risk factor; Confirmed patients; Covid 19*; *Died; Retrospective*

ABSTRAK

Covid-19 merupakan penyakit yang cepat menular sehingga menyebabkan pandemi di seluruh negara. Ada beberapa faktor risiko yang menjadikan Covid-19 menginfeksi seseorang menjadi lebih parah. Penelitian ini bertujuan untuk mengetahui faktor risiko pasien terkonfirmasi Covid-19 yang meninggal dunia di RSUP Dr. Kariadi. Penelitian ini merupakan retrospective study. Populasi adalah seluruh pasien terkonfirmasi Covid-19 di RSUP Dr. Kariadi. Sampel penelitian adalah seluruh pasien terkonfirmasi Covid-19 yang terdiri dari pasien meninggal dan sembuh tercatat sejak bulan Maret sampai Juni 2020. Data diperoleh dari formulir screeening dan dianalisis menggunakan uji Chi Square dengan tingkat kepercayaan 95%. Hasil peneltian menemukan enam belas faktor risiko pasien terkonfirmasi Covid-19 meninggal, yaitu umur (p= 0,000; OR= 8,803; 95% CI 3,982-19,462), wiraswasta (p= 0,041; OR= 14,894; 95% CI 1,12-198,65), petani/pedagang (p= 0,029; OR= 25,625; 95% CI 1,40-469,25), riwayat kontak (p= 0,000; OR= 12,923; 95% CI 6,163-27,097), demam (p= 0,000; OR= 4,877; 95% CI 2,647-8,984), sesak napas (p=0,000; OR= 17,018; 95% CI 8,523-33,977), batuk (p= 0,009; OR= 2,178; 95% CI 1,205-3,935), lemah lesu (p=0,010; OR= 2,282; 95% CI 1,205-4,323), pilek (p= 0,002; OR= 0,180; 95% CI 0,054-0,600), diabetes (p=0,000; OR= 9,767; 95% CI 3,932-24,263), gangguan paru kronik (p= 0,001; OR= 6,360; 95% CI 2,164-18,690), hipertensi (p= 0,043; OR= 2,436; 95% CI 1,008-5,887), keganasan (p=0,001; OR= 9,647; 95% CI 2,413-38,579), penyakit jantung (p= 0,000; OR= 12,226; 95% CI 2,4-62,294), gangguan neurologis (p=0,008; OR= 6,057; 95% CI 1,650-22,232), and gangguan imunitas (p=0,031; OR= 1,625; 95% CI 1,186-113,899). Dibutuhkan penanganan yang adekuat untuk mencegah kematian pada pasien terkonfirmasi Covid-19 yang memiliki faktor risiko.

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***Kata kunci**: Faktor resiko*; *pasien terkonfirmasi; Covid-19; meninggal; retrospektif*

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INTRODUCTION

Covid-19 is a communicable disease first reported in Wuhan, China in December 2019. Covid-19 known as Novel Coronavirus caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The first spread to other countries occured from 2020.[1] In comparison with SARS and MERS, Covid 19 is more contagious resulting in global pandemic.[2,3] Cases of pneumonia with unknown aetiology was announced as a Public Health Emergency of International Concern (PHEIC) in the end of January 2020 and declared as pandemic on 11 March 2020.[4]

Covid-19 was predicted to have mortality rate lower than SARS and MERS, in fact its mortality rate is 2%[2] - 2.3% or 20 times greater than common influenza.[4] Covid-19 cases is more prevalent among older people having comorbidities such as cardiovascular disease, diabetes, hypertension[2], chronic respiratory disease and cancer.[4,5,6]

Transmission of Covid-19 can occur between people with or without symptoms. Virus can survive for more than 72 hours resulting is fast contagion.3 So far, Covid-19 spreads through air when performing aerosol-generating medical procedures where virus can survive for 3-16 hours. In this case, health practitioners such as laboratory technicians have greater risk for Covid-19 exposure. Outside medical facilities, Covid-19 is found in the group of people in the crowded rooms such as restaurant and fitness centre without adequate ventilation.[7]

The fast spread of the virus results in fast growing of new cases. Worldwide, it was confirmed 172.558 positive cases and 3.921 death on 30 June 2020.[8] In Indonesia, the first case was reported on 3 March 2020. It was then found 56.385 cases involving 24.806 healed cases and 2.876 death cases on 30 June 2020. In Central Java, it was confirmed 3.836 cases involving 1.159 healed cases and 150 death cases.[9] It suggests the mortality rate of Covid-19 in Indonesia and Central Java is about 5.1% and 3.9% respectively. It is significantly greater than global mortality rate (Case Fatality Rate 2.3%) and South East Asian mortality rate (CFR 2.1%).[8]

The high confirmed cases and mortality rate are a result of host and virus factors. The virus ability to defeat host immune systems serve as determinant of infection severity. Inadequate host immune response results in virus mutation and more severe host tissue destruction. It is compounded with the virus ability to evade from host immune and replicate thus unrecognised by host immune.[6] Data indicate Covid-19 is more prevalence among elderly and those with comorbidities. The mortality rate is higher among older people.[10]

The presence or absence of symptoms is not the sole hint as a study from China found 12.6% infection occurs pre-symptomatic and some cases confirmed without symptom (asymptomatic).[11] Covid-19 patients without symptom may transmit viruses to other people. People in group in certain environment are at high risk for infection such as dormitory, prison, other closed environment, and public facilities such as bus stop, bus station, airport and shopping centre.[7]

People having close contact with Covid-19 patients or taking care of Covid-19 patients have heightened risk for Covid-19 exposure.[12] Besides, smoking is associated with heightened disease severity and Covid-19 related mortality.[13,14] Other factors such as vitamin D availability is considered playing important role for decreasing virus infection risk. It is known

that Covid-19 cases are increasing during winter where 25-hidroxyvitamin D concentration is low in human body.[15] Conversely, countries in the south hemisphere with dry season tend to have low case numbers. Race is also considered having relationship with percentage of Covid-19 related cases and death cases.[16]

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.

Dr. Kariadi Hospital is a referral hospital in Central Java having role and responsibilities for handling Covid-19 patients involving those who are suspected, having history of close physical contact with patients, confirmed Covid-19 patients and healed patients. The number of Covid-19 patients is predicted to keep increasing and end in uncertain time. The higher death cases may occur as more cases are found, particularly when several risk factors present. Covid-19 can impact significantly human life sustainability as supported by a study by Trias-Llimós dan Bilal found Covid-19 lowers life expectancy 1.9 and 1.6 years for male and female respectively in Madrid, Spain.[17] It encourages authors to investigate risk factors of Covid-19 confirmed dead patients in Dr. Kariadi Hospital.

MATERIALS AND METHODS

It is a retrospective study. The population involves all Covid-19 confirmed patients at Dr. Kariadi Hospital. Research samples involve all Covid-19 confirmed patients both healed and died patients from March to June 2020. The inclusion criteria involved Covid-19 confirmed patients both died and healed patients confirmed based on swab test. The exclusion criteria involved patients both in inpatient and outpatient settings still waiting for swab test results. Data was gathered from screening forms filled by patients or health practitioners interviewing patients on arrival (secondary data). Data was analysed with Chi-square with confidence interval of 95% using SPSS version 25. This research has gone through the review stage and has received approval from the Health Research Ethics Committee Dr. Kariadi Hospital No. 561 / EC / KEPK-RSDK / 2020.

RESULTS AND DISCUSSION

Covid-19 confirmed patients are 277 patients involving 59 died and 218 healed cases from March to June 2020. Research findings are shown at Table 1.

Bivariate test shows sixteen risk factors of Covid-19 confirmed died patients involving age (p= 0.000; OR= 8.803; 95% CI 3.982-19.462), entrepreneur (p= 0.041; OR=14.894; 95% CI 1.12-198.65), farmer/trader (p= 0.029; OR= 25.625; 95% CI 1.40-469.25), contact history (p= 0.000; OR= 12.923; 95% CI 6.163-27.097), fever (p= 0.000; OR=4.877; 95% CI 2.647-8.984), dyspnea (p=0.000; OR= 17.018; 95% CI 8.523-33.977), cough (p= 0.009; OR= 2.178; 95% CI 1.205-3.935), lethargic (p=0.010; OR= 2.282; 95% CI 1.205-4.323), cold (p= 0.002; OR= 0.180; 95% CI 0.054-0.600), diabetes (p=0.000; OR= 9.767; 95% CI 3.932-24.263), COPD (p= 0.001; OR= 6.360; 95% CI 2.164-18.690), hypertension (p= 0.043; OR= 2.436; 95% CI 1.008-5.887), cancer (p=0.001; OR= 9.647; 95% CI 2.413-38.579), heart disease (p= 0.000; OR= 12.226; 95% CI 2.4-62.294), neurological disorders (p=0.008; OR= 6.057; 95% CI 1.650-22.232), and immune disorders (p=0.031; OR= 1.625; 95% CI 1.186-113.899).

In confirmed died patients we found that the cause of death was mostly caused by respirato ry failure 40 cases (67.80%) and the rest caused by cardiovascular / MOD 19 cases (32.20%). Furthermore, the days to develop critical all for deadly patient are 6-7 days with the shortest

day 0 days and the longest day 28 days. Treatment given to Covid-19 confirmed patients consists of non-pharmacological and pharmacological therapy. Non-pharmacological therapy includes chest X-ray examination, monitoring for signs such as tachypnea, oxygen saturation, lymphopenia, progressive CRP and progressive lactic acidosis, and management of critical cases, respiratory failure, hypoxemia and ARDS. Pharmacological therapy are giving vitamin C, vitamin B1, zinc, azithromycin, antiniotics according to clinical conditions, chloroquine sulfate, hydroxycortisone injection, and antivirals (Favipiravir, umifenovir, remdesivir, and oseltamivir).

Table 1. Risk factors of Covid-19 confirmed death and healed patients

Risk factors	Total (n=277)	Number of death cases (n=59)	Number of healed cases (n=218)	p- value	OR (95% CI)
Age	38.99±14.954	53.27±13.249	35.12±12.925		
≥ 60	32 (11.6)	20 (33.9)	12 (5.5)	0.000	8.803 (3.982-19.462)
≤ 59	245 (88.4)	39 (66.1)	206 (94.5)		
Gender					
Male	145 (52.3)	36 (61.0)	109 (50.0)	0.133	-
Female	132 (47.7)	23 (39.0)	109 (50.0)		
Occupation					
Civil servant	169 (61.7)	11 (19.0)	158 (73.1)	0.922	-
Private sectors	30 (10.9)	7 (12.1)	23 (10.6)	0.265	-
Entrepreneur	23 (8.4)	12 (20.7)	11 (5.1)	0.041	14.894 (1.12-198.65)
Farmer/trader	8 (2.9)	7 (12.1)	1 (0.5)	0.029	25.625 (1.40-469.25)
Labour/driver/odd jobs	6 (2.2)	5 (8.6)	1 (0.5)	0.199	-
Unemployed/house wife/student	38 (13.9)	16 (27.6)	22 (10.2)	0.126	-
N/A	3	1	2		
Traveling history					
Yes	30 (11.5)	7 (13.2)	23 (11.0)	0.813	-
No	232 (88.5)	46 (86.8)	186 (89.0)		
N/A	15	6	9		
Contact history					
Yes	180 (70.6)	12 (25.0)	168 (81.2)	0.000	12.923
No	75 (29.4)	36 (75.0)	39 (18.8)		(6.163-27.097)
N/A	22	11	11		
Symptoms					
Fever	97 (35.0)	38 (64.4)	59 (27.1)	0.000	4.877 (2.647-8.984)
dyspnea	64 (23.1)	40 (67.8)	24 (11.0)	0.000	17.018 (8.523-33.977)
Cough	132 (47.7)	37 (62.7)	95 (43.6)	0.009	2.178 (1.205-3.935)
Lethargic	60 (21.7)	20 (33.9)	40 (18.3)	0.010	2.282 (1.205-4.323)
Nauseous vomit	28 (10.1)	9 (15.3)	19 (8.7)	0.139	-
Diarrhea	22 (7.9)	8 (13.6)	14 (6.4)	0.072	-
Shore throat	58 (20.9)	7 (11.9)	51 (23.4)	0.053	-
Headache	39 (14.1)	6 (10.2)	33 (15.2)	0.330	-
Cold	53 (19.1)	3 (5.1)	50 (22.9)	0.002	0.180 (0.054-0.600)
Loss of consciousness	3 (1.1)	2 (3.4)	1 (0.5)	0.054	-
Symptom duration (days)	5.82 ± 6.899	5.63 ± 5.622	5.91 ± 7.502		
≥ 6	55 (34.2)	17 (29.3)	38 (36.9)	0.330	-
≤ 5	106 (65.8)	41 (70.7)	65 (63.1)		
comorbidities					
Diabetes	24 (8.7)	16 (27.1)	8 (3.7)	0.000	9.767 (3.932-24.263)
COPD	15 (5.4)	9 (15.3)	6 (2.8)	0.001	6.360 (2.164-18.690)
Hypertension	24 (8.7)	9 (15.3)	15 (6.9)	0.043	2.436 (1.008-5.887)
Cancer	10 (3.6)	7 (11.9)	3 (1.4)	0.001	9.647 (2.413-38.579)
Heart disease	8 (2.9)	6 (10.2)	2 (0.9)	0.000	12.226 (2.4-62.294)
Neurological disorders	10 (3.6)	6 (10.2)	4 (1.8)	0.008	6.057 (1.650-22.232)
Immune disorders	4 (1.4)	3 (1.1)	1 (0.5)	0.031	11.625 (1.186-113.899)
Pregnancy/childbirth	6 (2.2)	1 (1.7)	5 (2.3)	0.779	-
Chronic kidney disease	1 (0.4)	0 (0.0)	1 (0.5)	0.602	-
Chronic liver disease	2 (0.7)	0 (0.0)	2 (0.9)	0.460	-
Others	6 (2.2)	1 (1.7)	5 (2.3)	0.779	-

DISCUSSION

The research results are showed sixteen risk factors of Covid-19 confirmed died patients involving age, occupation (entrepreneur, farmer/trader), contact history, symptoms (fever, dyspnea, cough, lethargic, cold), comorbidities (diabetes, COPD, hypertension, cancer, heart disease, neurological disorders and immune disorders) ($p<0.05$). Meanwhile, gender, traveling history and duration of symptoms were not risk factors for death in Covid-19 confirmed patients ($p>0.05$).

Age is one of the death-contributing factors among Covid-19 confirmed patients especially in the older age group. Onder et al.[18] found a higher case fatality rate in the age group over 80 years in Italy and China (20.2% and 14%) compared to other age groups. Nearly all studies have found a different mean age in patients with confirmed Covid-19. Zhou et al.[19] found mean death ages of Covid-19 patients is 69 years (63-76 years) and heightened along with age progression (p= 0.0043; OR= 1.10; 95 % CI 1.03-1.17). Harlem[20] found most Covid-19 positive cases in the age group of 25-44 years (28% and 31% respectively) and 45-64 years (26% and 27% respectively) in the high case country group (n= 178.469) and in the medium case country group (n= 178.196). Nie et al.[21] Found mean ages of Covid-19 confirmed patients is 43$\pm$15.09 years. They also found a relationship between ages and Covid-19 severity (p= 0.003; OR=:1.026; 95% CI 1.009-1.043). Lai et al.[22] state characteristic of the first 100 Covid-19 cases in Hong Kong with the greatest proportions are among those aged 45 years (76%) and is increasing along with age progression ($p<0.001$). Gemes et al.[23] predicted one out of five people in Sweden are at risk of severe Covid-19 known from prognostic factors aged older than 70 years (14.1%).

This study found most Covid-19 confirmed patients are male, but it does not affect the numbers of death cases. These findings are similar with a study by Nie et al. stating that most Covid-19 patients are male accounting for 377 people (56.2%) and this result is not statistically significant ($p>0.05$).[21] It is also in line with WHO that the percentage infection distribution in male is greater than female (51% vs 47%). WHO states the caused by different female against viruses and infections.[24] Alobuia et al. found female respondents were at least 85% more likely to have high practice scores compared to males ($p < 0.001$) in action against Covid-19.[25]

The risky occupation during this study is farmer/trader and entrepreneur. In many cases, farmers are traders too. These findings are similar with that found in Henan, China showing that the greatest cases occurred among farmers (21.2%) and labours (15.2%). Death cases among farmers are around 0.3%.[21] Mutambudzi et al. categorize the entrepreneur as other essential workers that had a higher risk of severe Covid-19 (RR= 1.60; 95% CI 1.05-2.45).[26] Furthermore, National Statistics state that occupations demanding direct interaction with many people is very risky. Farmers/traders and entrepreneur are types of occupations demanding direct interaction with many people without knowing whether they are infected by Covid-19 or not.[27]

Contact history in this study is defined as direct contact with a suspected, probable or confirmed Covid-19 patients. Findings show great number of died patients without any contact history. Conversely, healed patients are found to have greater contact history. Centers for Disease Control and Prevention (CDC) stipulates close contact as a risk factor mainly among people living in the same house with a confirmed case without physical distancing.[28] A study by Nie et al. found a relationship between direct contact with infected patients and Covid-19 severity (p= 0.039; OR= 0.456; 95% CI 0.213-0.976). In addition, Nie et al. found visitation to crowded places such as hospitals and traditional markets augment positive cases. However, the sources of contagion of some Covid-19 positive cases are still unknown.[21]

This study found the presence of symptoms serves as risk factors of Covid-19. Confirmed death cases show more symptoms than healed cases. From ten symptoms, seven symptoms show a higher percentage in death cases than healed that is fever (64.4 vs 27.1), dyspnea (67.8 vs 11.0), cough (62.7 vs 43.6), lethargic (33.9 vs 18.3), nauseous vomit (15.3 vs 8.7), diarrhea (13.6 vs 6.4), and loss of consciousness (3.4 vs 0.5). Sanyaolu et al.

showed the percentage of common symptoms similar with this study, that is fever (88.8%), dry cough (68%) and fatigue (33%), productive cough (28.5%), SOB (17%), muscle pain (14.4%), sore throat (11.4%), headache (10.2%), diarrhea (4.4%), nausea and vomiting (4.1%), rhinorrhea (3.2%), abdominal pain (0.16%), and chest pain (0.11%).[29] The high case of patients with symptoms is tightly related with ages and comorbidities. The risk for diseases severity increases along with age progression resulting in paediatrics tend to show no symptom (asymptomatic) compared with adults.[24] Although Byambasuren et al. found 6-41% asymptomatic cases,[30] but Qu et al. found some symptoms such dyspnea ($p<0.001$), shortness of breath ($p<0.01$) and chest distress ($p<0.05$) were correlated with death.[31]

This study found no difference in symptom duration between death cases and healed cases. This indicates that the duration of symptoms is not a risk factor for death in confirmed Covid-19 patients. However, the duration of symptom in this study was about 6 days, both in death cases (0 to 12 days) and healed cases (2 to 13 days). This study similar with Sanyaolu et al. found symptoms of Covid-19 appear 5 days (2 to 14 days).[29]

The presence of comorbidities can worsen patient conditions particularly those indicated for Covid-19. Zhou et al. found 91 patients (48%) have comorbidities and significantly correlated to Covid-19 related death ($p<0.5$). The comorbidities in death cases and healed cases are hypertension (48% vs 23%; $p=0.0008$), diabetes (31% vs 14%; $p=0.0051$), coronary heart disease (24% vs 1%; $p=<0.0001$), chronic obstructive lung disease (7% vs 1%; $p=0.047$), chronic kidney disease (4% vs 0%; $p=0.024$), and other (20% vs 8%; $p=0.016$).[19] Harlem found three most common chronic diseases involving hypertension (33% and 25 % respectively), obesity (28% and 15% respectively) and diabetes (15% and 8% respectively).[20] Not only adults, comorbidities also can be fatal among paediatrics. Oualha et al. found 19 paediatrics (70%) aged between 1 month and 18 years with comorbidities (neurological, respiratory, and sickle cell disease). Of five died paediatrics, two were died as a result of comorbidities.[32] A meta-analysis by Patel found 21% paediatrics has comorbidities such as asthma, immunosuppression and cardiovascular disease. The mortality rate of children that were hospitalized with Covid-19 was 0.18%.[33]

There are so many risk factors that are likely to contribute to the occurrence of death in confirmed Covid-19 patients while the risk factors examined in this study were only a small part. This is a limitation of this study. Apart from the risk factors in this study, other risk factors such as education level, smoking behavior, nutritional intake, physical activity, socioeconomic, body mass index (BMI) category, lifestyle-related factors (alcohol consumption) and other factors needs to be researched. Almost no confirmed cause of death for Covid-19 patients who died was caused by only one factor. This is the same as that found by Sanyaolu et al. that older patients with Covid-19, especially those 65 years old and above, who have comorbidities, are more likely to develop a more severe course and increased admission rate into the intensive care unit (ICU) and mortality from the COVID-19 disease.[29]

CONCLUSION

Age, occupation (entrepreneur and farmer/trader), contact history, symptoms (fever, dypsnea, cough, lethargic and cold), and comorbidities (diabetes, COPD, hypertension, cancer, heart disease, neurological disorders, and immune disorders) were risk factors of Covid-19 confirmed died patients in Dr. Kariadi Hospital. Meanwhile, gender, traveling history and duration of symptoms were not risk factors for death in Covid-19 confirmed patients in Dr. Kariadi Hospital. Adequate handling is needed to prevent death in patients with confirmed Covid-19 who have risk factors.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Original Article

Influence of TLR-8 Gene Polymorphisms (rs3764880 and rs3788935) Associated to Pulmonary Tuberculosis in Kupang, Indonesia

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ABSTRACT

Toll-like receptor 8 (TLR-8) is known as part of intracellular signaling transduction for bacterial phagocytosis. Mycobacterium tuberculosis (Mtb) is intracellular pathogenic bacteria that is recognized by this receptor, and genetic variation of TLR-8 might alter susceptibility of the host towards pulmonary tuberculosis (PTB). This study aimed to determine whether TLR-8 gene polymorphisms were associated to PTB in Kupang, Indonesia. This case-control study compared demographic and clinical data between 115 PTB patients and 115 controls, then two TLR-8 single nucleotide polymorphisms (rs3764880 and rs3788935) were explored using the GoldenGate® Genotyping for VeraCode® / BeadXpress Illumina®. There is no significant difference between sex distribution of patient vs control groups. The polymorphisms (rs3764880 and rs3788935) are in Hardy-Weinberg Equilibrium in this population (p > 0.05). The distribution of major vs minor genotypes and alleles of TLR-8 polymorphisms in PTB patients were as followed: rs3764880 (GG vs GA vs AA, 50.0% vs 21.4% vs 28.6% ; G vs A, 60.9% vs 39.1%) and rs3788935 (GG vs GA vs AA, 53.0% vs 21.7% vs 25.3%; G vs A, 62.9% vs 37.1%). Neither genotypes nor alleles were associated with PTB in this population (P > 0.05). Besides, when the analyses were stratified by gender, none of the alleles of polymorphism in both genders were associated with PTB cases. None of the TLR-8 polymorphisms have associated the risk of developing PTB in Kupang, East Nusa Tenggara population (as opposed to other studies in different ethnic groups). These might reflect the diversity of genetic polymorphisms in eastern Indonesia populations, suggesting different genetic backgrounds with western part of Indonesia.

Keywords: *Eastern Indonesia; Genetic polymorphisms; Pulmonary Tuberculosis; Toll-Like Receptor 8*

ABSTRAK

Toll-like receptor 8 (TLR-8) dikenal sebagai reseptor pathogen intraseluler terkait pensinyalan transduksi intraseluler setelah bakteri difagositosis. Mycobacterium tuberculosis (Mtb) adalah bakteri patogen intraseluler yang dikenali oleh reseptor ini. Variasi genetik pada gen TLR-8 memiliki kemungkinan asosiasi terhadap kerentanan tuberkulosis paru pada manusia. Penelitian ini bertujuan untuk mengetahui apakah polimorfisme gen TLR-8 berhubungan dengan kasus tuberkulosis paru di Kupang, Indonesia. Studi kasus-kontrol ini membandingkan data demografis dan klinis pada 115 pasien PTB dan 115 kontrol. Selanjutnya, dua polimorfisme nukleotida tunggal TLR-8 (rs3764880 dan rs3788935) dieksplorasi pada dua kelompok tersebut menggunakan GoldenGate® Genotyping for VeraCode® / BeadXpress Illumina®. Tidak ada perbedaan yang signifikan antara distribusi jenis kelamin pada kelompok pasien dan kelompok kontrol. Polimorfisme (rs3764880 dan rs3788935) berada dalam Ekuilibrium Hardy-Weinberg dalam populasi ini (p> 0,05). Distribusi genotipe dan alel mayor vs minor polimorfisme TLR-8 pada pasien PTB adalah sebagai berikut: rs3764880 (GG vs GA vs AA, 50.0% vs 21.4% vs 28.6% ; G vs A, 60.9% vs 39.1%) dan rs3788935 (GG vs GA vs AA, 53.0% vs 21.7% vs 25.3%; G vs A, 62.9% vs 37.1%). Baik genotipe maupun alel di atas tidak berasosiasi dengan tuberkulosis dalam populasi ini (P > 0,05). Selain itu, ketika analisis dikelompokkan berdasarkan jenis kelamin, tidak Ditemukan adanya hubungan antara genotipe maupun alel polimorfisme pada kedua jenis kelamin terahadap kasus tuberkulosis paru.

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Pada penelitian ini tidak satupun dari polimorfisme TLR-8 terkait dengan risiko kasus PTB di populasi Kupang, Nusa Tenggara Timur (dibandingkan dengan studi lain di kelompok suku yang berbeda yang menunjukkan adanya keterkaitan polimorfisme TLR-8 terhadap kasus PTB). Hal ini mungkin mencerminkan keragaman polimorfisme genetik pada populasi Indonesia bagian timur, menunjukkan latar belakang genetik yang berbeda dengan Indonesia bagian barat.

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

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INTRODUCTION

Tuberculosis still becomes a leading cause of morbidity and mortality among infectious diseases group, especially in low- and middle-income countries.[1] In 2015, the tuberculosis incident reached 10.2 million cases, and the mortality was 1.3 million cases globally.[1] Indonesia is ranked the third most prevalent countries in the world and about 845.000 new active tuberculosis cases emerged in 2018.[2]

Although M. tuberculosis (Mtb) is highly infectious, only 5-10 per cent of people infected with the bacilli had clinical manifestations; others remained to maintain latent infection status.[3] This might reflect different gene expression of innate and adaptive immune system controlling the disease expression. Recently, genetic polymorphisms in various loci had been investigated, showing some relation with complex susceptibility and disease expression of pulmonary tuberculosis (PTB).[4] Some of them were genes expressing toll-like receptors (TLRs), which have essential roles in recognizing pathogen by innate immunity frontline including Mtb.[5] One of these receptors, TLR-8, becomes one of the interesting genes to be explored in relation to PTB. Recent investigations showed that connection of TLR-8 polymorphisms in PTB susceptibility differed among race, gender, and age.[5–7]

Previous study has shown that TLR-8 has association with the PTB in Jakarta (western part of Indonesia), and other studies in Pakistan, Russia, South Africa, and Turkey described similar results.[8-10] However, some papers have also reported that these polymorphisms do not have significant Kupang, the capital city of East Nusa Tenggara province in Indonesia, has high PTB cases with positive acid-fast bacilli sputum examination (incidence rate 210 cases per 100,000 population in 2013).[13] Despite environmental and socioeconomic risk factors play essential role of PTB prevalence,[14-16] genetic predisposition might also affect this disease becomes highly close contact with dairy cows. prevalent in this area. Since the genetic background of people in East Nusa Tenggara (as more closely related to Melanesian and Polynesian people) is different with Java as western part of Indonesia (which has high relationship with Austronesian ancestry), we were intrigued to investigate how TLR-8 association might be differed between these groups. Therefore, this study was aimed to explore the association of TLR-8 gene polymorphisms towards PTB in Kupang, Indonesia.

MATERIALS AND METHODS

Patient recruitment and sample collection

This retrospective, case-control study was part of a bigger research conducted in Prof. Dr. W. Z. Johannes General District Hospital, Kupang, East Nusa Tenggara, Indonesia between January to September 2012. Case group comprised of adult (more than 15 years old) diagnosed as post-primary pulmonary tuberculosis based on clinical manifestation and radiographic thorax evaluation, then confirmed by positive sputum smear using Indonesian guideline for tuberculosis diagnosis and treatment.[17]

On the other hand, control groups comprised of healthy individuals with no clinical symptoms of PTB and proven negative sputum smear for acid-fast bacilli. Subjects with severe comorbidities, i.e. diabetes mellitus, asthma, cardiovascular disease, cancer, and autoimmune disease like systemic lupus erythematosus, were excluded. Before Genotypic examination was held in Institute of Tropical Diseases, Universitas Airlangga, Surabaya. enrolment in this study, subjects were explained about the study procedure and written informed consents were obtained. This study was reviewed and approved by the Ethical Committee Board from Faculty of Medicine, Universitas Padjadjaran (no. 136/UN6.C2.1.2/KEPK/PN/2012).

The subjects answered questionnaires regarding their demography and pulmonary TB data. Their venous blood then was collected in EDTA-tubes and stored in 4^{o}C before transported to Bandung, West Java, Indonesia. Routine blood profile tests were done using Hematology Analyzer Sysmex ® XT-2000i (Illnois, USA), Tokyo Boeky TRX 7010 (Tokyo, Japan), and ABX Pentra 400 (Horiba Medical, Kyoto, Japan). The samples were also examined for the human immunodeficiency virus (HIV) reactivity using Alere DetermineTM dipsticks (Alere Scarborugh Inc, Maine, USA). Subjects with reactive-HIV tests and random blood sugar level ≥200 mg/dL were also excluded.

Genotyping of TLR-8 gene polymorphisms

Genomic DNA in each sample was extracted using QIAamp DNA Blood Minikit, Qiagen according to its protocol, Several single nucleotides from TLR-8 gene were analyzed using the GoldenGate ® Genotyping Assay VeraCode ® / BeadXpress (Illumina, San Diego, CA, USA) according to the manufacturer's protocol.[18] The concentrations of DNA extracted from the samples were measured using spectrophotometer at absorbance 260 nm / 280 nm, and only DNA with concentration higher than 250ng/ml was included for further process according to the protocol (Illumina®).

In brief, the first step is DNA activation which enables DNA genomic samples to bind to paramagnetic samples, then hybridization was followed. Then, the BeadXpress ® Reader identifies microbead code and fluorescent signal. A laser beam goes through each of VeraCode ® microbeads to produce a unique code image during scanning. Illumina's GenomeStudio ® software (San Diego, CA, USA) analyzed the data generated in this process, then allele and genotype frequencies as well as distribution in each SNPs were counted and compared.

Statistical Analysis

Data statistic was performed in Microsoft Excel 365 (Microsoft Corp., Redmond, WA, USA). Demographic and clinical data in this study was examined using Kolmogorov-Smirnov test to see whether they are normally distributed. The comparison between numeric and categoric variables within groups were analyzed using Student t-test or Pearson chi-square, respectively. . In female subjects, genotype and allele frequencies were counted based on values predicted by Hardy-Weinberg equilibrium (HWE) using the Haldane exact test. Two SNPs were excluded due to deviation from HWE, and the remaining SNPs (rs3764880 and rs3788935) underwent further analysis. Analyses of genotype and allele frequencies of the SNPs among cases and control groups were compared using Pearson Chi's Square or Fischer's exact tests as appropriate, then odd ratios (OR) with 95% confidence intervals (95% CI) were also calculated.

RESULTS

A total of 230 subjects were enrolled in this study, comprised of 115 cases and 115 controls. The median of ages and gender distribution did not differ significantly between cases and control groups. Body-mass index and hemoglobin concentration were significantly lower, whereas leukocyte and thrombocyte counts were higher in PTB patients (Table 1). Among PTB patients, 79.1% percent of them

were newly diagnosed as pulmonary TB and the rest of them were either relapsed, defaulted, or failure to treatment category (Table 1).

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Table 1. Demographic and clinical characteristic of population study

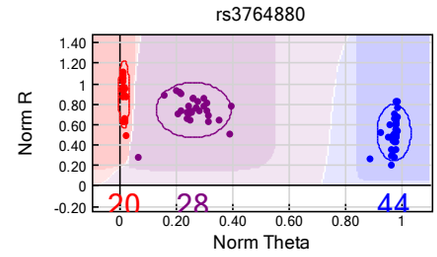
Parameter	TB Patients n=115	Control n=115	*P* value
Clinical characteristic			
Age, median (IQR)	34 (23)	35 (18)	0.543
Male, n (%)	58 (50.4%)	49 (42.6%)	0.234
BMI in kg/m^2, mean ± SD	15.38 ± 2.4	20.57 ± 2.6	<0.001*
Haemoglobin in gr/dL, mean ± SD	11.06 ± 1.9	13.24 ± 1.9	<0.001*
Leucocyte count in x10^3 cells/mm^3, median (IQR)	10.46 (4.95)	8.25 (3.34)	<0.001*
Thrombocyte count, x10^3 cells/mm^3 median (IQR)	387 (197)	260 (94)	<0.001*
Anemia, n (%)	92	31	<0.001*
Registration group			
Newly Diagnosed †	91	n/a	n/a
Relapsed / Defaulted ‡	22	n/a	n/a
Failure §	2	n/a	n/a

Notes: Test conducted in independent T-test or Mann U-Whitney test as appropriate. **P* values were significant if < 0.05. † Newly diagnosed cases have never had treatment for TB, or have taken anti-TB drugs for less than 1 month.

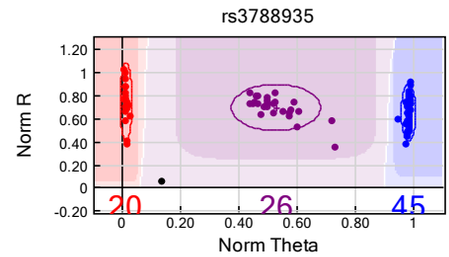
‡ Relapsed is defined as the cases had been treated completely and cured, but now it is relapsed; Defaulted is defined as patients had been declared loss to follow up before now it is treated again. § Failure is defined as those who have previously been treated for TB but the treatment failed at the end of their most recent course of treatment.

Abbreviations: BMI, Body Mass Index; IQR, Interquartile Range.

The frequencies of genotypes in one plate of 96 individuals were described for each polymorphism as shown using Genome Studio ® (Figure 1). Analysis of the genotype distribution of these polymorphisms of TLR-8 in this population showed no significant association with the risk of PTB (P > 0.005) (Table 2). Since the TLR-8 gene is located in chromosome X and might exhibit gender-inequality pattern distribution, sex-stratified analyses were also performed both in genotype and allele frequencies Again, no association was observed between these polymorphisms with the presence of PTB in the male and female subgroup (P > 0.005) (data not shown).



(a)



(b)

Figure 1. Distribution of SNP TLR-8 gene in Kupang population. As shown using Illumina's Genome Studio ®, (a) rs3764880 and (b) rs3788935. Every single dot represents one individual genotype in one plate for 96 individuals. Pink areas represent minor allele genotypes, purple area as heterozygote genotype, whereas blue areas represent major allele (wild type) genotype, respectively.

Table 2. Genotype and allele distributions of TLR-8 polymorphisms in Kupang population

Gene Polymorphisms	Cases, n (%)	Controls, n (%)	HWE P-value*	Chi-square P-value†
rs3764880				
Genotype				
GG or G-	42 (50.0)	30 (50.8)	0.066	0.264
AG	18 (21.4)	17 (28.8)		
AA or A-	24 (28.6)	12 (20.3)		
Allele				
G	81 (60.9)	62 (62.0)		0.865
A	52 (39.1)	38 (38.0)		
rs3788935				
Genotype				
GG or G-	44 (53.0)	27 (45.8)	0.164	0.653
AG	18 (21.7)	19 (32.3)		
AA or A-	21 (25.3)	13 (22.0)		
Allele				
G	83 (62.9)	58 (58.6)		0.508
A	49 (37.1)	41 (41.4)		

Notes: * *P* value was calculated in female population, *P* values > 0.05 indicate no deviation of the genotype from equilibrium. † Genotype chi-square calculation between dominant and intermediate vs recessive variants. *P* values were significant if < 0.05

Abbreviations: HWE: Hardy-Weinberg Equilibrium, CI: Confidence Interval

DISCUSSION

The linkage of PTB susceptibility and genetic polymorphisms had been studied intensively in recent decades.[5,19,20] TLR-8 gene was included in these interests due to its important part in macrophage endosomal pathogen sensing.[21,22] TLR-8 was firstly known as its function in response toward viral nucleic acids.[19] However, its role in recognizing various bacteria in macrophage endosome and induction of IFN-β had been uncovered lately.[19,21,22] Ex-vivo experiments showed that monocytes activated with living mycobacteria could induce follicular helper T-cell via TLR-8. Further real-time Polymerase Chain Reaction (PCR) analysis found that TLR-8 expressions were up-regulated in TB patients during their acute phase.[21] Whereas the exact mechanisms have still to be elucidated, several studies depicted an intricate and complex association between TLR-8 polymorphisms and tuberculosis susceptitibility in different gender distribution.[6] Davila et al in 2008 showed that these two TLR-8 SNPs (rs3764880 and rs3788935) were in perfect linkage disequilibrium.[8] This study further revealed that minor A-allele in rs3764480, which is located in Exon 1 and could change start codon in the transcription, as well as rs3788935 located in the regulatory region had a profound influence for increasing PTB for adult male in both Jakarta and Russia population.[8] Salie et al found that A-allele in rs3764880 was attributed to increased odds of developing tuberculosis in South African males.[9] A study conducted with Dalgic et al also identified a strong association of A-allele of rs3764880 with PTB susceptibility in male pediatrics patients.[7] On the other hand, Kobayashi et al found no significant association between any of TLR-8 polymorphisms and PTB susceptibility in several ethnicities of South East Asia (Javanese, Sundanese, and Vietnamese).[11] Hasheme-Shari et al in 2014 also revealed that rs3764880 was not risk factor for tuberculosis susceptibility in the Iranian population.[12] Interestingly, Bukhari et al found out that G-allele, rather than A-allele of rs3764880, was attributed to increased risk of PTB incidence and bacterial load in male Pakistani patients.[10] Most of these studies showed no similar association found in female population subsets, excepts in the South African population for three TLR-8 polymorphisms (rs3761624, rs37647879, and rs3764880).[9]

This study, according to our best knowledge, was the first case-controlled report regarding the TLR-8 genetic variation study concerning PTB susceptibility in East Nusa Tenggara, eastern part of Indonesia. None of these TLR-8's polymorphisms associated with PTB, which was in accordance with Kobayashi et al results but differed with Davila et al study.[8,11] Whereas Davila et al study were conducted in Jakarta (a metropolitan city which has heterogeneous ethnicities)[8], this study population was located

in Kupang, East Nusa Tenggara which could be assumed to have relatively homogenous ethnicities and genetic pools rather than Jakarta. Kupang city is located in Timor Island in East Indonesia, and the Timor Island inhabitants were postulated to be 'melting-pot' ancestor genes coming from the Melanesian and Polynesian population (via Sahul continental shelf) and Austronesia (via Sundaland continental shelf).[23,24] This unique 'melting pot' genetic background is further supported by Tumonggor et al study, who found that the maternal loci of West Timor population are dominated by Asian origin while paternal loci are dominated from Melanesian (Papuan) origin.[25] Therefore, this study findings might be caused by distinct genetic characteristics compared with western Indonesia studies.

CONCLUSION

There is no associated risk of having TLR-8 polymorphism in pulmonary TB occurrence among the population in Kupang, Nusa Tenggara Timur (as opposed to previous studies conducted in other different ethnic populations). Further studies in genetic polymorphisms should be explored to elucidate the susceptibility of people in these population to Mtb infection to understand more about the interaction of this microorganism and genetic host variability.

CONFLICT OF INTEREST

The authors declare no conflicts of interest in this work.

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Original Article

Intestinal Parasitic Infection, The Use of Latrine, and Clean Water Source In Elementary School Children At Coastal And Non-Coastal Areas, Sumenep District, Indonesia

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ABSTRACT

Inadequate latrine and water source cause transmission of intestinal parasitic infection, particularly in children. There is a lack information about it and it is needed to be investigated. This study aimed to compare the prevalence of intestinal parasitic infection, the use of latrine and clean water source in elementary school children at coastal and non-coastal areas in Sumenep District, Indonesia. An analytic observational study with cross sectional design was conducted in Dasuk Timur Elementary School located at coastal area, and Kolor II Elementary School at non-coastal area, Sumenep district, in January 2020. Intestinal parasites in students' stools were identified by microscopic examination using wet direct smear stained with lugol. The use of latrine and water sources were analyzed with questionnaire. A total of 68 children stools were collected from both elementary schools. Worm infections were not found. Thirty-one children (31/44, 70.5%) from Dasuk Timur Elementary School and eight children (8/24, 33.3%) from Kolor II Elementary School were infected with intestinal protozoan and significant difference (P=0.003, Chi-square test). Blastocystis hominis was highly found in stools of Dasuk Timur Elementary School's students (31/44, 70.5%) and significantly different from Kolor II Elementary School's students (P<0.0001, Chi-square test). Three children (3/44, 6.8%) from Dasuk Timur Elementary School were still practicing open defecation. Dasuk Timur Elementary School's students suffered from intestinal parasitic infection were mostly using non-piped water source (20/31, 64.5%) and were significantly different between two elementary schools (P=0.015, Fisher's exact test). Prevalence of intestinal parasitic infections in children was found higher in coastal than non-coastal area due to the commonly use of unclean water sources and inadequate latrine.

Keywords: *Intestinal Parasitic Infectio; Clean Water Source; Latrine, Elementary Children; Coastal Area*

ABSTRAK

Jamban dan sumber air yang tidak layak menyebabkan transmisi infeksi parasit usus, terutama pada anak. Sedikit informasi terkait jamban, sumber air dan infeksi parasit usus pada anak, sehingga perlu untuk diteliti. Penelitian ini bertujuan untuk mengidentifikasi perbedaan prevalensi infeksi parasit usus, penggunaan jamban, dan sumber air bersih pada anak sekolah dasar di daerah pesisir dibandingkan dengan bukan pesisir di Kabupaten Sumenep, Indonesia. Penelitian observasional analitik dengan desain cross sectional dilaksanakan di SDN Dasuk Timur berlokasi di daerah pesisir, dan SDN Kolor II berlokasi di daerah bukan pesisir Kabupaten Sumenep pada bulan Januari 2020. Parasit usus dalam tinja anak sekolah dasar diidentifikasi dengan pemeriksaan mikroskopis dari sediaan hapusan tinja basah yang tercat dengan larutan lugol. Penggunaan jamban dan sumber air dianalisis dengan kuesioner. Sebanyak 68 tinja anak dikumpulkan dari kedua sekolah dasar. Kecacingan tidak ditemukan. Sebanyak 31 anak (31/44, 70.5%) SDN Dasuk Timur dan 8 anak (8/24, 33.3%) SDN Kolor II terinfeksi protozoa usus dan berbeda chisquare test). Blastocystis hominis ditemukan banyak dalam tinja anak SDN Dasuk Timur (31/44, 70.5%) dan

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berbeda bermakna dengan anak SDN Kolor II (P<0.0001, Chi-square test). Tiga anak (3/44, 6.8%) dari SDN Dasuk Timur masih melakukan defekasi di tempat terbuka. Anak SDN Dasuk Timur yang terinfeksi parasit usus kebanyakan menggunakan sumber air non-pipa (20/31, 64.5%) dan berbeda bermakna antara kedua sekolah dasar (P=0.015, Fisher's exact test). Prevalensi infeksi protozoa usus pada anak ditemukan lebih tinggi di daerah pesisir dibandingkan di daerah bukan pesisir karena penggunaan sumber air tidak bersih dan jamban yang tidak layak.

Kata kunci: *Infeksi Parasit Usus; Sumber Air Bersih; Jamban; Anak Sekolah Dasar; Daerah Pesisir*

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INTRODUCTION

Parasitic infections are caused by intestinal helminth and protozoa that are very common in developing country, such as Indonesia. Parasitic infections can cause high morbidity and mortality in endemic area[1]. Intestinal parasitic infections are estimated to occur in 3.5 billion people around the world and the majority occur in children[2].

The intestinal helminth infections can cause iron deficiency anemia, impaired mental function and cognitive delevelopment that affect to children growth and development[3]. Intestinal protozoan infections also impact growth and development in children[2]. Children who consume contaminated food and water can lead to intestinal parasitic infections. Moreover, enviromental and economy factors, such as poor sanitation, poverty, and lack of education, also contribute to intestinal parasitic infections. Children have active period of playing and moving, then they forget to wash their hands that affect to the intestinal parasite transmission[4].

Based on Law Number 27 Year 2007 about Management of Coastal Areas and Small Islands, coastal areas are transitional areas between land and marine ecosystems which are affected by changes on land and sea. Indonesia is the largest archipelago in the world that consists of around 18,110 islands with coastline of 108,000 km5. This makes coastal areas in Indonesia a hope for people to fulfill life necessities. However, the environment will be damaged as the development blooms in coastal area. The damaged environment can trigger health problems and makes the disease transmission easier, such as water pollution, littering, and defecate in the open place [6].

The prevalence of parasitic infection in coastal area were reported by a study in Tanawangko, Tombariri Sub-district, Minahasa District found that 4.3% elementary school children were infected with Ascaris lumbricoides [7]. Another study conducted in coastal area of Wori Sub-district, North Minahasa District showed that 4.7% children suffered from intestinal helminth infections and 15.5% children were infected with with intestinal protozoa.[8] In addition, previous research in coastal area of Makassar City reported that the prevalence of intestinal helminth infections was 59.3% in children.[9]

Therefore, the incidence of intestinal parasitic infection is still quite high in Indonesia. Sumenep Regency is located at the eastern part of Madura Island and has a coastline length of 577.76 km. It can increase the risk of intestinal parasitic infections in coastal area.[10] A study conducted in Aeng Merah III Elementary School, Batuputih Sub-district, Sumenep District showed that 55.6% of 14 children who defecated on the ground and 44.4% of 20 children who defecated in latrine were infected with intestinal nematodes in 2014.[11] The prevalence of intestinal parasitic infection in elementary school children at Sumenep District has not yet been

investigated further. Thus, the identification of prevalence of intestinal parasitic infection with the use of latrine and clean water sources in elementary school children at coastal area and non-coastal area of Sumenep District, Indonesia were conducted in this study.

MATERIALS AND METHODS

Study Site and Population

An observational analytic study with cross sectional design was conducted in coastal area, Dasuk Timur (DT) Elementary School, and non-coastal area, Kolor II (KII) Elementary School, of Sumenep District, Indonesia in January 6-13, 2020. Sumenep district lies in the eastern part of Madura Island. The study sites is shown on Figure 1. Dasuk Timur School is 0.07 km from seashore and Kolor II School is 6.32 km. The distance of Dasuk Timur School from Kolor II School is 16 km.

Elementary school children in 1-4 grade, who were willing and allowed by their parents, were included in this study. Their stools and the questionnaires were collected. Sterilized-clean tool pots were distributed to children in both elementary schools. Children, who submitted stools pots and also fulfilled the questionnaire, were analyzed.

Data Collection

The stool samples of elementary school children were collected and preserved by 10%formalin solution. Stool samples were examined with direct smear using 1% lugol solution under light microscope with 200x magnifications for identifying intestinal helminth, and 1000x magnifications with immersion oil for detecting intestinal protozoa. The stool examination was performed in the Laboratory of Medical Parasitology Department, Faculty of Medicine, Universitas Airlangga, Surabaya. The use of latrine and clean water sources were determined by using questionnaires. Questionnaire was guided by researchers and teachers to the children. The latrine types consisted of household toilet, public toilet, and open air defecation in river, sea, or the bush. The clean water sources were composed by piped and non-piped water sources. The non-piped water sources consisted of river, sea, pond, and draw or artesian well water. The collected data were analyzed using 25.0 version SPSS program. The Chi-square test were used when

Ethical Consideration

This study was declared by Ethics Committee of the Faculty of Medicine, Universitas Airlangga (Number of 221/EC/KEPK/FKUA/2020).

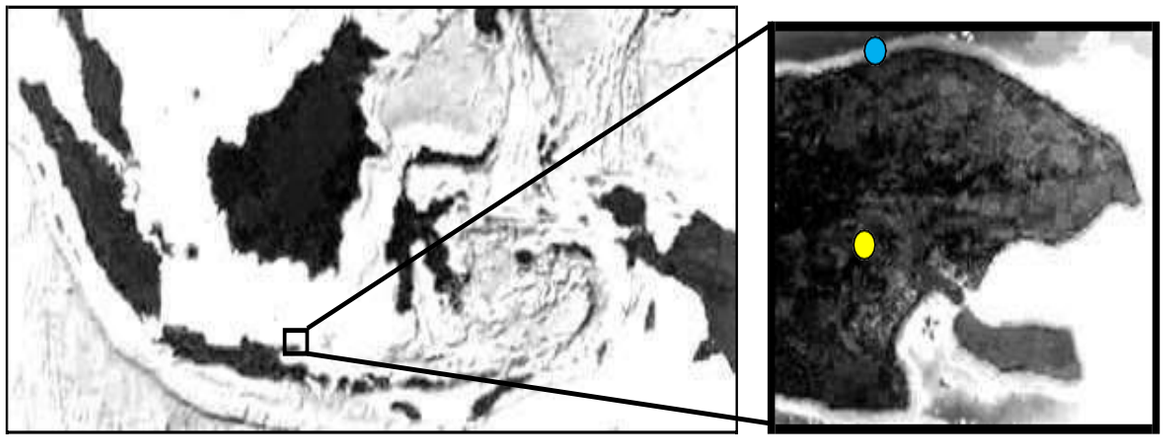

Figure 1. Study sites are located in Sumenep District, Madura Island, Indonesia that are in Dasuk Timur Elementary School (the blue circle) and Kolor II Elementary School (the yellow circle).

RESULTS

Characteristics of Subjects

Elementary school children from grade 1st to 4th were voluntarily to participate this study by filling data using questionnaire and submitting their stools that were totally of 68 children (68/155, 43.9%) from both elementary schools (see on Figure 2).

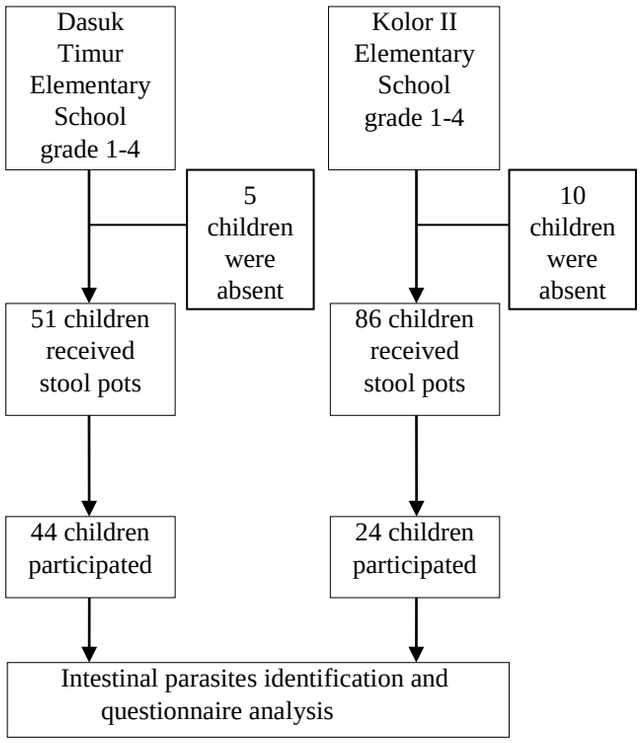

Figure 2. Flow diagram of sample collection

Most samples were obtained from Dasuk Timur Elementary School (44/68, 64.7%). The oldest participants (11-year old) were only found in Dasuk Timur Elementary School. However, there was no significant difference between the distribution of age from both schools. The distribution of children infected with intestinal parasites according to sex and age did not show a significant difference between the two elementary schools (see on Table 1).

Intestinal Parasitic Infections

The collected stool samples were examined with a direct smear or wet mount. Intestinal helminth infection was not found in children from both elementary schools (see on Table 2). Children infected with intestinal protozoan parasites were highly shown in Dasuk Timur Elementary School (31/39, 79.5%).

Table 1. The Characteristic of elementary school children

Characteristic	Study Site: Dasuk Timur Elementary School N (%)	Study Site: Kolor II Elementary School N (%)	*P* value
Sex			
Male	25 (56.8)	10 (41.7)	0.232*
Female	19 (43.2)	14 (58.3)	
Age (y.o)			
6	3 (6.8)	5 (20.8)	
7	8 (18.2)	5 (20.8)	
8	7 (15.9)	5 (20.8)	0.017+
9	10 (22.7)	7 (29.2)	
10	13 (29.5)	2 (8.3)	
11	3 (6.8)	0	
Infected Children Sex			
Male	15 (48.4)	5 (62.5)	0.695*
Female	16 (51.6)	3 (37.5)	*
Age (y.o)			
6	1 (3.2)	2 (25)	
7	7 (22.6)	1 (12.5)	
8	4 (12.9)	0	0.311+
9	8 (25.8)	4 (50)	
10	9 (29)	1 (12.5)	

Latrine

Three children (3/44, 6.8%) from Dasuk Timur Elementary School were still practicing open defecation in places such as a river, sea, or bushes, while all children from Kolor II Elementary School were defecating in the latrine. However it was not statistically significant between both elementary schools (p=0.336, Fisher exact test) (see on Table 4).

A total of 29 infected children from Dasuk Timur Elementary School used household toilet (29/31, 93.5%), while all Kolor II Elementary School children who suffered from intestinal parasitic infections used household toilet (8/8, 100%). It was no a significant difference found between two elementary schools (see on Table 4).

Table 2. Intestinal protozoan in stools of elementary school children

Intestinal Parasitic Infection		Study Site: Dasuk Timur Elementary School n=44 (%)	Study Site: Kolor II Elementary School n=24 (%)	*P* value
Intestinal Helminth				
Positive		0	0	
Negative		44 (100)	24 (100)	
Intestinal Protozoan				
Giardia lamblia	Single	0	0	0,536**
	Mix	2 (4.5)	0	
Entamoeba coli	Single	0	1 (4.2)	0,413**
	Mix	5 (11.4)	0	
Blastocystis hominis	Single	25 (56.8)	5 (20.8)	**<0,0001**
	Mix	6 (13.6)	0	
Cryptosporidium spp.	Single	0	2 (8.3)	0,121**
	Mix	0	0	
Negative		13 (29.5)	16 (66.7)	**0,003***

* P value is calculated using Chi-square test. P≤0.05 was significant.
** P value is calculated using Fisher's exact test. P≤0.05 was significant.

The infected stool samples consisted of 33 samples (33/39, 84.6%) with single infection and 6 samples (6/38, 15,8%) with mixed infection. *B. hominis* infection was found more frequently in Dasuk Timur Elementary School children (31/31, 100%) compared to Kolor II Elementary School children (5/8, 62,5%) and it was significantly different (*P*<0,05, *Chi-square test*) (see Table 2). Seventeen children (17/36, 45.7%) were infected with *B. hominis* by density of 1/20 field (see on Table 3).

Table 3. Blastocystis hominis density

Number of *Blastocystis hominis**	No. Infected Samples n (%)
1/20 fields	17 (47.2)
2/20 fields	4 (11.1)
3/20 fields	4 (11.1)
4/20 fields	1 (2.8)
5/20 fields	2 (5.5)
7/20 fields	5 (13.9)
10/20 fields	1 (2.8)
11/20 fields	1 (2,9)
40/20 fields	1 (2,9)
Total	36 (100)

*Number of B. hominis was counted by the field of 1000x magnifications with immersion oil in total 20 fields.

The intestinal protozoa were identified tha were cysts of *G. lamblia*, cysts of *E. coli* vacuolar type of *B. hominis* and oocyst o *Cryptosporidium spp*. (see on Figure 3).

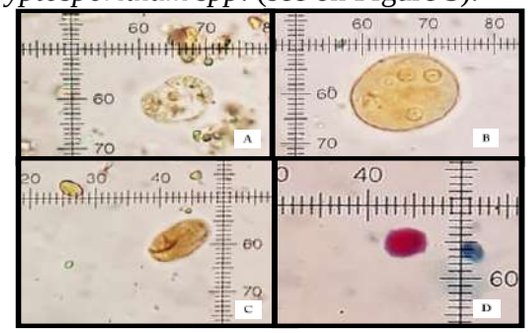

Figure 3. The morphology of intestinal protozoa in children's stool samples were (A) vacuolar type of B. hominis; (B) cyst of E. coli; (C) cyst of G. lamblia; and (D) oocyst of Cryptosporidium spp (modified Ziehl Neelsen stain). Minimal length is 1 micrometer.

Table 4. Latrine usage of elementary school children

Latrine	Study Site: Dasuk Timur Elementary School N (%)	Study Site: Kolor II Elementary School N(%)	*P* valu
Household Toilet	40 (90.9)	22 (91.7)	
Public Toilet	1 (2.3)	2 (8.3)	0.336
Open Defecation	3 (6.8)	0	
Infected Children			
Household Toilet	29 (93.5)	8 (100)	
Public Toilet	1 (3.2)	0	1.000
Open Defecation	1 (3.2)	0	

* P value is calculated using Fisher's exact test. P≤0.05 was significant.

Clean Water Sources

Non-piped water sources were mostly used by children from Dasuk Timur Elementary School compared to children from Kolor II Elementary School (28/44, 63.6% vs 2/24, 8.3%) and there was a significant difference between two elementary schools (P<0,05, Chi-square test) (see Table 5).

Non-piped water sources were mostly used by infected children from Dasuk Timur Elementary School compared to infected children from Kolor II Elementary School (20/31, 64.5% vs 1/8, 12.5%) and it was a significant difference between two elementary schools (P<0,05, Chi-square test) (see on table 5). The non-piped water sources in Dasuk Timur village are open draw well, artesian well, river, a pond and sea water, while in Kolor II

village is artesian well water (see on Figure 4).

Figure 4. The non-piped water types are A) an open draw well, B) an artesian well, and C) a pond with outfall.

Table 5. Clean water sources of elementary school children

Clean Water Source	Study Site: Dasuk Timur Elementary School N (%)	Study Site: Kolor II Elementary School N (%)	*P* value
Piped Water	16 (36.4)	22 (91.7)	**<0,0001***
Non-Piped Water	28 (63.6)	2 (8.3)	
Infected Children			
Piped Water	11 (35.5)	7 (87,5)	**0.015****
Non-Piped Water	20 (64.5)	1 (12,5)	

* P value is calculated using Chi-square test. P≤0.05 was significant.
** P value is calculated using Fisher's exact test. P≤0.05 was significant.

DISCUSSION

The present study determined that sex and age had the same probability to be infected with intestinal parasites. Molina[12] and Saputra[13] stated that there was no statistical difference in intestinal parasitic infections based on age and sex. Another study conducted in Sanandaj City showed that sex and age did not have a significant difference in intestinal parasitic infection[4]. Sex and age did not affect the incidence of intestinal parasitic infections at Dasuk Timur Elementary School and Kolor II Elementary School.

The present findings found no intestinal helminth infections in children from both elementary schools. The zero prevalence of intestinal helminth infection in children might due to the regular consumption of oral anthelmintic medicine. Based on interview to teachers, anthelmintic medicine, albendazole, is regularly consumed by children in their schools twice per year.The albendazole was provided by public health service or local public health center. Albendazole was last taken by children in September 2019 or 4 months before collecting the stools samples. Previous studies also showed the reduction of intestinal helminth infection in elementary school students after providing regularly albendazole and health education such as in Bunduduk elementary school Central Lombok[14], and Pagi Paseban elementary school Central Jakarta[15]. The prevalence of intestinal helminth infections in elementary school children can be eliminated by regular consumption of oral anthelmintic and education of intestinal helminth infections.

Blastocystis hominis was found the most in children's stools both from Dasuk Timur elementary school (31/31, 100%) and Kolor II elementary school (5/8, 62,5%) and significantly different (P<0,05, Chi-square test). It indicates that Dasuk Timur elementary school children are more at risk to be infected with *B. hominis.* In addition, they were without symptoms and did not have a history of diarrhea. Asymptomatic *Blastocystis* infection might due to rare number of *B. hominis* in their stools. It was reported that finding >.5 parasites per high- power field (400 magnifications) is associated with the presence of gastrointestinal disease[16]. Asymptomatic Blastocystis infection occurred in elementary school children in coastal and non-coastal areas. They could be carriers who were able to contaminate *B. hominis* into environment, particularly in the poor personal hygiene and sanitation.

Most of Dasuk Timur elementary school children used non-piped water sources and they were carrying the *B. hominis* in their stools. In addition, people in Dasuk Timur village including elementary children used to the wells water for their drink. Some of them used to without boiling wells water for drink (based on interviews with several children and teachers). *B. hominis* infection belongs to waterborne disease and transmitted by the fecal-oral route,

such as through food and water contaminated with feces containing B. hominis and poor sanitation in the community.[17,18] A study in Sanandaj City reported that a high prevalence of intestinal protozoan infection among school children occurred and the use of drinking water sources from unprotected wells was a risk factor of intestinal parasitic infection[4].

It was also found in Lao PDR that people used the water sources from mountain and wells water were infected with either intestinal helminth or protozoa.[19] This fact confirmed that unboiling non-piped water source for drink is potential to transmit the *B. hominis* into children living in Dasuk Timur village.

Poor sanitation facilities in coastal areas can also contribute to spreading the intestinal parasites infection, such as the inadequate supply of clean water, inadequate latrine, improper waste disposal, and littering.[6,20] Furthermore, a study in Karangasem District, Bali showed that 34% of elementary school children were infected with *B. hominis* and most of the children were still practicing poor sanitation, shared water with animals, and had a lack of household toilets[21]. Our study showed that the most children stools carrying *B. hominis* used the house toilets for defecation. Only two elementary children carrying *B. hominis* in their stools used to either open defecation or public toilet and they are living in Dasuk Timur. Nevertheless, they could contaminate the water source. Ironically, they still used unboiling non-piped water source for drink in Dasuk Timur village, so *B. hominis* transmission occurred more in Dasuk Timur than in Kolor II village. Therefore, the non-piped water source needs to be investigated further, whether contains *B. hominis* or not, in order to cut off the B. hominis transmission. Thus, coastal areas with non-piped water sources, without boiling water for drink, and still doing the open defecation can increase the risk of *B. hominis* infection.

Our findings showed that *G. lamblia* and *E. coli* were found more frequently in Dasuk Timur Elementary School children's stools compared to Kolor II Elementary School children's stools. These protozoan parasites also belong to water borne disease.[22,23,24,25] It showed that Dasuk Timur Elementary School children were more at risk to be suffered from intestinal protozoan infection because most of the children in the coastal area still used non- piped water sources. Gabbad et al revealed that difficulty accessing clean water in Elengaz, Khartoum, Sudan caused children suffering from intestinal parasites.[18] A study in a rural area of Boyer-Ahmad, Iran represented that the prevalence of intestinal protozoan infection was 37.5% with 9 species of protozoa found in stool samples, including G. lamblia, B. hominis, E. coli, and Endolimax nana. This high prevalence of intestinal protozoan infection might due to water shortages during decresed level of rainfall in Iran, which caused poor sanitation.[26] Recent study in Kenya stated that source of water for drinking was a major determinant for the risk of intestinal protozoan infections in children under 5 years with diarrhea.[27] Lack of access to clean water sources is one of the risk factors of intestinal protozoan infection.

CONCLUSION

Prevalence of intestinal parasitic infection was found higher in coastal than non-coastal area due to commonly use unclean water source and inadequate latrine.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Review Article

Plasmodium falciparum Breath Metabolomics (Breathomics) Analysis as a Non-Invasive Practical Method to Diagnose Malaria in Pediatrics

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ABSTRACT

Children under 5 years of age are particularly vulnerable to malaria. Malaria has caused 445,000 deaths worldwide. Currently, rapid diagnostic tests (RDTs) are the fastest method to diagnose malaria. However, there are limitations that exist such as low sensitivity in detecting infections with low parasitemia. Practical, non-invasive and high ability tests to detect parasite are needed to find specific biomarkers for P. falciparum infection to determine the potential of P. falciparum 4 thioether in breathomics analysis by GC-MS as a practical non-invasive method in diagnosing malaria in pediatrics. Literature reviews from Google Scholar and ProQuest were published no later than the last 5 years. The concept of breathomics is that the breath's volatile organic compounds (VOCs) profile is altered when the health condition changes. Breath samples from individuals infected with P. falciparum malaria were taken by exhalation. Through GC-MS analysis, it was found that 4 thioether compounds (allyl methyl sulfide (AMS), 1-methylthio-propane, (Z) -1-methylthio-1-propene and (E) -1-methylthio-1-propene) underwent a significant change in concentration during the infection. Based on experiments conducted on mice and humans, the breathomics method is known to be able to detect parasitemia levels up to <100 parasites/µL, has a sensitivity level of about 71% to 91% and a specificity of about 75% to 94%. The discovery of 4 thioether compounds by GC-MS is a strong indication of malaria, because it has the potential for high sensitivity and specificity, and the detection power exceeds the ability of RDTs.

Keywords: *Breath metabolomics; malaria; Plasmodium falciparum; volatile organic compound*

ABSTRAK

Anak di bawah usia 5 tahun sangat rentan terkena malaria. Penyakit ini telah menyebabkan 445.000 kematian di seluruh dunia. Saat ini, rapid diagnostic tests (RDTs) merupakan metode yang paling cepat dalam mendiagnosis malaria. Namun, masih terdapat kelemahan seperti sensitivitas yang rendah dalam mendeteksi pasien dengan parasitemia rendah. Pemeriksaan praktis, non-invasif dengan daya deteksi parasit tinggi dibutuhkan untuk menemukan biomarker yang spesifik terhadap infeksi P. falciparum. Mengetahui potensi 4 thioether dari P. falciparum dalam analisis breathomics melalui GC-MS sebagai metode praktis non-invasif dalam mendiagnosis malaria pada pediatrik. Tinjauan pustaka dari Google Scholar dan ProQuest dengan kriteria rilis paling lama 5 tahun terakhir. Konsep breathomics adalah profil SOV dalam napas berubah ketika terjadi perubahan kondisi kesehatan. Sampel nafas dari individu yang terinfeksi malaria akibat P. falciparum diambil dengan menghembuskannya. Melalui analisis GC-MS, ditemukan bahwa 4 thioether (allyl methylsulfide, 1-methylthio-propane, (Z)-1-methylthio-1-propene, dan (E)-1-methylthio-1-propene) mengalami perubahan konsentrasi secara signifikan selama infeksi berlangsung. Berdasarkan percobaan yang dilakukan pada tikus dan manusia, metode breathomics diketahui mampu mendeteksi level parasitemia hingga <100 parasit/µL dan memiliki tingkat sensitivitas sekitar 71% hingga 91% dan spesifisitas sekitar 75% hingga 94%. Penemuan 4 senyawa thioether melalui GC-MS menjadi indikasi kuat terhadap penyakit malaria karena memiliki potensi tingkat sensitivitas dan spesifisitas yang tinggi, serta daya deteksi melebihi kemampuan RDTs.

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Kata kunci: *Breath metabolomics; malaria; Plasmodium falciparum; volatile organic compound*

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INTRODUCTION

Children under 5 years of age are one of the most vulnerable populations in malaria. This disease has caused 445,000 deaths worldwide.[1] Based on World Health Organization (WHO) data in 2017, the incidence of malaria in Southeast Asia is still quite high.[2] According to the latest World malaria report, released on 30 November 2020, there were 229 million cases of malaria in 2019 compared to 228 million cases in 2018. The estimated number of malaria deaths stood at 409 000 in 2019, compared with 411 000 deaths in 2018.[1] In addition, malaria is still a public health problem, because it can reduce the quality of life and increase the economic burden; furthermore, it has the potential to cause a plague. Malaria is caused by Plasmodium spp infection such as Plasmodium falciparum; the vector is the anopheline mosquitos such as Anopheles gambiae.[3]

Recently, malaria diagnostic tests were carried out by examining thick and thin blood smears, rapid diagnostic tests (RDTs), polymerase chain reaction (PCR) and serological tests (antibodies).[4] Currently, thick and thin blood smears are still the gold standard in diagnosing malaria. However, this examination requires a blood sample, which is an invasive process especially for pediatric patients.[5]

RDTs are currently the most effective method for diagnosing malaria on a large scale. However, RDTs have a low sensitivity in detecting asymptomatic patients; low levels of parasitemia may give inaccurate results due to cross-reactions to autoantibodies (such as the rheumatoid factor in the case of the HRP2 test).[4,5] In addition, this method has a limited shelf life, and somewhat qualitatively less sensitive than laboratory-based quantitative tests.[6]

Therefore, a non-invasive, fast and simple examination method is needed to increase the effectiveness and efficiency of implementing malaria diagnostic tests clinically. Technological advances have led to new techniques, namely the breath metabolomic test for biological sample research.[7,8] From 2010 to 2015, there were two types of metabolomic tests, invasive (using blood and tissue samples) and non-invasive (using saliva, urine, feces and respiration samples).[9] Although urine and fecal samples can be used as noninvasive biological samples, the sampling process might be uncomfortable, leading to instability of sample quality.[10] Breath sampling is another non-invasive method that does not require any discomfort.[11]

At present, non-invasive examinations through expiratory breathing (breath metabolomics/breathomics) are being developed.[9,10,11] In *P. falciparum* malaria patients, specific volatile organic compounds (VOCs) have been found in their inhalation-expiration, namely 4 thioether compounds (allyl methyl sulfide (AMS), 1-methylthio-propane, (Z)-1-methylthio-1-propene and (E)-1-methylthio-1-propene) which have never been established as another disease biomarker. The VOCs analysis method of exhalation is practical and beneficial due to its ability to detect low levels of parasitemia, which could not be done by RDTs.[9,10,11] In addition, this method is non-invasive and suitable for children and HIV/AIDS patients who are at high risk of developing opportunistic infections. It won't cause any discomfort and the results of the study can be obtained immediately.[12,13]

Through this review, the authors deliver the VOCs' potential of P. falciparum in breathomics analysis via GC-MS as a practical non-invasive method for detecting malaria, especially in pediatric patients.

The Role of Volatile Organic Compounds (VOCs) in *Plasmodium falciparum* as an attractant for *Anopheles*

Malaria parasites produce volatile signals recognized by mosquito vectors that attract mosquitoes to facilitate the transmission process. Vectors of malaria have special interest in infected individuals.[14] Several research projects have been conducted to study the chemical signals produced by the malaria parasite to attract the malaria mosquitoes to bite the host. This study aims to develop a non-invasive diagnostic tool to detect malaria in pediatric patients.

Studies of host and vector interactions show that several VOCs play an important role in attracting mosquitoes to infected humans.[14,15] The study used the headspace solid-phase micro-extraction/gas chromatography-mass spectrometry (HSPME GC-MS) analysis method on VOCs composition of extracellular vesicles and supernatants of ultracentrifugation (SNUs), performed on P. falciparum cultures at both high and low parasitemia levels.[16] The concentration of VOCs were detected by this method are 1,2,3-propanetriol and diacetate (diacetin). The supernatant analysis, however, gave off 56 VOCs, with pentane 2,2,4-trimethyl being present in all the SNUs of uninfected erythrocytes but absent from the parasite-infected ones.[16]

In both infected and uninfected individual red blood cells, 18 VOCs were obtained with elevated levels of 1,2,3-propanetriol, diacetate (diacetin) in infected extracellular vesicles. Diacetin is an insect-attracting compound found in plants.[15,16] Based on HSPME GC-MS analysis and supernatant, diacetin was found in the majority of infected erythrocytes and was found only once in uninfected erythrocytes. In supernatant analysis, 56 VOCs were found. Pentane 2,2,4-trimethyl was present in all SNUs of uninfected erythrocytes and was not found in infected erythrocytes.[17] Hexanal, a mosquito attractant compound, was the only VOC present in all samples from SNUs of infected erythrocytes. This shows that the component was formed when red blood cells were infected. This hexanal component is considered as an An. gambiae attractant for low malaria transmission.[18,19]

Although this VOC has been detected in Plasmodium vinckei culture in vitro, there are no reports of hexanal in *P. falciparum* infection.[20] These compounds may form during peroxidation of cell lipid membranes, and when red blood cells are under stress conditions such as when they were infected.[21]

Another study showed that mosquito attraction behavior can also be influenced by host odor. The study compared the chemical composition and attractiveness of *Anopheles coluzzii* to a *P. falciparum* infected individual's skin odor, because there were differences in the composition of the skin odor between infected and uninfected individuals.[22,23] These positive samples were collected within a mean time of two days after the parasites passed from the liver to the peripheral blood, with low parasite counts in the asexual phase and absence of gametocytes.[23] Several identified volatile compounds (2- and 3-methylbutanal, 3-hydroxy-2-butanone and 6-methyl-5-hepten-2-one) in this phase are known to influence mosquito behavior.[24] In addition, the skin microflora also plays a role in changing the composition of odor by increasing the production of 2- and 3-methylbutanal and 3-hydroxy-2-butanone emissions.[25]

Research showed that *An. gambiae* can respond to VOCs in the form of terpenes and their derivatives (10-carbon monoterpene such as pinene and limonene) at low concentrations produced by *P. falciparum*. Therefore, it can bite infected individuals to continue the malaria transmission process.[23,24] *An.gambiae* detects VOCs via signals that pass through a ligand voltage channel known as odorant receptors (AgORs). A VOC, which is a low

pressure isoprenoid and hydrocarbon compound, is produced by non-photosynthetic plastid organelles (apicoplast) via the methylerythritol phosphate (MEP) route during the intraerythrocytic period. In addition, plants also carry out the production process of terpenes by apicoplast. At low concentrations, terpenes can directly mediate the attractiveness of Anopheles spp.[24]

P. falciparum produces distinctive terpenes annotated as 15-carbon sesquiterpene (4,5,9,10-dehydroisolongifolene) and its close derivative (8,9-dehydro-9-formyl cycloisolongifolene). Additionally, each infected sample contained at least one 10-carbon monoterpene. This monoterpene annotation varies between samples, but is included in compounds containing the structural compounds limonene and pinanediol (alpha-pinene derivatives). [24]

To evaluate whether terpenes in samples infected with malaria parasites were produced de novo by the parasites, researchers used fosmidomycin, a phosphonic acid antibiotic that blocks the first specific enzyme of the MEP pathway, deoxyxylulose phosphate reductoisomerase. High terpene concentrations usually repel mosquitoes, while pinene and limonene at low concentrations can attract the attention of *An. gambiae*. This study shows that *P. falciparum* generates a repertoire of VOCs that serve as interspecies chemical signals that modulate the attractiveness of mosquitoes to hosts.[24,25]

Apart from terpenes, there are several other sodium orthovanadate SOV compounds in patients infected with malaria that have the potential to attract mosquitoes, namely 2- and 3-methylbutanal, 3-hydroxy-2-butanone, 6-methyl-5-hepten-2-one, carbon dioxide, isoprene, acetone, benzene, cyclohexanone, and 4 thioether, allyl methyl sulfide, 1-methylthio-propane, (Z)-1-methylthio-1-propene, (E)-1-methylthio -1-propene Methyl undecane, dimethyl decane, trimethyl hexane, nonanal, tridecane, α-pinene monoterpenes and 3-carene.[25]

Analysis of Volatile Organic Compounds (VOCs) in Vitro

In vitro analysis to detect VOCs based on the research of Wong et al used two methods, through solid phase micro-extraction (SPME) and purge and trap/thermal desorption (PTTD), which is connected to gas chromatography-mass spectrometry (GC-MS).[17,18] Trophozoite stage parasitemia with a level of > 5% were flowed into 1% hematocrit with 1% O_2 and 5% CO_2 environment via SPME. Furthermore, PTTD optimization is carried out so that the environment is more supportive of parasite life. This is done by circulating 5% O_2 and 5% CO_2, as well as maintaining the level of parasitemia at 1% hematocrit by 20%. This is compared to other methods that usually only maintain a 5% hematocrit parasitemia level of 5%.[17,18,19] In addition, the use of containers in the shorter PTTD method and wider base area also increases the mass of parasites and the resulting VOCs so that the parasite suspension can be increased from 18 mL (using a volumetric flask) to 50 mL.[17]

In the VOCs analysis through headspace analysis, there were 100 chemical compounds in the control group and the *P. falciparum* infected group. No specific biomarkers to identify *P. falciparum* were found in two groups. The VOCs obtained from the two methods showed no significant difference. There are no specific compounds that can be obtained from *P. falciparum* even though the analytical method used is different.[20]

Because of the limitations of this in vitro study, in vivo studies to detect VOCs from breathing are suggested to provide more specific SOV results that can be used as biomarkers of infectious disease.[17,21]

Profile of Volatile Organic Compounds (VOCs) in Malaria-Infected Mice

The evaluation topic of VOCs as an indicator of certain diseases using GC-MS has become popular among in vivo research.[25,26,27] SOV can be formed either due to parasite metabolism or host response to infection.[28]

This discovery later became the development of a diagnostic tool with the help of gas sensor arrays (GSA). GSA contains selective sensors formed from a collection of 11 quartz microbalance gas sensors (QMB) where the material consists of a solid layer of porphyrin.[28,29] The gas sample can then be flowed into this device via a miniature diaphragm pump.[27,28,29]

Based on the research, which uses GC-MS and GSA in mice infected with the *Plasmodium berghei*, parasite, VOCs were correlated with the infection.[27] Although parasite concentration did not have a strong correlation with the number of SOVs, this study focused more on the changes in VOC patterns in infected mice. The SOVs found from *P. berghei* infection were 2-nitro-1,4 benzenedicarboxamide, nonanal, 2,6-bis (1,1-dimethylethyl)-4-(1-oxopropyl) phenol and 2-methyl-2-propanamine.[28] Furthermore, in order to increase the ability of ASF in identifying Plasmodium infection in mice, the partial least squares discriminant analysis (PLSDA) algorithm was used, so that the sensitivity increased to 91% while the specificity increased by 75%.[28,29]

4 Thioether Compounds as Specific Expiratory Respiratory Specimens for Human Malaria Infection Biomarkers

Breath analysis offers an inexpensive and fast diagnostic test for several types of diseases. The results of the exhaled breath reflect the composition of the VOCs contained in blood. The concept of breathomics is that the VOCs composition profile in the breath changes when there is an infection or metabolic abnormality. The VOCs' composition of exhaled breath has the potential to assess not only the presence or absence of disease, but also the severity, progression and response to treatment. However, many aspects still exist at the methodological level that must be improved and optimized to achieve this goal.[30]

Exhalation results can be used as an alternative diagnostic sample compared to more invasive blood.[28,29,30] Based on a study, breath samples taken from individuals infected with *P. falciparum*, malaria can be used to detect the presence of typical VOCs associated with this infection.[30] The health worker will take a sample of the patient's breath by asking the patient to exhale a little and briefly (saying "ha"), then stop for a moment and continue the normal expiration on the cardboard tube between their lips until it's finished. By this method, the alveolar air is stored in the bag of the tool.[27,28] Then, using GC-MS, 9 compounds were found with varying concentrations during malaria infection, namely carbon dioxide, isoprene, acetone, benzene, cyclohexanone, allyl methyl sulfide, 1-methylthio-propane, (Z)-1-methylthio-1-propene and (E)-1-methylthio-1-propene.[30]

Among these compounds, 4 thioether compounds (allyl methyl sulfide, 1-methylthio-propane, (Z)-1-methylthio-1-propene and (E)-1-methylthio-1-propene) experienced a significant change in concentration during infection.[31] These four compounds have never been determined as biomarkers for other diseases, so they can potentially become specific biomarkers for *P. falciparum* malaria.[30,31] These four compounds are also reported to be low in healthy people.[32]

Berna *et al.* conducted a cohort experiment in 2 groups, where the difference was only in the administration of antimalarial drugs. The first group was given OZ439, which is a fast-acting synthetic drug, while the second group was given slower-acting piperaquine.[12] 4 thioether compounds might not be detected or barely detected when there was no infection. Four days later after the infection, the level of 4 thioether compounds increased and exceeded a maximum level of 6.5 hours after being given the drug.[12] The maximum level obtained after drug administration, indicates the presence of crushed erythrocytes (ruptured schizonts) so that merozoites spread out and infect the red blood cells, alongside parasite death, thus stimulating the maximum release of thioether.[13,14] Of the four thioether compounds for the cohort 1 group, (Z)-1-methylthio-1-propene reach the highest increase, 100 times at drug administered group than at initial period when there was no infection. Meanwhile, for cohort 2, 1-methylthio-propane reached the highest increase, about 90 times. After that, the level of VOCs in both cohort groups decreased

with clearance of parasitemia.[14,15]

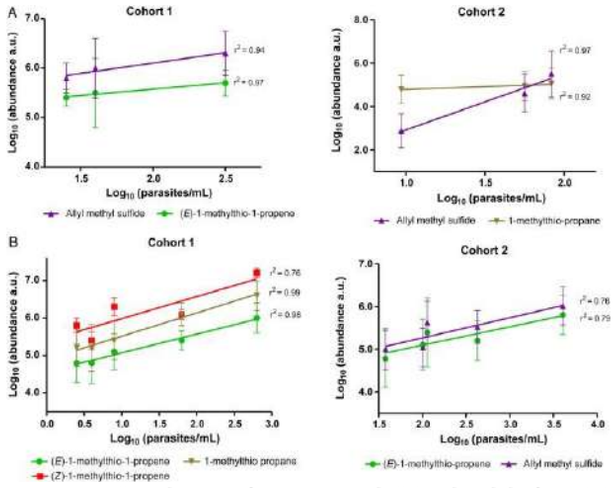

Figure 1. Correlation of parasite and SOV level before (A) using Allyl methyl sulfide and 1-methylthio-1-propene and after (B) using 1-methyltio-1- propene and 1-methylthio propane antimalarial treatment in 2 cohorts.[14] The thiother data were subjected to a phase shift of 24 hours, which revealed a direct correlation between parasitemia and volatile levels. In cohort 1, a fast-acting syhnthetic ozonide drug was used on day 7, and in cohort 2, a slower-acting piperaquine drug was administered on day 8. Points denote means and standard deviations.

As the result of the study above, it can be seen that the increase in VOC levels is directly proportional to the increase in blood parasitemia levels (Figure 1). In addition, the changes in VOCs of infected patients with low levels of parasitemia indicate that breath specimen analysis is a promising sensitive method in diagnosing malaria patients.[28,29,31] This method is able to detect parasitemia below the threshold level of rapid diagnostic tests against P. falciparum through PfHRP2 biomarkers (<100 parasites/μL).[31,32]

Another study conducted by Schaber et al., which analyzed breathomics using the breathprint method, was successful in diagnosing malaria infection in more than 80% of children with fever symptoms.[29] In addition, it shows 94% specificity and 71% sensitivity to Plasmodium falciparum. A higher sensitivity value is required to apply this diagnostic method widely. Nonetheless, these studies prove that breathprint analysis can be further refined to develop a safer and less invasive diagnostic tool. Such tests can be applied at a larger population level to monitor changes in malaria prevalence in endemic areas.[28,29]

Various Methods in Detecting 4 Thioether Compounds as a Breathomics Analysis Tool

Four thioether compounds that are *P. falciparum*-specific biomarkers can be detected using various techniques. Techniques include mass spectrometry (MS), e.g. gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and proton transfer reaction mass spectrometry (PTR-MS); ion mobility spectrometry (IMS); differential mobility spectrometry (DMS); electronic nose device; colorimetric tests: infrared spectroscopy (IR spectroscopy), e.g. selected ion flow tube-mass spectrometry (SIFT-MS), fourier transform-infrared (FTIR) spectroscopy and ring-down cavity spectroscopy.[31,32]

Mass spectrometry interacts with ionizing molecules to produce charged molecules or molecular fragments; it further measures the mass to charge ratio, which can be used in conjunction with chromatographic separation techniques. LC-MS is an analytical method that combines the features of liquid chromatography and mass spectrometry to identify compounds. PTR-MS is a very sensitive technique for online monitoring of VOCs.[32] PTR-MS has high sensitivity (into the low pptv range) and a fast response time (in the 40-100 ms time regime). IMS is an analytical technique used to separate and identify ionized molecules in a gas phase, based on their mobility in a carrier buffer gas. This technique can be combined with mass spectrometry and/or chromatographic separation techniques.[33] DMS is distinguished by the difference between mobility in high and low electric fields because the value of ion mobility depends on the strength of the applied field. This method is easy to use, sensitive, fast and relatively selective. However, the use of mass spectrometry has several limitations, including the large size of the instrument and the difficulties to carry (non-portable). It is suggested that future device designs can be made more portable and easier to use by health workers clinically. In addition, processing sample data by mass spectrometry requires multiple data processing and analysis software,

as well as manual checking of raw data. This takes a long time, so it is recommended for researchers to develop simpler software to process the diagnosis results from breathomics.[34]

Currently, a portable version of mass spectrometry has been developed in a device called an electronic nose (E-nose) that detects the VOCs' composition of the breath sample.[35] This technique is also known as "electronic sensing" or "e-sensing". The E-nose includes three main parts: the sample delivery system, detection system and compute system. The detection system, which consists of a sensor set, is a 'reactive' part of the instrument. When in contact with VOCs, the sensor reacts and undergoes a change in electrical properties. On the E-nose, each sensor is sensitive to some volatile molecules, but each has a specific way and it depends on the sensor used.

This tool is a handful size so it's easy to carry everywhere. In addition, this tool can be used multiple times, then it is expected to have lower costs and more accurate and less invasive results compared to RDTs. This advantage makes this tool ideal to be used en masse, especially for screening.[36] Changes in the VOCs' composition of exhaled breath provide relevant information for diagnosing several diseases other than malaria, including cardiovascular disease, neurodegenerative diseases, oncogenic diseases, infectious diseases, diabetes mellitus and liver and kidney diseases.[37,38]

CONCLUSION

A practical, non-invasive method of examining malaria with a high level of sensitivity and specificity will be beneficial for a clinician to diagnose malaria, especially in pediatric patients. By using the VOCs' analysis method of exhaled gas (breathomics) from patients living in endemic areas with risk factors and clinical symptoms of malaria caused by Plasmodium falciparum, hopefully it can help to identify the *P. falciparum* parasite. The discovery of 4 thioether

CONFLICT OF INTEREST

There is no conflict of interest of this study.

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Original Article

Validity of Method for MTBC and NTM Detection in FNAB Specimens from Tuberculous Lymphadenitis Using Microscopy, XPERT MTB / RIF and Culture Method

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ABSTRACT

Mycobacterium tuberculosis and Nontuberculous Mycobacteria usually cause infection in tuberculous lymphadenitis. To improve accuracy of the detection MTB and NTM bacteria it is necessary to select valid methods. This study aims to compare validity of diagnostic methods from FNAB specimens for determining tuberculous lymphadenitis patients. a descriptive observational laboratory study involved 35 samples were obtained from tuberculous lymphadenitis patients in Dr. Soetomo Hospital Surabaya East Java. All specimens examined Ziehl-Neelsen staining microscopy, Xpert MTB/RIF , culture method Middlebrook7H10 solid media and MGIT as Gold standard. Identification of MTB dan NTM with SD Bioline TB Ag MPT64 and niacin paper strip BD . Used diagnostic test 2x2 to analyze sensitivity, specificity, negative predictive value and positive predictive value. Ziehl-Neelsen staining microscopy Sensitivity 83,33 % and Specificity 95,65% of , PPV 90,91%and NPV 91,67%, Diagnostic Accuracy 91,43 % . Xpert MTB/RIF Sensitivity 75% and Specificity 95,65% , PPV 90 % and NPV 88 %, Diagnostic Accuracy 88,57 % with 95% CI (Confidence Interval) . Characteristics female dominated 23/35 (65.7%) while Male numbered 12/35 (34.3%), age range distribution of TB lymphadenitis patients is highest in young adults 17 years to 25 years as many as 15/35 (42.9%) the second highest is the age group of 36 years to 45 years by 8/35 (22.9%), Clinicial presentation are mostly lymph node enlargement in cervical 37% patients other locations supraclavicular ,mamae. Clinical symptoms mostly lymphadenopathy 31,5% and other lymphadenopathy with fever. Microscopy method still have the good validity shoul be conjunction with the molecular rapid tests and culture as gold standard in determining the diagnosis of TB lymphadenitis.

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

ABSTRAK

Mycobacterium tuberculosis dan Non Tuberculous Mycobacteria merupakan penyebab infeksi pada limfadenitis tuberkulosis. Untuk meningkatkan akurasi isolasi bakteri MTB dan NTM diperlukan pemilihan metode yang valid. Penelitian ini bertujuan membandingkan validitas metode diagnostik deteksi MTB dan NTM serta karakteristik pasien limfadenitis TB dari spesimen FNAB . Penelitian dilakukan di Rumah sakit Dr. Soetomo Surabaya jawa timur bersifat deskriptif observasional diperoleh sebanyak 35 sampel FNAB pasien limfadenitis TB . pemeriksaan yang dilakukan adalah Pemeriksaan mikroskopis menggunakan pewarnaan Ziehl-Neelsen, tes cepat molekuler Xpert MTB/RIF, kultur media padat Middlebrook7H10 . Standar emas pada penelitian ini menggunakan metode kultur media cair MGIT. Identifikasi MTB dan NTM dilakukan dengan SD Bioline TB Ag MPT64 dan niasin paper strip BD. Analisis sensitivitas, spesifisitas,nilai duga negatif dan nilai duga positif menggunakan uji diagnostik tabel 2x2 . . Pemeriksaan mikroskopis pewarnaan Ziehl-Neelsen dengan CI (Confidence Interval) memiliki 95% memiliki nilai sensitivitas 83,33 % dan spesifisitas 95,65%, nilai duga positif 90,91%, nilai duga negatif 91,67%, diagnostik akurasi 91,43 %. Metode diagnostik tes cepat molecular Xpert MTB/RIF sensitivitas 75 % , spesifisitas 95,65 %, nilai duga positif 90 %, nilai duga negatif (88 %, dan diagnostik akurasi 88,57 %. Karakteristik perempuan mendonina-

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si sebanyak 23/35 (65.7%) laki-laki berjumlah12/35 (34.3%), 17 tahun sampai 25 tahun sebanyak 15 orang 15/35 (42.9%) terbanyak kedua adalah kelompok usia 36 tahun sampai 45 tahun sebanyak8/35 (22.9%). Pasien dengan gejala benjolan pada leher sebanyak 37%, lokasi lain supraklavikula, mamme. gejala klinis paling banyak mengalami gejala klinis pembesaran kelenjar getah bening saja sebanyak 31,5%,dan gejala klinis lainnya berupa pembesaran kelenjar getah bening dan febris Pemeriksaan metode mikroskopik masih memiliki validitas yang baik , pemeriksaan yang dilakukan dengan dukungan tes cepat molekular dan kultur sebagai gold standar dapat membantu menegakkan diagnosis limfadenitis TB.

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

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INTRODUCTION

Tuberculosis is an infectious disease that is still a health problem in the world because of the high burden of mortality and morbidity. World Health Organization (WHO) reported in 2017 number of TB cases are currently 254 per 100,000 or 25.40 per 1 million population.[1] Indonesia has the number of new cases 420,994 cases in 2017,[2] in 2018 Estimated 10.0 million range (9.0 – 11,1 million) people ill with TB Incidence of extra pulmonary TB cases in the world estimated increase 14% from total of 6.4 million TB cases in 2017. Detection of Tuberculous lymphadenitis is quite difficult because the symptoms are often not typical, course of infection depends on patient risk factors and definitive diagnostics can be established based on the discovery of microbes causing infection.[3]

This study aims to compare validity of diagnostic methods specimens examined Ziehl-Neelsen staining microscopy, Xpert MTB/RIF, culture method Middlebrook7H10 solid media and MGIT as Gold standard from FNAB specimens for determining tuberculous lymphadenitis patients.

Microscopic examination with Ziehl-Neelsen staining method is first examination to identify acid fast bacilli (AFB) using a binocular microscope but rarely positive results because of paucibacillary nature of MTB bacteria in tissues.[5] Ziehl-Neelsen staining microscopy Sensitivity range 78,3%- 83,33 % 20. Molecular test Xpert MTB/RIF Assay (Cepheid, USA) is a diagnostic tool recommended by WHO and available in various health care facilities in Indonesia, detect DNA MTB as well as mutations in the rpoB gene that cause resistance to rifampicin.[6] The relevant literature.

Culture method from specimen is still a gold standard in growing bacilli with sensitivity range 70% to 80%. Advantage culture examination to avoid risk of false negative results and obtain MTB isolates for identification. Positive results do not always indicate the presence of living or viable microorganisms. A quick and accurate diagnosis is very important so that there is no over diagnose or under diagnose. Moreover patient can get TB treatment immediately and provide other benefits, such as reducing disability and death rates and preventing TB transmission to others.[7]

MATERIALS AND METHODS

This research is a descriptive observational laboratory testing the validity. FNAB spesimens were taken from April 2019 until June 2019 at the anatomical pathology laboratory.

Material culture method using Microbiology Systems-.BD BACTEC™ MGIT 960 System . Identification of MTB dan NTM with SD Bioline TB Ag MPT64 and niacin paper strip from Becton Dickson company.

Method Ziehl-Neelsen staining microscopy procedure using fuchsine staining 3 minutes, alcohol acid solution staining for 30 seconds and methylene blue staining for 30 seconds, Xpert MTB /Rif using aspirate

samples according manufacture ‘s protocol sample reagent was added in a 2:1 ratio to unprocessed falcon tube, incubation 15 minute at room temperature. And add 2 ml material to cartridge and loaded to Genexpert machine. Culture was put up after decontamination samples on media slopes following the standard protocolMicrobiology Systems-.BD BACTEC™ MGIT 960 System. Used diagnostic test 2x2 to analyze sensitivity, specificity, negative predictive value and positive predictive value.

Ethical Clearance This study received approval from the health research ethics committee of Dr. Soetomo Hospital Surabaya with the statement of ethical clearance of 1232 / KEPK / V / 2019.

RESULTS AND DISCUSSION

Demographic data obtained from medical records April to June 2019 most lymphadenitis patients came from Surabaya with 21/35 (60%) and 14/35 outside Surabaya (40%). No data available incidence of tuberculous lymphadenitis disease at the hospital Dr. Soetomo therefore needs further epidemiological research. Characteristics of gender female dominated as many as 23/35 (65.7%) while Male numbered 12/35 (34.3%) in line with other studies women dominated as many as 120 people (58.8%).[8,9, 10]

Female factors are more dominant because of several factors such as differences in biological, hormonal, social, environmental and different behavior from men. Male and female immune systems can be indicative of underlying causes for various patterns of disease in women. Socially in developing countries, women are more vulnerable because they have low economic status, which makes it late for someone to come to a health care center.[10] The age range distribution of TB lymphadenitis patients is highest in young adults 17 years to 25 years as many as 15/35 (42.9%) the second highest is the age group of 36 years to 45 years by 8/35 (22.9%). Based on the background of the work status there were most 16 /35 employees (46%), the second highest was the non working group.

Because some people have been exposed to people who are suspected of having tuberculosis. Other studies also get the same distribution in the age range of 22 years to 44 years due to the productive age affects the high risk of TB.[11] TB lymphadenitis cases can occur 60 to 80% in people with HIV because TB is an opportunistic infection in people with HIV-AIDS and this disease is found so that in patients with suspected TB lymphadenitis should be screened for HIV co-screening.

Clinical presentation lymph node enlargement mostly in cervical 37% patients other locations supraclavicular mamae. Clinical symptoms mostly lymphadenopathy 31,5% and other lymphadenopathy with fever. Clinical symptoms are one of the conditions for establishing a diagnosis of TB lymphadenitis, but in other studies it was found that clinical syndromes cannot be used as a single basis in determining the diagnosis of TB lymphadenitis because there are many variations in results because it is difficult to determine when the patient first experiences a complaint, subjectivity patients various.[12] Mostly lymph node enlargement sites in collie area, Other studies also have the same data which is located in the cervical area.[13]

Totally 35 specimens were examined 11/35 (31.4%) showed positive smear acid fast bacilli (AFB) and 24/35 (68.6%) negative similar to the results of other studies a total of 120 patients affected by TB lymphadenitis as many as 26 samples (21.7%) found positive smear and 94 samples (78.3%) did not find smear,[14] MTB bacilli were rare found in lymph node tissue because of its Paucibacillary nature, low positivity depends on the average number of aspirate aspirates FNAB 1000 to 10,000 / ml sample, Liquid Culture method MGIT and solid culture method showed positive results 12/35 (34,3 %) and negative 23/35 (65,7%) (Figure 1) (Figure 2), to confirm presence of mycobacteria from positive culture using rapid identification tests SD Bioline TB Ag MPT64 (Figure 3) and niacin paper test showed positive MTBC species 10 /12 (84%) and 2/12 (16%) reported as NTM species.

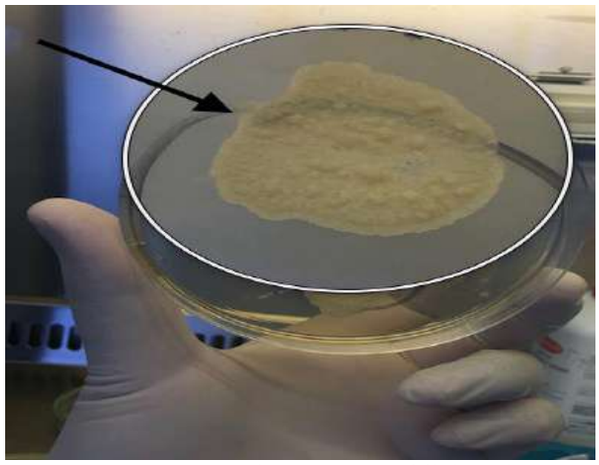

Figure 1. MTB colonies on Middlebrook7H10 medium

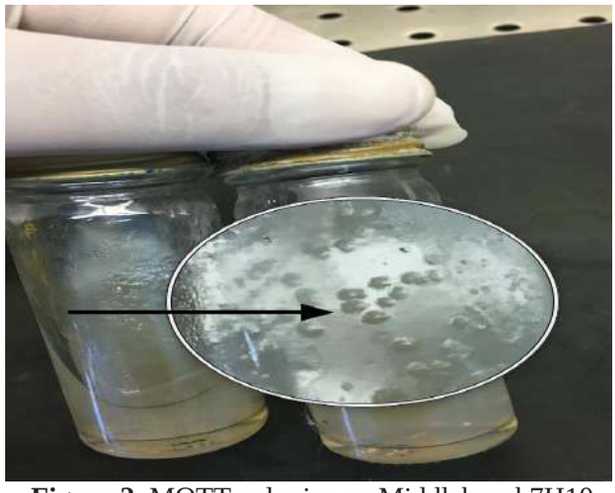

Figure 2. MOTT colonies on Middlebrook7H10 medium

The culture method is a Gold standard examination aimed at isolating MTB bacteria from samples of patients suspected of having TB lymphadenitis. The sensitivity value is quite high because the number of bacteria from 10 to 100 bacilli / ml from concentrated specimens can be detected.[15] Examination of culture methods at the Clinical Microbiology Laboratory Dr. Soetomo uses the Microbiology Systems -.BD BACTEC ™ MGIT 960 System diagnostic tool. Process of decontamination can potentially cause the death of MTB bacilli, too acidic and too alkali conditions can also cause MTB bacilli death and failure to thrive.[16] Other studies comparing the Loweinsten Jensen and Middlebrook 7H10 solid growth media found differences in the growth period of Middlebrook faster than the growth in Loweinsten Jensen media.[17] The sensitivity of the MPT 64 TBAg test kit in differentiating MTB and MOTT 100%.[18]

The results of the examination Molecular test Xpert MTB / RIF Assay (Cepheid, USA) 35 patients with suspected TB lymphadenitis showed that 10/35 (28,69%) patients were positively detected by the M. tuberculosis bacterial gene and negative results in 25/35 (71,4%) . Results of the reading of the detected MTB GeneXpert can be known quantitatively the level of MTB detection using Xpert MTB/RIF tool and categorized as follows: MTB detected low rif resistance not detected 8 samples (80%) and 2 sampels (20%) MTB detected very low rif resistance not detected.

The validity test results in this study used the analysis of the sensitivity and specificity of the FNAB specimens by using the Wilson diagnostic analysis table with 95% confidence interval microscopic method of Ziehl-Neelsen staining (ZN), comparison with the gold standard Culture method MGIT obtained a sensitivity value of 83.33% the ability of Ziehl-Neelsen microscopic staining methods to identify with smear positive results in TB lymphadenitis patients is quite high. Specificity Value of 95.65% with 95% CI means that the ability to find out negative and true results of no AFB in Tuberculous lymphadenitis patients is 95.65%. A positive predictive value of 90.91% means that the probability of AFB being present on microscopic examination if the results of a positive diagnostic test is 90.91%. A negative estimate value of 91.67% means that the probability of not having AFB if the diagnostic test is negative is 91.67%. Diagnostic accuracy of 91.43%. In line with other studies namely sensitivity of 83% and specificity of 98%.[19]

		Culture MGIT	
		Positive	Negative
microscopic	Positive	10	1
	Negative	2	22

Table 1. The result of diagnostic test

Sensitivity value: 83,33%
Specificity Value: 95,65% with 95% CI
positive predictive value of 90.91%: 90,91%
negative estimate value of 91.67%: 91,67%
Diagnostic accuracy of 91.43%.: 91,43 %

The validity test results in this study use the Wilson Wilson diagnostic analysis Table 1 with 95% CI method of molecular Xpert MTB / RIF rapid test comparison with the MGIT culture method as the gold standard. The results of this study obtained a sensitivity value of 75% which means that the ability of the examination of the rapid molecular Xpert MTB / RIF method in identifying MTB bacterial DNA in TB lymphadenitis patients is 75%.Specificity Value of 95.65% with 95% CI means that the ability to find negative and true results of absence of MTB bacterial DNA in TB lymphadenitis patients is 95.65%, positive predictive value of 90% means that the probability of MTB bacterial DNA if positive diagnostic test results is 90%. An estimated negative value of 88% means that the probability of the absence of MTB bacterial DNA if the diagnostic test is negative is equal to 91.67%. Diagnostic accuracy of 88.57%. Specificity (95.65%) in this study is in line with other studies where the specificity of Xpert MTB / RIF is 91%.[20,21,22] Regarding the negative results of the molecular nuclei acid amplification test need procedure of specimen collection . Although the sensitivity value of this study is lower than the specificity value, a high enough specificity value of 95.65% can help in establishing the diagnosis that the patient did not have TB lymphadenitis.

CONCLUSION

TB lymphadenitis diagnostic examination can penot be done only rely on one method in establishing the diagnosis of TB lymphadenitis The distribution of MTB and NTM is very helpful in providing therapy based on the causative agent for TB lymphadenitis infection.

CONFLICT INTEREST

There is no conflict interest of this paper.

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Original Article

Correlation between Climate Factors with Dengue Hemorrhagic Fever Cases in Surabaya 2007 – 2017

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ABSTRACT

Dengue Hemorrhagic Fever (DHF) is a disease caused by dengue virus. DHF is mediated by the mosquito vector, the Aedes mosquito. The proliferation of dengue vector is influenced by many factors, one of which is climate factors. DHF is one of the main public health problems in Indonesia. Cases of dengue were first discovered in 1968 in the city of Jakarta and Surabaya. Currently Surabaya is one of the dengue endemic areas in Indonesia. . The case of DHF in the city of Surabaya can be said to be still quite high compared with another city in Indonesia, although there is a decrease in the number from year to year. When examined, many factors influence the high number of dengue cases in Surabaya, one of which is climate factor. Climate factors play a role in the proliferation of DHF vectors. Therefore, this study aims to examine for 10 years, namely in 2007 - 2017 whether there is a correlation between climate factors with dengue cases in the city of Surabaya., which in this study the climate factors used are rainfall, average temperature, and average air humidity. This research uses an analytical method namely Spearman on the SPSS software version 20. The results obtained that the case of DHF in the city of Surabaya has no relationship with climatic factors such as rainfall and average temperature with a significance value of the relationship p> 0.05. While the climate factor that has a relationship with DHF cases in Surabaya City is air humidity with a significance value of p <0.05 and has a positive relationship with the value of r = + 0.190. It can be concluded that not all climate factors have a relationship with the DHF case in Surabaya in 2007 - 2017, which has a relationship with the DHF case is air humidity.

Keywords: *DHF case; climate factors; humidity; Surabaya; 2007 - 2017*

ABSTRAK

Demam Berdarah Dengue (DBD) merupakan penyakit yang disebabkan oleh virus dengue. DBD diperantarai oleh vektor nyamuk yaitu nyamuk Aedes. Perkembangbiakan vektor demam berdarah ini dipengaruhi oleh banyak faktor salah satunya adalah perubahan iklim. DBD merupakan salah satu masalah kesehatan utama masyarakat di Indonesia. Kasus demam berdarah pertama kali ditemukan pada tahun 1968 di Kota Surabaya. Saat ini Surabaya merupakan salah satu daerah endemis DBD di Indonesia. Kasus DBD di Kota Surabaya sendiri dapat dikatakan masih cukup tinggi apabila dibandingkan dengan kota lain di Indonesia walaupun terlihat ada penurunan jumlah dari tahun ke tahun. Apabila ditelaah, banyak faktor yang mempengaruhi masih tingginya kasus DBD di Kota Surabaya, yang salah satunya adalah faktor iklim. Faktor iklim berperan dalam perkembangbiakan vektor DBD. Maka dari itu, penelitian ini bertujuan untuk meneliti selama 10 tahun, yaitu tahun 2007 – 2017 apakah ada hubungan antara faktor iklim dengan kasus DBD di Kota Surabaya, yang pada penelitian ini faktor iklim yang digunakan adalah curah hujan, suhu rata-rata, dan rata-rata kelembaban udara. Penelitian ini menggunkan metode analitik yaitu Spearman pada perangkat SPPS versi 20. Didapatkan hasil bahwa kasus DBD di Kota Surabaya tidak mempunyai hubungan dengan faktor iklim berupa curah hujan dan suhu rata-rata dengan nilai signifikansi hubungan p>0.05. Sedangkan faktor iklim yang memiliki hubungan dengan kasus DBD di Kota Surabaya merupakan kelembaban udara dengan nilai signifikansi p<0.05 serta memiliki hubungan yang positif dengan nilai r = + 0.190. Dapat disimpulkan tidak semua faktor iklim mempunyai hubungan dengan kasus DBD Kota Surabaya tahun 2007 – 2017, yang memiliki hubungan dengan kasus DBD adalah kelembaban udara.

Kata kunci: *Kasus DBD; Faktor iklim; Kelembaban udara, Surabaya, 2007 - 2017*

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INTRODUCTION

Dengue hemorrhagic fever (DHF) is a disease caused by dengue virus carried by female Aedes mosquitoes, especially Aedes aegepty and a few Aedes albopictus.[1] Dengue is widespread in the tropics and subtropics, including Indonesia. Dengue is one of the main health problems in Indonesia.[2] DHF cases first appeared in Indonesia, namely in Jakarta and Surabaya in 1968.[3] DHF Incidence Rate (IR) in Indonesia from 1968 - 2015 continue to increase.[4,5,6,7,8,9] Dengue cases found in all provinces in Indonesia.[10] One of the things that influences this phenomenon is the climate change. Climate change causes changes in rainfall, temperature, humidity, and air direction, thus affecting the terrestrial and oceanic ecosystems and also health.[11] Climate change has a role in DHF vector.[12]

Aedes mosquitoes live in urban habitat and breed specifically in containers. Water needs for breeding is very important. It reach its peak during the rainy season.[13] This mosquito tend to bite in the morning until noon.

DHF has a strong correlation with the climate because the incidence of DHF usually happens on the beginning and the end of the rainy season.[14] Very high rainfall influences the population of mosquitoes. Increased rainfall intensity refers to the increasing place of mosquitoes to breed, resulting in increasing mosquitoes population. Increasing the mosquito population increases the risk of female mosquitoes carrying the pathogens which will transmit to the next host.[15] Aedes mosquito reproduction cycle will be shorter at temperatures higher than 32°C so that the mosquito population will multiply with increasing temperature [16]. Warm temperatures also accelerate the metabolic process so that the frequency of biting will increase.[17] The maximum temperature for mosquito growth is 25-27°C. [18]

Humidity affects the flight behavior and host search, mosquito life span and mosquito reproduction.[17] High humidity helps the process of mosquito metabolism which will indirectly increase the frequency of biting.

MATERIALS AND METHODS

This research is an analytical study that uses secondary data in the form of institutional administrative data, namely the report of the Meteorology Climatology and Geophysics Agency (BMKG) and the Surabaya City Health Office with a cross-sectional approach. The sampling technique in this study uses a total sampling technique. The data were taken is the BMKG of Surabaya City weather report in 2007-2017 and the Surabaya City Health Office report on the incidence of dengue cases in 2007-2017. The collected data is grouped by month in each year and is written using tables and graphs and analyzed descriptively and tested statistically the correlation using Spearman method on the SPSS software version 20.

RESULT AND DISCUSSION

DHF Cases Profile in Surabaya

In Surabaya, the incidence of DHF in the 2007-2017 period as a whole has decreased in numbers although not stable. In below, figure 1 show the number of DHF cases in Surabaya from 2007 until 2017.

Figure 1. Number of DHF cases in Surabaya in 2007 – 2017

From the data obtained a significant increase occurred in 2010, 2013, and 2016. If it is associated with the time of the El Nino occurrence, in those years the El Nino events that occur in the moderate and strong category. From previous study, there was an increase in the incidence of DHF when the El Nino with the same category occur.

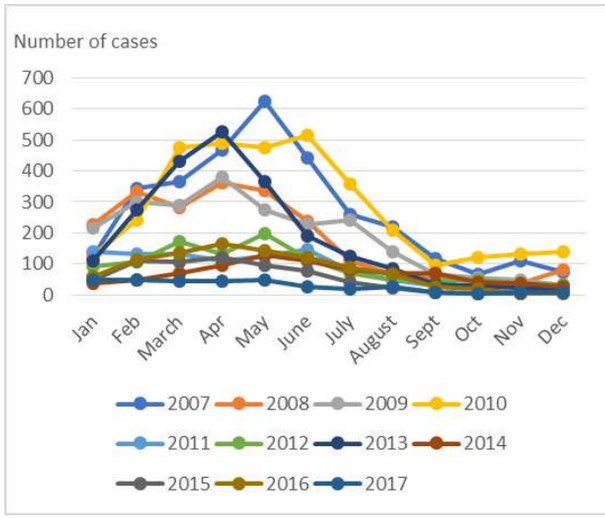

Figure 2. Cases of DHF per month each year

Judging from the graph above in Figure 2, the same pattern was formed in 2007 - 2009. Starting from 2011 to 2017 the number of dengue cases began to decrease so that the pattern formed had changed from before. Whereas in 2010, 2013, and 2016 have different patterns from other years. Overall, from September to December the number of dengue cases has always been lower than in previous months.

Rainfall Distribution of Surabaya

Surabaya has monsoonal rain type that is influenced by west and east monsoon winds where the peak of the rainy season occurs in January, and the peak of the dry season occurs in August.[19]

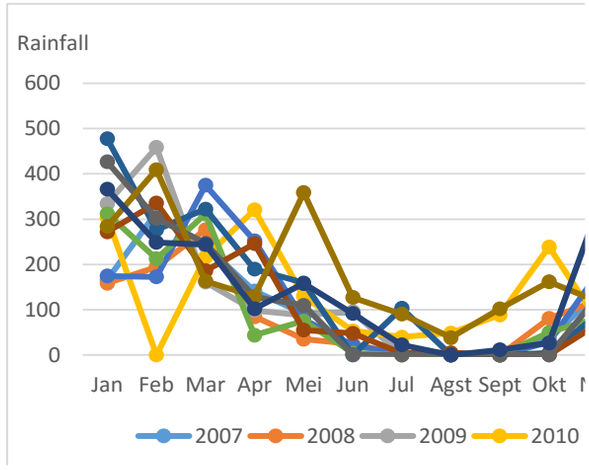

Figure 3. Rainfall per month each year

Based on Surabaya City rainfall data for 2007-2017 in Figure 3, December to March were months with high rate of rainfall, while July to September were months with low rainfall. However, in 2010, 2013 and 2016 there was a change in the pattern in which high rate of rainfall occurred throughout the year even in the month that was supposed to be the peak of the dry season, making the accumulation of rainfall in those years the highest among the other years.

Average Temperature Distribution of Surabaya City

The average temperature of Surabaya City for the last 11 years is within normal range when compared to the 30-year data with an average value of 28,62ºC.

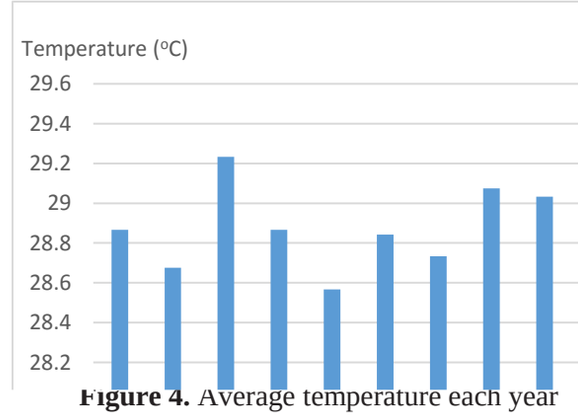

Figure 4. Average temperature each year

From the graph in Figure 4, 2011 became a year with the lowest average temperature with a value of 28,6oC, while 2016 was a year with the highest average temperature of 29,4ºC.

Humidity Distribution of Surabaya City

The humidity of the city of Surabaya for the last 11 years is within normal range when compared to the 30-year data with a value of 74,33.

From the graph in Figure 5, 2009 became the year with the lowest humidity with a value of 69,58 and 2017 became the year with the highest average humidity with a value of 76,92.

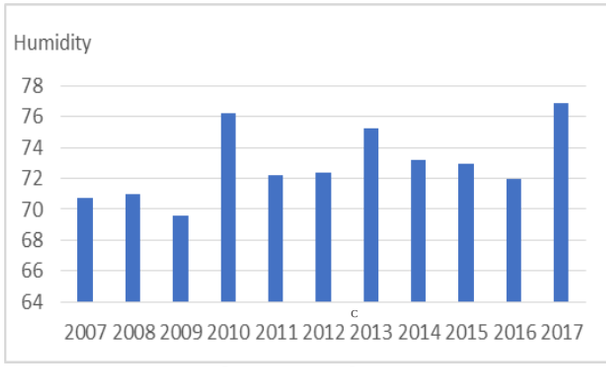

Figure 5. Humidity in Surabaya City per year

Correlation between Rainfall with DHF Cases

The effect of rainfall on the incidence of DHF cases is complex, because it is influenced by several other factors.[20] Rainfall has an influence on the vector growth, which is the density of adult mosquitoes. High rainfall intensity will cause the breeding site of adult mosquitoes to increase, which in turn increase the density of mosquitoes.[15] However, in a short period, heavy rain will destroy mosquito larvae and reduce the survival rate of female mosquitoes.[16]

Table 1. Spearman Correlation Test Result

Variable	Mean ± SD	p
Rainfall	138,265 ± 131,269	0,159
DHF Cases	136,71 ± 135,560	

From the result of published study in Table 1, the correlation test using Spearman obtained relationship significance of p >0.05. It can be interpreted that there was no correlation between rainfall and the incidence of DHF cases in Surabaya in 2007-2017. However, it must be noted that incidence of DHF cases is influenced by other factors besides than rainfall, such as humidity, evaporation of water, wind speed, and cloudiness.[20]

These results supported previous study in Surabaya which showed no significant fluctuations in certain months of the year regarding the number of dengue cases. These results are also similar with studies in other influencing factors.[20] In addition, changes in rainfall patterns can also affect human behavior which will later affect lifestyle that further affect the dynamics of Aedes mosquito populations, for example, a change in water storing habit.[20] However, studies assessing the correlation of rainfall with the incidence of DHF is not suitable to use the Spearman method. Spearman is suitable for measuring linear and static relationships, while the correlation between weather and DHF events is neither linear nor static.[18] This can happened because from the previous study, the correlation between rainfall and the incidence of DHF has several conditions, such as regular rain that may cause an increase in dengue cases, whereas heavy rainfall does not. [18,21] So, the correlation bertween DHF cases and rainfall are not linear nor static. The results of this study supported previous studies in the city of Surabaya which showed no significant fluctuations in certain months of the year regarding the number of dengue cases.

Correlation between Temperature and DHF Cases

Based on previous study, temperature has a role in the transmission cycle of dengue virus.[21] Research in Thailand and Singapore showed that there was a correlation between temperature and the incidence of DHF cases. [22,16]

Table 2. Spearman Correlation Test Result

Variable	Mean ± SD	p
Temperature	28,909 ± 0,739	0,066
DHF Cases	136,71 ± 135,560	

Correlation test results in Table 2 showed the relationship significance of p >0.05 which means there was no correlation between temperature and the incidence of DHF in the city of Surabaya in 2007-2017. Similar to correlation of rainfall with the incidence of DHF cases, the relationship with temperature is also not a linear relationship or static, thus making this method not suitable for this case.[18] It can be seen that the temperature data used is the average temperature, whereas the temperature is not only measured from the

average value but there is also a minimum and maximum temperature. This minimum or maximum temperature value may also affect the presence or absence of its relationship with the incidence of DHF.

Research that uses an epidemiological approach states that in certain months, high temperatures will cause mosquito populations to increase with low virus transmission, which usually causes an increase in virus transmission under conditions of high rainfall, low temperatures, and high humidity.[23]

Correlation between Humidity with DHF Cases

Humidity affects the flight behavior of mosquitoes by increasing the metabolism of the mosquito's body which then increase the biting behavior.[24]

Table 3. Spearman Correlation Test Result

Variabel	Mean ± SD	p
Humidity	73,48 ± 5,614	0,029
DHF Cases	136,71 ± 135,560	

The correlation test results in Table 3 showed the relationship significance of $p < 0.05$ which means there was a significant correlation between the two variables. The strength of the relationship between the two variables is very weak and the direction of the relationship is positive ($r = +0.190$). It can be interpreted that the higher the humidity, the higher the incidence of DHF.

Similar to current study which showed that air humidity has a relationship with the incidence of DHF cases through the effect on the density of the dengue virus vector, the Aedes aegepty mosquito and the external incubation period of the dengue virus itself, thereby increasing its transmission. [23]

Correlation between ENSO and DHF Cases

Based on the available data, year of 2010, 2013 and 2016 were the year with high rainfall accumulation followed by an increase in the incidence of dengue cases. Previous studies showed that there was a correlation between the increase in the incidence of DHF cases with the phenomenon of El-Nino-Southern Oscillation (ENSO) which is a cycle of sea surface temperature in the Pacific Sea. From the results of studies in Venezuela, 2009 - 2010 were the year with moderate El-Nino category, while 2014-2016 were the year with strong El-Nino or Mega Nino. [24] Within those 3 years, there was a recorded climate phenomenon that does not usually occur in Surabaya. During those years, dengue fever cases in the city of Surabaya also showed an increase in number. This result is linear with the previous study, that there is a significant relationship between ENSO and dengue incidence.[25]

CONCLUSION

The climate factor which has an analytical correlation with the DHF case in Surabaya in 2007 - 2017 is humidity, while the climate factor such as rainfall and temperature does not have an analytical correlation with the DHF incidence rate. There is an influence of the El-Nino phenomenon on the number of DHF cases in Surabaya in a certain year.

CONFLICT OF INTEREST

There is no conflict of interest of this study.

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Review Article

Genital Tract Infection during Pregnancy and its Association with Preterm Delivery

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ABSTRACT

Genital tract infection (GTI) remains a significant health concern. It is estimated that in 2016, there were 370 million people who suffer from chlamydia, gonorrhea, and trichomoniasis; and 708 million others suffer from genital herpes and condyloma acuminatum. It has been reported that in pregnant women, GTI is associated with preterm delivery. The mechanisms of GTI-associated preterm delivery need to be further understood to prevent neonatal mortality and morbidity that could be the risk factor for neonates' growth and development disorders. This article aims to describe various types of GTI and the associated pathogenesis causing preterm birth. A literature search was conducted to retrieve recent articles published in English from online databases including Pubmed, ScienceDirect, and Google Scholar. This literature study found that GTI evokes inflammatory responses that trigger several mechanisms leading to preterm delivery. The inflammatory responses in GTI include the production of proinflammatory cytokines and robust activation of neutrophils. The key mechanisms that stimulate preterm delivery in GTI include the events of early uterine contraction, preterm premature rupture of membranes, and induction of cervical ripening; which are under normal circumstances in a full-term pregnancy, those mechanisms are regulated by progesterone and prostaglandin levels along with suppression of the inflammatory responses. In conclusion, this paper has described the underlying mechanisms of preterm delivery in pregnant women with ISG. However, such mechanisms remain unclear in candida and gonococcal infection; thus, prompting the need for further studies.

Keywords: *Genital tract infection; sexually transmitted infection; preterm delivery; preterm birth; pregnancy*

ABSTRAK

Infeksi saluran genital (ISG) masih menjadi masalah kesehatan yang penting. Pada tahun 2016, diperkirakan terdapat 370 juta orang yang menderita klamidia, gonore, dan trikomoniasis; dan 708 juta orang lainnya menderita herpes genital dan kondiloma akuminata. Telah dilaporkan bahwa ISG pada wanita hamil berhubungan dengan kasus persalinan preterm. Mekanisme terjadinya persalinan preterm yang berhubungan dengan ISG perlu dipahami secara lebih mendalam untuk mencegah mortalitas dan morbiditas neonatus yang merupakan faktor risiko terjadinya gangguan tumbuh kembang. Artikel ini bertujuan untuk mendeskripsikan berbagai jenis ISG dan patogenesis yang berkaitan dengan terjadinya kelahiran preterm. Data didapatkan melalui penelusuran literatur terkini yang diterbitkan dalam bahasa Inggris pada database online Pubmed, ScienceDirect, dan Google Scholar. Hasil penelusuran literatur menunjukkan bahwa ISG akan menimbulkan respon inflamasi yang memicu terjadinya beberapa mekanisme yang menyebabkan persalinan preterm. Respon inflamasi tersebut meliputi produksi sitokin-sitokin proinflamasi dan aktivasi neutrofil yang masif. Mekanisme utama yang menstimulasi terjadinya persalinan preterm pada ISG meliputi kontraksi dini uterus, ketuban pecah dini sebelum kehamilan genap bulan, dan pematangan serviks; yang normalnya pada kehamilan yang genap bulan, hal-hal tersebut diatur oleh kadar progesteron dan prostaglandin serta penekanan respon inflamasi. Sebagai kesimpulan, makalah ini menjelaskan mekanisme yang mendasari terjadinya persalinan prematur pada kasus kehamilan dengan ISG. Namun, mekanisme tersebut belum

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sepenuhnya jelas pada infeksi kandida dan gonokokus, sehingga dibutuhkan penelitian lebih lanjut.

Kata kunci: *infeksi saluran genital; infeksi menular seksual; persalinan preterm; kelahiran preterm; kehamilan*

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INTRODUCTION

Preterm delivery is an important health problem as this condition can increase neonatal mortality and morbidity. Preterm delivery defines as parturition that happens between 20 weeks and 37 weeks of gestation.[1] The rates of preterm birth ranges from 5% to 18% globally and are estimated to be 15 million cases every year.[2] Neonates who are born preterm are at a higher risk for developing respiratory distress, hypothermia, hypoglycemia, and sepsis compared to full-term babies. Those who are born preterm may also develop cognitive and behavioral impairment later in life.[3,4] A previous report highlighted the significant contribution of preterm delivery in neonatal mortality, causing 1 million neonatal deaths in 2015.[5]

Untreated genital tract infection (GTI) plays an important role in causing preterm delivery. The exact number of pregnant women who suffer GTI is unclear as the condition is often underdiagnosed, especially in resource-limited settings. Nonetheless, there is a growing concern with regards to GTI in pregnancy because the condition poses a risk for preterm premature rupture of membranes (PPROM), preterm contraction, and cervical ripening that could lead to preterm delivery.[6]

Various agents can cause GTI. For example, GTI could be caused by viruses (condyloma acuminatum, genital herpes), bacteria (chlamydia, gonorrhea, and bacterial vaginosis), fungi (vulvovaginal candidiasis), and protozoa (trichomoniasis).[7,8] In 2016, 370 million people had sexually transmitted infections caused by *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* whereas 708 million cases were caused by viruses with condyloma acuminatum accounted for 291 million cases and genital herpes was observed in 417 million cases.[9,10] In addition, 4.5% to 50% of women worldwide had bacterial vaginosis and 134 millions women suffer from vulvovaginal candidiasis annually.[11,12]

Due to the high burden of GTI in the population and the significant complications of prematurity, GTI-associated preterm delivery is regarded as an important subject of consideration. This paper presents the results of literature search in Pubmed, ScienceDirect, and Google Scholar to deepen our knowledge about the underlying mechanisms of GTI causing preterm delivery. The literature search was limited to recent articles published in English.

PATHOGENESIS OF GTI-ASSOCIATED PRETERM DELIVERY

Condyloma acuminatum

Human papillomavirus (HPV) is the causative agent for condyloma acuminatum. This virus can infect cells of genital mucosa and induce epithelial proliferation by the action of viral proteins. Viral protein E5, E6, and E7 enhance epithelial growth via stabilization of epidermal growth factor, binding and degrading cellular p53 protein, and inactivation of cellular retinoblastoma protein, respectively.[13-15] HPV infection manifests as epidermal growth that commonly appears on vulva, vagina, and cervix. The shape of the lesions can be papular, flat or pedunculated.[16]

The E5 and E7 viral proteins generate local immunosuppression through the reduction in toll-like receptors (TLRs) expression, reduction in surface expression of major histocompatibili-

ty complex class I (MHC I), induction of T-regulatory cells attraction, and upregulation of immunosuppressive genes.[17,18] The decrease of the local immunity may facilitate the normal flora of the lower genital tract to gain ascending access and generate bacterial infection to the upper genital tract.[19]

The subsequent bacterial infection generates inflammatory responses via the nuclear factor-kappa B (NF-κB) pathway and increases the risk of preterm delivery through two mechanisms. First, inflammation causes an upregulation of prostaglandin E2 synthesis that plays a vital role in inducing uterine contraction.[20,21] Second, inflammation increases the production of matrix-degrading enzymes that responsible for PPROM and cervical ripening.[22] These events will lead to preterm delivery.

Genital herpes

Genital herpes is caused by the herpes simplex virus (HSV) that could be either HSV-1 or HSV-2 with classically HSV-2 being the more common etiologic agent.[23] Typical manifestations of genital herpes are painful papules that develop into vesicles, ulcerates, or form a crust within the course of the disease.[24] common etiologic agent.[23] Typical manifestations of genital herpes are painful papules that develop into vesicles, ulcerates, or form a crust within the course of the disease.[24]

Untreated HSV infection is a risk factor for preterm delivery. Similar to HPV, HSV infection could lead to a decrease in TLRs expression.[17]

TLRs, especially TLR-4 and TLR-5, are essential in initiating an innate immune response against bacterial antigens. It has been reported that a decrease of TLRs expression in genital herpes is associated with an increased risk of *Escherichia coli* ascending infection.[25] - β (IL-β), IL-6, IL-12, chemokine (C-X-C motif) ligand 1 (CXCL1), monocyte chemoattractant protein-1/ chemokine (C-C motif) ligand 2 (MCP-1/CCL2), macrophage inflammatory proteins-1α/β (MIP-1α/β), and chemokine regulated upon activation, normal T cell expressed and presumably secreted that are released during bacterial infection are considered as the prime movers for remodeling and rupture of the fetal membrane as well as cervical ripening.[17,26]

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 HSV infection can change the structural collagen fibers of cervical tissue by increasing the synthesis of hyaluronic acid and facilitating the proliferation of epithelial cells. The increasing amount of hyaluronic acid weakens the cervical collagen network and the proliferation of cervical epithelial cells increases the tissue sensitivity to 17β-estradiol (E2); both events are associated with cervical ripening that intitiate a key process in delivery.[27,28]

Chlamydia infection

(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 Chlamydia infection is a sexually transmitted disease caused by *C. trachomatis*. This bacteria is an intracellular obligate pathogen with two different forms during its life cycle; i.e., the elementary body as the infectious phase and the reticulate body as the replicative phase.[29] The clinical manifestations of chlamydia infection in women include cervicitis, vaginitis, or urethritis. Chlamydia infection is sometimes accompanied by increased cervical secretion, lower abdominal pain, post-coital bleeding, and dysuria.[30]

A previous prospective cohort study showed that chlamydia infection during pregnancy is associated with preterm delivery before 32 weeks and 35 weeks of gestation.[31] The infection of the upper genital tract could trigger inflammatory responses affecting the placenta along with both maternal and fetal membranes. The involvement of transplacental and

transmembrane leads to chorioamnionitis, an inflammatory condition involving chorion, amnion, and placenta that could trigger preterm delivery.[32] Chorioamnionitis causes upregulation of inflammatory cytokines such as IL-6, IL-8, and tumour necrosis factor-α (TNF-α) in the membranes that facilitates the production of prostaglandin and metalloprotease, resulting in membrane rupture and uterine contraction.[33,34]

The inflammatory responses are the results of the host's direct response to bacterial infection or as the results of the host's response to the heat shock protein produced by the bacteria.[35] Chlamydial heat shock protein (hsp60) is produced by the chlamydial reticulate body and released into extracellular milieu. The protein has an antigenic epitope that could trigger the host's immune responses. It could also induce the production of IL-6 as well as adhesion molecules such as E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1).[29,36] In pregnant women with *C. trachomatis* infection, hsp60 can be isolated from the epithelial cells of the genital tract and placental tissue, suggesting its association with placental inflammation. The sign of placental inflammation is more commonly observed in women with confirmed chlamydia infection compared to those uninfected.[32] Furthermore, IgG antibody associated with placental hsp60 is only detected in women with preterm delivery.[37]

Gonorrhea

Gonorrhea is a disease caused by the diplococci gram-negative *N. gonorrhoeae*.[38] Women infected with this bacteria will develop cervicitis with purulent cervical discharge, dysuria, and lower abdominal pain.[39] Pelvic inflammatory disease (PID) could arise as a complication of gonorrhea infection which causes fallopian tube damage, resulting in women's infertility.[40] The bacteria are resistant against most antimicrobial agents.[41] The currently recommended treatment regimen for gonorrhea is dual therapy with either or cefixime plus azithromycin.[42] However, it has been reported that the bacteria are resistant to third-generation cephalosporins.[43,44]

N. gonorrhoeae infection has been known to be involved in the occurrence of preterm delivery although the underlying mechanisms have not been demonstrated clearly and the association between the entities has not been found as commonly as other types of GTI.[26] A previous retrospective cohort study reported that gonorrhea is associated with an increased risk of spontaneous preterm birth.[45] Another study supports this finding by reporting the involvement of gonorrhea in preterm labor and PPROM but not preterm delivery.[46] Another retrospective cohort study also failed to demonstrate a relationship between preterm delivery and gonorrhea despite finding a trend toward chorioamnionitis during the third trimester of pregnancy with gonorrhea.[47] These findings suggest that gonorrhea's role in inducing chorioamnionitis is time-specific.

A strong neutrophilic inflammatory response may explain the link between *N. gonorrhoeae* infection and preterm delivery as the bacteria is known to cause a robust response of neutrophils. The bacteria possess opacity (Opa) proteins that act as adhesins and bind to the human's surface protein of the family carcinoembryonic antigen-related cell adhesion molecules (CEACAMs).[48] Opa proteins could also evoke inflammatory responses by promoting chemokines production such as MIP-1α, MIP-2, CXCL-1, and TNF-α on CEACAMs-expressing neutrophils.[49] In addition, the bacterial lipo-oligosaccharide (LOS) and the released peptidoglycan fragments would activate TLRs, thereby drive the production of inflammatory cytokines such as IL-1, IL-6, IL-8, and IL-17.[48] These inflammatory responses are believed to cause subsequent events that lead to myometrial contraction, membranes rupture, and cervical ripening that induce preterm delivery.[26]

Trichomoniasis

Trichomoniasis is caused by the protozoan parasite *T. vaginalis*. The infection causes local inflammation as a result of the host's immune

response to the attachment of the parasite to mucosal tissue.[50] The clinical manifestations of trichomoniasis include dysuria, pruritus, and frothy yellowish or greenish vaginal discharge.[51] The disease is associated with preterm birth and other perinatal morbidities such as PPROM and small for gestational age infants.[52]

Lipophosphoglycan (LPG) is the major adhesion molecule of *T. vaginalis* and it is recognized by TLR-4. The antigenic molecules stimulate an abundant production of IL-8 by activating the pathways of NF-κB, extracellular signal-regulated kinases 1 and 2 (ERK1/2), and mitogen-activated protein kinase 1 and 2 (MEK1/2).[53] LPG induces the production of inflammatory cytokines in TLR-4 independent manner by binding to galectin-3 on vaginal epithelial cells. The activation of galectin-3 has been shown to trigger IL-8 expression.[54,55] IL-8 promotes neutrophil migration and activation that leads to collagenase and elastase secretion within the cervix, a key role in cervical ripening that induces the delivery process.[26]

The protozoa could also be parasitized by *Mycoplasma hominis* and transmit the bacterial infection to the human host following treatment with metronidazole.[56,57] Mycoplasma can migrate into the upper genital tract and cause chorioamnionitis. The presence of mycoplasma in amniotic fluid is a predictive factor for preterm delivery.[58,59] Mycoplasma infection causes an increase of inflammatory markers in amniotic fluid, including TNF-α, IL-6, IL-8, and matrix metalloproteinase-8 (MMP-8). [60]

Bacterial vaginosis

Bacterial vaginosis (BV) is a condition when there is a shift in the vaginal microbiome in which the population of lactobacillus is significantly depleted and the vaginal microbiome is dominated by anaerobic polymicrobial organisms such as *Gardnerella vaginalis, Atopobium vaginae, Peptostreptococcus spp., Prevotella spp.*, and other BV-associated bacteria.[61,62] It has been reported that there is a decrease in the growth of hydrogen peroxide-producing lactobacillus during HSV-2 infection. [63] The exact mechanism of lactobacillus depletion in HSV-2 infection remains unknown but the infection causes dysbiosis in vaginal flora, resulting in BV. BV typically presents with minimal signs and symptoms so that the diagnosis is frequently missed. The diagnosis of BV can be made by using Amsel criteria as follows: (1) increased homogenous and thin vaginal discharge; (2) vaginal pH of >4.5; (3) positive whiff test or amine aroma generated after KOH treatment; and (4) clue cells in microscopy examination. The distinctive amine smell or fishy odor is an important clinical indicator for BV. The diagnosis of BV is confirmed when there are minimally 3 criteria present.[64]

The bacteria causing BV could ascend to the upper genital tract and cause inflammatory responses. The local inflammatory responses induce the secretion of prostaglandin that promotes early uterine contraction as well as metalloproteinase enzymes that cause PPROM.[20,65]

Vulvovaginal candidiasis

Vulvovaginal candidiasis is an inflammatory condition of the vulva and vagina caused by fungi from the Candida genus with *Candida albicans* being the most common etiologic agent.[66] The condition is characterized by white curdy vaginal discharge, erythema of the vulva and vagina, dysuria, and dyspareunia.[67] The diagnosis of vulvovaginal candidiasis could be made by either stained or unstained wet mount microscopy can be used as a confirmatory diagnostic test. The diagnosis is confirm when blastospores and pseudohyphae are present. If neither is present, the sample must undergo culture to ascertain diagnosis.[68]

It has been demonstrated that women with vulvovaginal candidiasis have a higher risk of chorioamnionitis and preterm delivery compared to those without the infection.[69,70] A previous meta-analysis showed that antifungal treatment of asymptomatic candidiasis in pregnant women is associated with a lower incidence of preterm birth compared to untreated cases.[71] It is uncertain how vulvovaginal candidiasis could lead to preterm birth but it has been suggested that vulvovaginal candidiasis increases the susceptibility of the host to ascending bacterial infection.[72]

It has been reported that the pathogenic *C. albicans* is a potent inducer of IL-10.[73] Thus, in vulvovaginal candidiasis, the host's immune response is being suppressed and an alteration of the vaginal normal flora develops, favoring bacteria to generate infection in the upper genital tract.[72,73] This subsequent bacterial infection drives the production of pro-inflammatory cytokines, thereby leads to the elevation of prostaglandin and the increased production of matrix-degrading enzymes, similar to the events following HPV and HSV infection that lead to preterm delivery.

Figure 1 summarizes the mechanisms involved in GTI-associated preterm delivery. The common pathway that results in early uterine contraction, PPROM, and cervical ripening is the production of proinflammatory cytokines. In particular, the immune response to chlamydia infection is triggered either by direct stimulation or as the subsequent response against heat shock protein produced by the reticulate body of chlamydia.[29,32,35,36] induced via robust neutrophils activation whereas in bacterial vaginosis, the production of proinflammatory cytokines occurs without a robust neutrophilic response.[26,48,49,53,65]

HPV, HSV, and *C. albicans* stimulate cytokines production by interfering cervical immune responses that facilitate bacterial infection.[17,19,27,72,73] The cytokines production is followed by an elevation in the levels of prostaglandin and matrix-degrading enzymes such as MMP-2, MMP-9, MMP-8, MMP-3, and MMP-10 that leads to PPROM, early uterine contraction, and cervical ripening.74 Similarly, robust activation of neutrophils can induce PPROM and cervical ripening. Altogether, early uterine contraction, PPROM, and cervical ripening are the key mechanisms leading to preterm delivery.[20,21,22,26,75-78] It is important to note, however, that the pathogenesis of preterm birth associated with candida and gonococcal infection is not clearly understood.

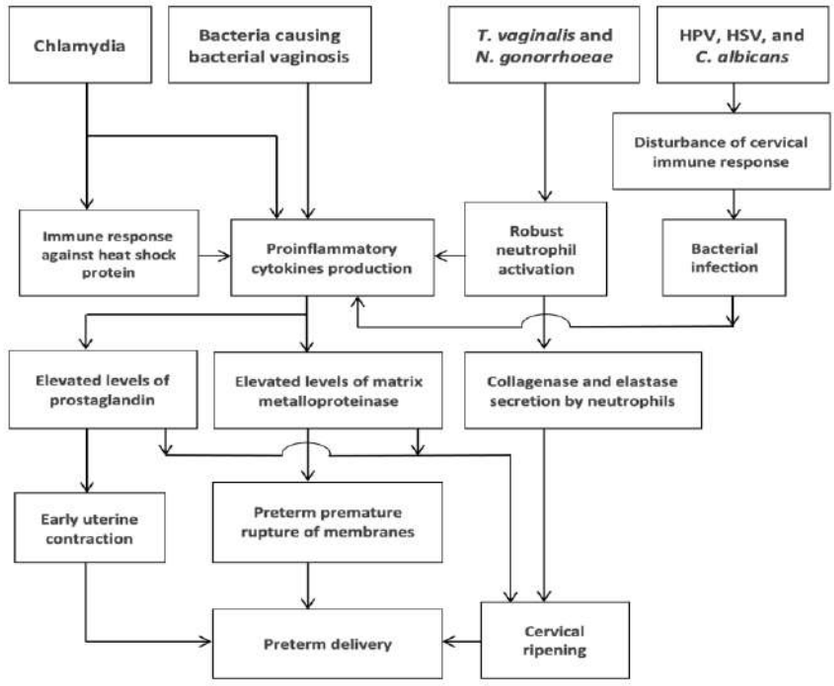

Figure 1. Summary of the mechanisms involved in GTI-associated preterm delivery

Immune Activation and Inflammatory Responses in Preterm Delivery

The production of proinflammatory cytokines in *T. vaginalis* and *N. gonorrhoeae* infection is

Early uterine contraction

Uterine contractility is maintained through progesterone withdrawal and an increase in

prostaglandin E2 level.[20] Progesterone increases cyclic adenosine or guanosine monophosphate (cAMP or cGMP) which in turn inhibit the release of intracellular calcium. Thus, progresterone withdrawal will initiate uterine contraction.[21]

Prostaglandin E2 causes myometrial contractions via prostaglandin prostanoid receptors (EP). There are four different subtypes of EP; i.e, EP1, EP2, EP3, and EP4 (Figure 2). When prostaglandin E2 is secreted, all the EP receptors are expressed continuously on different anatomical sites, producing different effects on the uterus during the delivery process. EP1 and EP3 are classified as contractility enhancers and they are expressed highest in fundal tissue. EP1 facilitates Ca^{2+} influx into myometrium whereas EP3 inhibits adenylate cyclase activity and cAMP production. EP2 and EP4 are located in the lower uterine segment and classified as muscle relaxants that increase cAMP molecules.[79,80] The difference in anatomical sites of EP expression and their effects on the uterus is important to allow passage of the fetus during the delivery process.

GTI stimulates the NF-κB pathway of inflammation, which in turn promotes prostaglandin synthesis by the upregulation of prostaglandin E synthetase and downregulation of 15-hydroxyprostaglandindehydrogenase.[20,21] Prostaglandin E synthetase is the enzyme required in the terminal step of the production of prostaglandin E2 while 15-hydroxyprostaglandin dehydrogenase is required in the prostaglandin inactivation pathway.[76,81] It has been reported that inflammatory signals may stimulate the expression of 20α-hydroxysteroid dehydrogenase (20α-HSD), an enzyme that promotes the inactivation of progesterone.[22] Thus, GTI-induced inflammation could lead to an increase in uterine contractility by altering progesterone-prostaglandin level, one of the factors that determine uterine contractility leading to parturition.

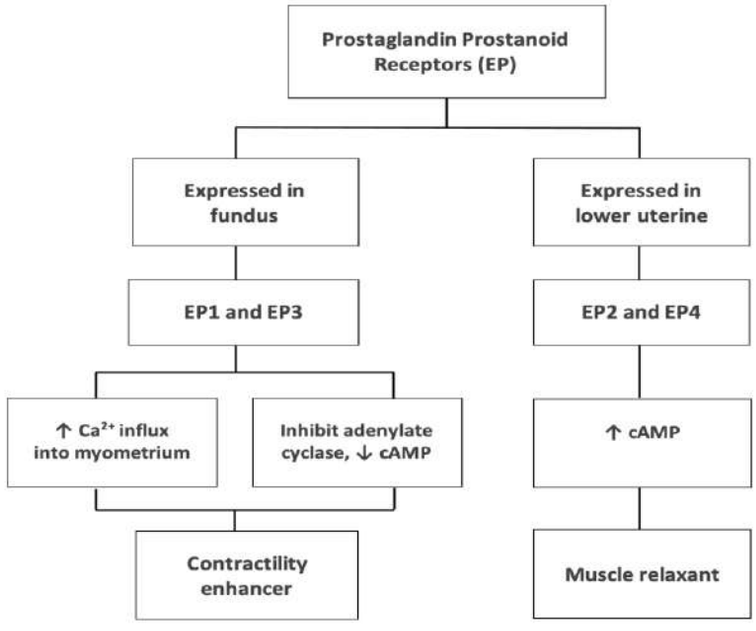

Figure 2. EP receptors and their effects on uterus

Preterm premature rupture of the membranes (PPROM)

Uterine contraction and the rupture of membranes are crucial steps to initiate the delivery process, which are controlled

In at least 35% of women with preterm delivery, contraction happened after the membranes rupture.[75,82] PPROM is a pre-labor rupture of the membranes occurring before 37 weeks of gestation.[77]

Infection is responsible for about 55% of PPROM by promoting the release of proinflammatory cytokines.[75] The major proinflammatory cytokines that contribute to PPROM is TNF-α. Proinflammatory cytokines induce amnion epithelial apoptosis and increase the synthesis of matrix-degrading enzymes such as matrix-metalloproteinase and caspase that stimulate the degradation of the membranes' extracellular matrix. The degradation of the membranes' extracellular matrix weakens the fetal membranes and eventually leads to membranes rupture.[78]

Cervical ripening

Cervical ripening is is an important phase to initiate vaginal delivery of the fetus. The condition is characterized by a gradual decrease in collagen concentration of the matrix and an increase in cervix extractability to dilate.[83] Cervical ripening is regulated by several factors, including progesterone level and inflammatory reaction.[84]

It has been reported that the administration of vaginal progesterone could reduce the risk of preterm birth.[85] Progesterone suppresses inflammatory responses by reducing macrophages activity, decreasing migration of neutrophils, and augmenting $CD4^+$ T regulatory activity. In addition, progesterone action through progesterone receptors (PRs) has been shown to repress Cx43 gene transcription. Cx43 gene plays a vital role in the initiation of full-term or preterm birth through cell communication to generate myometrial contraction. Progesterone withdrawal thereby promotes the activation of this gene and leads to the generation of myometrial contraction.[22,86]

Inflammatory responses generated from GTI could induce cervical ripening through the following mechanisms. First, GTI-induced inflammation promotes the migration of neutrophils and monocytes into the extracellular matrix of the cervix. These activated cells secrete matrix-degrading enzymes such as matrix metalloproteinase and collagenase into the extracellular milieu of the cervix. As the consequence, cervix remodeling occurs and results in the decrease of matrix collagen and an increase in cervix extractability to dilate.[6,84]

The predominating cytokines that are associated with cervical ripening in GTI-induced inflammation include IL-1, IL-6, and TNF-α. A significant elevation of chemoattractant molecules such as CXCL-2, IL-8, and MCP-1 is also observed as well as an increase in prostaglandin levels.[87,88] Subsequently, inflammatory responses withdraw progesterone action by decreasing progesterone level. In addition, IL-1β promotes the expression of 20α-HSD enzyme in cervical fibroblasts. This enzyme catalyzes the metabolism of progesterone into its inactive form, thereby reducing progesterone level.[89] The decrease of progesterone level is a key mechanism that promotes cervical ripening and uterine contractility, thus initiating delivery.

CONCLUSION

Understanding factors that contribute to preterm delivery is important in order to deliver appropriate management. Our review shows that GTI-induced inflammatory responses are involved in the initiation of preterm delivery; i.e., early uterine contraction, PPROM, and cervical ripening. The underlying mechanisms of preterm delivery in candida and gonococcal infection, however, are not fully understood. Therefore, further studies in this area are needed.

CONFLICT OF INTEREST

There is no conflict of interest of this study.

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Original Article

Diagnosis Based on Detection of CXCL10 in Urine as Biomarker for The Determining Diagnosis of Active Lung Tuberculosis

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ABSTRACT

Tuberculosis diagnosis is an important component in decreasing TB incidence and prevalence. Because of the difficulty to collect sputum in some cases, urine specimens are used as it is easier to garner. One of the biomarkers in urine that can be used to diagnose pulmonary TB is IP-10, which can be represented by the CXCL10 gene. The study aims to determine the accuracy of diagnosis based on detection of the CXCL10 gene in urine as a biomarker for the patients with suspected pulmonary TB in Dr. Soetomo Hospital in Surabaya from November 2019 until March 2020. Thus, this is an observative laboratory research with a cross-sectional study. CXCL10 gene was examined using PCR for 36 urine samples, and then, the data, together with the medical records of clinical manifestations of pulmonary TB, GeneXpert MTB /RIF, blood count, and thorax radiograph, were processed using IBM SPSS Statistics 26. The results of the GeneXpert MTB/RIF and thorax radiograph criteria show positive results of pulmonary TB, which were 44.4% and 69.4% respectively. CXCL10 gene was not found in all urine of healthy people (negative), while 2.8% (1/36 samples) positive CXCL10 gene was found in a patient with positive GeneXpert, also with negative clinical manifestations and urine culture. In this study, the accuracy of diagnosis based on detection of the CXCL10 gene in urine for diagnosis of active pulmonary TB was 2.8%. Future research is needed to improve the methods, among them are bigger size of urine samples and clearer medical history of patients.

Keywords: *Tuberculosis; CXCL10; Biomarker; Urine; Diagnosis*

ABSTRAK

Diagnosis tuberkulosis merupakan komponen penting dalam menurunkan insiden dan prevalensi TB. Karena sulitnya mengumpulkan dahak pada beberapa kasus, spesimen urin digunakan karena lebih mudah didapatkan. Salah satu biomarker dalam urin yang dapat digunakan untuk mendiagnosis TB paru adalah IP-10 dengan cara mendeteksi keberadaan gen CXCL10. Penelitian ini bertujuan untuk mengetahui akurasi diagnosis berdasarkan deteksi gen CXCL10 dalam urin sebagai biomarker untuk diagnosis pasien TB Paru di RSUD Dr. Soetomo Surabaya dari November 2019 hingga Maret 2020. Oleh karena itu, penelitian ini termasuk penelitian laboratorium observatif dengan studi cross-sectional. Pemeriksaan gen CXCL10 dilakukan menggunakan PCR, kemudian data, bersama dengan hasil rekam medis manifestasi klinis TB paru, GeneXpert MTB/RIF, menghitung darah, dan rontgen dada, diolah menggunakan IBM SPSS Statistics 26. Hasil kriteria GeneXpert MTB/RIF dan rontgen dada menunjukkan hasil positif masing-masing 44,4% dan 69,4% TB paru. Semua urine orang sehat menunjukkan hasil gen CXCL10 negatif, didapatkan hasil sebesar 2,8% gen CXCL10 positif dalam urin pasien dengan GeneXpert positif dengan manifestasi klinis dan kultur urin negatif. Dalam penelitian ini akurasi diagnosis berdasarkan deteksi gen CXCL10 dalam urin untuk diagnosis TB paru aktif adalah 2,8%. Penelitian lebih lanjut dibutuhkan untuk meningkatkan metode yang digunakan, terutama agar menggunakan lebih banyak sampel urin dan riwayat pasien yang lebih jelas.

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Kata kunci: *Tuberkulosis; CXCL10; Biomarker; Urin; Diagnosis*

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INTRODUCTION

Tuberculosis (TB) is a pulmonary infectious disease caused by Mycobacterium tuberculosis and one of the top 10 causes of death as well as the number one cause of death from infection in the world. In 2017, 1.3 million people died from TB, and 10 million people were infected with TB.[1] Again, in 2018, as many as 1.3 million people died from TB and 10 million people were infected.[2] Indonesia is one of the 20 countries with the most TB cases, with 845 thousand people infected and 563 thousand diagnosed, and 98 thousand of them died from TB in 2018. Indonesia is included in High Burden Countries (HBC), countries with a high burden of TB based on 3 indicators, namely TB, HIV-TB coinfection, and MDR-TB. As Indonesia is included in all indicators, TB becomes one of the main health problems in Indonesia.[2]

In Indonesia, the detected and reported TB cases were 53% in 2017.[1] And then, it increased to 67% in 2018.[2] Of the unreported cases, 29% were detected but not reported and 18% were not detected at all. Java and Bali are the regions with the highest number of unreported TB cases, which is at 42%. Puskesmas as primary care in Indonesia has 15% of unreported cases, relatively lower than cases not reported by hospitals, which reaches 65%, and the highest by a combination of general practitioners, clinics, and laboratory practices was 96%.[1] Indonesia targets to increase TB disease control by decreasing the number of people with TB disease from 293 people per 100,000 population in 2013 to 245 people per 100,000 population in 2019 (4). Indonesia also targets TB elimination by 2035 and TB-free Indonesia by 2050.[5]

Diagnosis is an important component in achieving the target of reducing TB incidence and prevalence. Diagnosis of pulmonary tuberculosis begins with clinical criteria, chronic cough symptoms for more than 2 weeks, accompanied by fever, night sweats, and weight loss. For a country with a high TB prevalence such as Indonesia, all patients with suspected pulmonary TB clinical criteria are immediately diagnosed with pulmonary TB disease. The problem is that not all patients showing symptoms of chronic cough proved to be Acid Resistant Basil (AFB) positive, and vice versa. Data shows that 10-25% of patients with positive smear do not show symptoms of cough.[3]

The most common laboratory microscopic examinations are the sputum smear using the Ziehl-Neelsen staining technique and the GeneXpert MTB/RIF Molecular Rapid Test.[3] Both of these methods have disadvantages, that it is difficult for the patient to pass sputum, and consequently, there have not been enough sputum specimens collected for examination. A method of examination using specimens that is easier to collect, such as urine, is needed. It is easier to ask the patient to urinate than to expel phlegm. Besides, urine collection is non-invasive, not too risky for the medical personnel involved, and requires no special equipment or expertise.

One of the biomarkers in urine that can be used for diagnosis of pulmonary TB is IP-10, or Interferon-gamma(IFN-γ)-inducible protein of 10 kDa, which is represented by the CXCL10 gene, a pro-inflammatory chemokine released by exposed cells with antigens and cause activated *T lymphocytes* to move toward

the site of inflammation. Inflammation in active pulmonary TB spreads inflammatory cells lymphogenously and haematogenously throughout the body, including the kidneys, to be excreted together with urine. Urinary IP-10 levels are significantly elevated in patients with pulmonary disease, be it TB or other infections. The level of IP-10 in the urine of pulmonary TB patients who were examined at the onset of the disease was higher than in patients who had recovered.[12] (Cannas et al., 2010). IP-10 levels increase in patients with active pulmonary TB and decrease when TB treatment is complete.[19] This study aims to analyze the accuracy of the diagnosis based on the detection of the CXCL10 gene in the urine of patients with suspected pulmonary TB. The results of this study are expected to determine the accuracy of the CXCL10 gene detection method in urine as a laboratory tool for diagnosis of pulmonary TB.

MATERIALS AND METHODS

This research was a laboratory observational study with a cross-sectional study design using primary data of the results of the CXCL10 gene examination using PCR and secondary data of medical records unit in Dr. Soetomo Hospital. Medical records include clinical manifestations of pulmonary tuberculosis, Molecular Rapid Test of GeneXpert MTB/RIF (Cepheid, Canada), laboratory examinations, complete blood count, physical examination, and radiological photos of the thorax. The research was conducted in the period of November 2019 - March 2020. Urine samples were collected from 36 pulmonary TB adult patients. Laboratory procedure for urine examination was urine processing using centrifugation, DNA extraction using TE buffer with boiling, and PCR optimization. The primer for PCR were 5'-TTCCTGCAAGCCAATTTTGTCC-3' for forward and 5'- for the reverse. GCAGCTGATTTGGTGACCAT-3' 3 urine Urine culture based on standard solid medium culture method, 200 µL sediment of urine processing, was inoculated in Middlebrook 7H10. The accuracy was determined by detecting the CXCL10 gene, which represented IP-10 protein in the urine of active pulmonary TB patients, and Nucleic Acid Amplification Tests (NAATs) method using PCR. The results was determined as positive if the band measured was 305 basepair (bp) and it matched with the primer set. The collected data were then processed with IBM SPSS Statistics 26. After processing the data, the next step was to analyze the data whether the existing research hypothesis were to be accepted or rejected. Data analysis was used to describe, understand, and explain the relationship between the variables studied.

RESULTS

Based on the study, there were 36 samples of patients with suspected pulmonary TB consisting of 20 (55.6%) men and 16 (44.4%) women. Most samples were found in the age range of 20-29 years old, i.e., 10 people (27.8%). The complete findings are: 4 people aged 10-19 (11.1%), 1 person aged 30-39 (2.8%), 6 people aged 40-49 (16.7%), 3 people aged 50-59 (8.3%), 8 people aged 60-69 (22.2%), and 4 people aged 70-79 (11, 1%). The results shows that there were 33.3% of patients with low BMI, 2.8% of patients with high BMI, and 63.9% of patients with normal BMI.

The majority of the research samples came from Surabaya with 20 people (55.6%), while 16 people (44.4%) came from outside Surabaya. The majority of the sample, 15 people (41.7%) of the 36, did not work, 10 of them were private workers (28.7%), 6 were students or students (16.7%), 3 were farmers (8.3%), while for merchants and regional senators (DPRD) were with the same number, each consisting of 1 person (2.8%) as can be seen in Table 1.

Table 1. Frequency distribution of patients with lung TB suspect based on characteristics in the DOTS clinic of Dr. Soetomo Surabaya in November 2019 - March 2020 period

Characteristics	Total	Percentage
Gender		
Male	20	55,6%
Female	16	44,4%
Total	36	100%
Age		
10-19	4	11,1%
20-29	10	27,8%
30-39	1	2,8%
40-49	6	16,7%
50-59	3	8,3%
60-69	8	22,2%
70-79	4	11,1%
Total	36	100%
Body Mass Index		
Normal	23	63,9%
Underweight	12	33,3%
Overweight	1	2,8%
Region		
Surabaya	20	55,6%
Outside Surabaya	16	44,4%
Total	36	100%
Job		
Student	6	16,7%
Private Worker	10	27,8%
Farmer	3	8,3%
Merchant	1	2,8%
DPRD	1	2,8%
No Job	15	41,7%
Total	36	100%

The comparison of the results of CXCL10 gene detection in urine with clinical manifestations has a specificity of 100%, a sensitivity of 6.6%, and an accuracy of 61.1% as can be seen in Table 2.

Table 2. Comparison of the results of the CXCL10 gene detection in urine with clinical manifestations

Detection of the CXCL10 Gene in Urine		Clinical Manifestation		Total
		Positive	Negative	
Positive	Total	1	0	1
	%	2,80%	0,00%	2,80%
Negative	Total	14	21	35
	%	38,90%	58,30%	97,20%
Total	Total	15	21	36
	%	41,70%	58,30%	100,00%

The comparison of the results of the CXCL10 gene detection in urine with physical examination has a specificity of 97.2% and sensitivity of 2,8% as can be seen in Table 3.

Table 3. Comparison of the results of the CXCL10 gene detection in urine with physical examination

Detection of the CXCL10 Gene in Urine		Physical Examination		Total
		Positive	Negative	
Positive	Total	0	1	1
	%	0,00%	2,80%	2,80%
Negative	Total	0	35	35
	%	0,00%	97,20%	97,20%
Total	Total	0	36	36
	%	0,00%	100,00%	100,00%

The comparison of the results of the CXCL gene detection in urine with the manifestations of laboratory tests of complete blood has a specificity of 100%, sensitivity of 11%, and accuracy of 77.7% as can be seen in Table 4.

Table 4. Comparison of CXCL10 gene detection results in urine with manifestations of complete blood count

Detection of The CXCL10 Gene in Urine		Complete Blood Count		Total
		Positive	Negative	
Positive	Total	1	0	1
	%	2,80%	0,00%	2,80%
Negative	Total	8	27	35
	%	22,20%	75,00%	97,20%
Total	Total	9	27	36
	%	25,00%	75,00%	100,00%

The comparison of the results of the CXCL gene detection in urine with the radiological results of the chest radiograph has a specificity of 100%, sensitivity of 4% and an accuracy of 33.3% as can be seen in Table 5.

Table 5. Comparison of the results of the CXCL gene detection in urine with the results of cadiological chest radiographs

Detection of the CXCL10 Gene in Urine		Chest Radiograph		Total
		Positive	Negative	
Positive	Total	1	0	1
	%	2,80%	0,00%	2,80%
Negative	Total	24	11	35
	%	66,70%	30,50%	97,20%
Total	Total	25	11	36
	%	69,50%	30,50%	100,00%

The comparison of the results of the CXCL gene detection in urine with the results of GeneXpert has a specificity of 100%, a sensitivity of 6.2%, and an accuracy of 58.3% as can be seen in Table 6.

Table 6. Comparison of CXCL10 gene detection results in urine with GeneXpert results

Detection of the CXCL10 Gene in Urine		GeneXpert		Total
		Positive	Negative	
Positive	Total	1	0	1
	%	2,80%	0,00%	2,80%
Negative	Total	15	20	35
	%	41,70%	55,60%	97,20%
Total	Total	16	20	36
	%	44,40%	55,60%	100,00%

The comparison of the results of the CXCL gene detection in urine with the results of urine culture has a specificity of 97.2%, a sensitivity of 0%, and an accuracy of 97.2% as can be seen in Table 7.

Table 7. Comparison of the Results of the CXCL gene detection in urine with the results of urine culture

Detection of the CXCL10 Gene in Urine		Urine Culture Result		Total
		Positive	Negative	
Positive	Total	0	1	1
	%	0,00%	2,80%	2,80%
Negative	Total	0	35	35
	%	0,00%	97,20%	97,20%
Total	Total	0	36	36
	%	0,00%	100,00%	100,00%

patients (10.8%) with MRSA carrier events as much as zero (0%).[18]

DISCUSSION

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.

Pulmonary tuberculosis is a disease caused by Mycobacterium tuberculosis. These bacteria are transmitted through droplets that enter the respiratory tract. The clinical symptoms are 3 weeks or more cough with phlegm, hemoptysis, fever, chest pain, weight loss, night sweats, and tightness. The results show that 91.7% of the patients had cough symptoms for 3 weeks or more. This is supported by a research conducted.[8] which states that 81% of patients had cough symptoms for 3 weeks or more

Low BMI is associated with the risk of developing pulmonary TB because it is correlated with malnutrition and susceptibility to infectious diseases. The results show that there were 33.3% of patients with low BMI, 2.8% of patients with high BMI, and 63.9% of patients with normal BMI. Research states that low BMI correlates with the risk of being infected with pulmonary TB, but not with extrapulmonary TB.[13] Research in Taiwan also states that low BMI increases the risk of infection and mortality of TB disease.[28] Another study in Korea shows that a high BMI lowers the risk of TB infection, but a very high BMI does not reduce the risk.[20] Meanwhile, research in China shows that high BMI and obesity are associated with the risk of TB infection, possibly because the excess cell adiposity weakens the immune system.[30]

The majority of patients showed that vital signs were outside the normal limits, and the results of the study showed that most of the patients had abnormal temperatures, which was in 33.3% of patients. Another study states

that 22.5% of 40 patients experienced increased body temperature as a result of TB disease.[24] The vital sign that was most often outside the normal limit was the respiratory rate, with 79.5% of 49 patients showing a respiratory rate that exceeded the normal limit.[26]

Complete blood count can be a parameter for diagnosis, prognosis, or response to pulmonary TB disease treatment.[27] The results show that 19.4% of patients had leukocytosis and 5.6% of patients had decreased hemoglobin (Hb). TB patients experienced decreased leukocytosis and Hb. Decreased hemoglobin is one of the hematological problems that often appears in TB patients.[23] There were 61.5% of TB patients with anemia, where 43% of them suffered from moderate and severe anemia, and 49% suffered from iron deficiency anemia and anemia of chronic diseases.[9]

A chest radiograph is an important examination for people with suspected pulmonary tuberculosis but showing negative results of smear examination. It can also be used to determine disease progression and evaluate responses to therapy. Radiological photos alone cannot diagnose pulmonary tuberculosis, it is also necessary to combine it with a physical examination and clinical symptoms.[3] The results show that there were 69.4% of patients with positive chest radiology results. In the right lung, the most found were infiltrates and infiltrates accompanied by fibrosis, each of 5 people (13.9%), pleural effusions as many as 4 people (11.1%), and cavities accompanied by infiltrates as many as 2 people (5,6 %). In the left lung, the most found was infiltrates as many as 5 people (13.9%) and infiltrates with fibrosis in 3 people (8.3%). The most common features on chest radiological radiographs include 55% consolidation, 26% pleural effusion, and 17% lung collapse (Appleton et al., 2017). Another study shows that the most commonly seen features were 45% non-specific apex, 33% normal apex, and 16% apex of the lung with infiltrates. When compared with patients whose culture results were negative, pulmonary TB patients showed 43% of infiltrates at the apex and 14% of cavities.[6] Pleural effusion was found in 38% of new cases of pulmonary TB patients.[10]

Research conducted in Nigeria shows that the use of GeneXpert for the diagnosis of pulmonary tuberculosis has better results than smear testing.[15] The results show that there were 44.4% of patients with positive GeneXpert results, and 2.8% of patients showed resistance to Rifampicin. Examination using GeneXpert is more accurate than occasional sputum examination with higher sensitivity and negative predictive value (NPV).[29] Another study conducted in China shows that examination with GeneXpert has a sensitivity of 94.6%, specificity of 82.9%, positive predictive value (PPV) 77.3%, and negative predictive value (NPV) of 96.1% so that it can be used for examinations that require a shorter time, are simpler, and more efficient.[23]

Research conducted in Iran shows that 3.1% of 162 pulmonary TB patients whose sputum test results were positive were also resistant to Rifampicin from the results of the GeneXpert examination.[7] Another study conducted in Ethiopia shows that 1 out of 14 pulmonary TB patients who were bacteriologically positive was also resistant to Rifampicin.[17]

In this study, the results show that none of the patients had positive urine culture results. Meanwhile, a study conducted on HIV positive pulmonary TB patients in Ethiopia shows that urine culture could help improve detection of the bacterium *Mycobacterium tuberculosis* in HIV positive patients. Of the 45 people, there were 14.5% positive culture patients in Lowenstein-Jensen media, 6% positive culture patients from urine smears, and 24.8% positive patients from RD9-based PCR examinations.[14] Another study conducted in India shows that 26.1% of the 46 patients with suspected pulmonary TB with positive sputum culture results also showed positive urine

culture results.[16]

The results of this study show that there were 2.8% of patients with positive urine detection of the CXCL10 gene. The detection accuracy of the CXCL10 gene in urine was 2.8% compared with clinical manifestation of pulmonary TB, chest radiograph, and GeneXpert. Active pulmonary TB patients have higher urine levels of the CXCL10 gene than healthy people. The detection of CXCL10 in urine using ELISA (Quanterix) has a sensitivity of 78% and a specificity of 94%.[22] The detection of the CXCL10 gene in serum has a sensitivity of 87.5% and a specificity of 78.9%.[21] Another study reveals that detection of CXCL10 in serum showed positive results in 87.5% of active pulmonary TB patients, 45.5% of latent TB, and 9.5% in control variables.[18]

The differences in the accuracy rate between this research and other studies may be caused by some reasons. The study from Petrone et al. in 2019.[22] was conducted using ELISA (Quanterix) to measure CXCL10 in urine, while this research was using PCR. Studies by Nonghanphithak et al. in 2017. [21] and Hong et al. in 2012.[18] measured CXCL10 in serum of TB patient, while this research measured it in urine to avoid invasive procedures.

If the detection of the CXCL10 gene in urine is to be compared with other tests, the highest sensitivity is shown by complete blood laboratory examination, which is 11%. The examination with the highest specificity are shown in the manifestation of clinical symptoms, complete blood laboratory tests, gene GeneXpert results, and radiological radiographs of the chest, which are 100% respectively. The examination with the highest accuracy is physical examination and urine culture result, which is 97.2%.

This study has limitations in that the samples representing low BMI and optimal BMI are not sufficient. BMI is also a determining factor for the production of the CXCL10 gene. Also, it was difficult to determine the duration of clinical symptoms that varied among the patients.

Overall, the accuracy of the method of diagnosing pulmonary TB based on detection of the CXCL10 gene in urine cannot be measured because of several reasons, i.e., lack of medical history, especially in treatment of anti-tuberculosis drugs, small sample size of active TB patients, and the difficulty to collect urine directly from hospitalized patients. Further research in cohort study is needed with more complete clinical variable data, a wider scope, more samples, a real-time PCR method to detect the CXCL10 gene in urine, and the Next Generation Sequencing (NGS) method. The validity of this research can also be increased by using 50 ml of the patients' first morning urine.

CONCLUSION

In this study, the accuracy of diagnosis based on detection of the CXCL10 gene in urine as a biomarker for diagnosis of active pulmonary TB is 2.8%. Future research is needed to improve the methods by increasing urine samples to 50 ml and using clear medical history of the patients especially the history of anti-tuberculosis drugs.

CONFLICT OF INTEREST

There is no confict of interest regarding this study.

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Indonesian Journal of
Tropical and Infectious Disease

INDEX

Vol. 9 No. 1 January-April 2021

AUTHOR INDEX

SUBJECT INDEX

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