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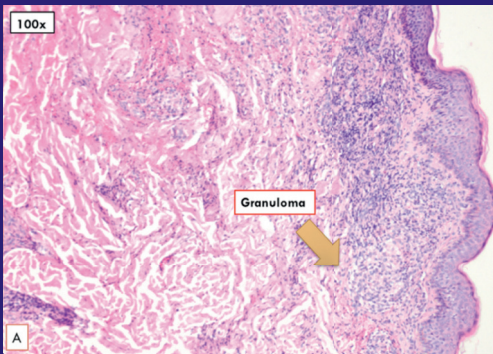

Prevalence of Trichomoniasis in Asymptomatic Pregnant Women Population in Bandung, West Java, Indonesia

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Vol. 7 ● No. 4 January-April 2019

IJTID

Indonesian Journal of
Tropical and Infectious Disease

Indonesian Journal of
Tropical and Infectious Disease

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Research Report

PREVALENCE OF TRICHOMONIASIS IN ASYMPTOMATIC PREGNANT WOMEN POPULATION IN BANDUNG, WEST JAVA, INDONESIA

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ABSTRACT

About 81% of pregnant women with trichomoniasis are asymptomatic, while trichomoniasis in pregnant women can increase the risk of complications, include premature rupture of membranes, preterm birth, and babies with low birth weight. Trichomoniasis can also increase the risk of other sexually transmitted infections (STIs) and human immunodeficiency virus (HIV) transmission. Trichomoniasis case in pregnant women could be influenced by demographic characteristics, the sexual behavior, and also the diagnostic method used. Until now, there is no data about prevalence of trichomoniasis in pregnant women in Indonesia. The aim of this research was to determine the prevalence of trichomoniasis in pregnant women in Bandung, West Java, Indonesia. A descriptive cross-sectional study was performed in December 2016 until January 2017. The study participants were 50 pregnant women who visit antenatal care to Obstetric and Gynecology Clinic of 'Rumah Sakit Khusus Ibu dan Anak Kota Bandung', and meet the inclusion and exclusion criteria, through consecutive sampling. The study participants had a history taking, venereological examination, and Trichomonas rapid test from vaginal swabs. Trichomoniasis in this study was diagnosed based on Trichomonas rapid test, a test that uses color immunochromatographic, capillary flow, dipstick technology, and has high sensitivity and specificity in diagnosing trichomoniasis. Almost all participants in this study were low risk pregnant women to have STI based on demographic characteristics and sexual behaviour. The positive Trichomonas rapid test result was found from one of 50 study participants. In conclusion, prevalence of trichomoniasis in pregnant women in Bandung was 2%. Trichomoniasis case in low-risk pregnant women population is still found.

Keywords: *pregnant women, trichomoniasis, trichomoniasis prevalence, Trichomonas rapid test, sexually transmitted infection*

ABSTRAK

Sekitar 81% ibu hamil dengan trikomoniasis tidak memberikan gejala, sedangkan kejadian trikomoniasis pada ibu hamil dapat meningkatkan risiko timbulnya berbagai komplikasi antara lain ketuban pecah dini, persalinan prematur, dan bayi dengan berat badan lahir rendah. Trikomoniasis juga dapat meningkatkan risiko terkena infeksi menular seksual (IMS) lain dan transmisi (human immunodeficiency virus) HIV. Kejadian trikomoniasis pada ibu hamil dapat dipengaruhi oleh karakteristik demografi, perilaku seksual, dan metode diagnostik yang digunakan. Sampai saat ini belum terdapat data mengenai prevalensi trikomoniasis pada ibu hamil di Indonesia. Tujuan penelitian ini dilakukan ialah untuk mengetahui prevalensi trikomoniasis pada ibu hamil di Bandung, Jawa Barat, Indonesia. Penelitian deskriptif dengan desain potong lintang telah dilakukan pada bulan Desember 2016 hingga Januari 2017. Peserta penelitian adalah 50 ibu hamil yang melakukan kunjungan kontrol kehamilan ke Poliklinik Kebidanan dan Kandungan Rumah Sakit Khusus Ibu dan Anak Kota Bandung, memenuhi kriteria inklusi dan eksklusi, berdasarkan urutan kedatangan. Pada peserta penelitian dilakukan anamnesis, pemeriksaan venereologis, dan tes cepat Trichomonas dari apus vagina Diagnosis trikomoniasis pada penelitian ini ditegakkan menggunakan tes cepat Trichomonas, yaitu suatu tes yang menggunakan teknologi 'dipstick' berbasis imunokromatografi warna, serta memiliki sensitivitas dan spesifitas yang tinggi untuk mendiagnosis trikomoniasis. Hampir seluruh peserta penelitian merupakan ibu hamil yang berisiko rendah terkena infeksi menular seksual berdasarkan karakteristik demografi

dan perilaku seksual. Hasil tes cepat Trichomonas positif didapatkan pada satu dari 50 peserta penelitian. Kesimpulan dari penelitian ini, prevalensi trikomoniasis pada populasi ibu hamil di Bandung sebesar 2%. Masih ditemukan kasus trikomoniasis pada populasi berisiko rendah.

Kata kunci: *ibu hamil, trikomoniasis, prevalensi trikomoniasis, test cepat Trichomonas, infeksi menular seksual*

INTRODUCTION

Trichomoniasis is sexually transmitted infection (STI) caused by a parasite, *Trichomonas vaginalis* (TV).[1,2] Based on the World Health Organization (WHO) meta analysis study in 2012, it is known that trichomoniasis is the world's most common non-viral STI and one of four curable STI.[3] In addition, the Center for Disease Control and Prevention (CDC) established trichomoniasis as one of the five neglected parasitic infections that became the priority of public health programs.[4]

In women, the disease causes inflammation primarily in the vagina,[1] causing clinical abnormalities of dense, foul-smelling, yellow vaginal discharge, which may be accompanied by abdominal pain and dysuria.[2,5,6] Based on a study which conducted in 2013 at antenatal care (ANC) clinics in Iran, it is known that only 19% of pregnant women with trichomoniasis are symptomatic.[7]

In sustainable development goals (SDGs) established by the WHO in 2015, maternal health as well as the prevention of Human Immunodeficiency Virus (HIV) infection/ Acquired Immunodeficiency Syndrome (AIDS) are the main targets.[8] If not adequately treated, trichomoniasis in pregnant women could cause complications in pregnancy, such as premature rupture of membranes (premature delivery), preterm labor, and low birth weight babies (LBW).[1,9,10] Trichomoniasis may also increase the risk of other STIs and HIV transmission.[4,6,7,11-13]

Based on prevalence studies which are conducted in 2005 from commercial sex workers population in ten cities/districts of Indonesia, namely Medan, Tanjung Pinang, Palembang, West Jakarta, Semarang, Banyuwangi, Surabaya, Bitung, Jayapura, and Bandung, the prevalence of trichomoniasis were between 3-33%, with the prevalence of commercial sex workers in Bandung was 18%.[9] There has been no report of trichomoniasis prevalence in pregnant women in Indonesia.

Clinical diagnosis of trichomoniasis in women is difficult because of variation in signs and symptoms and the similarity to other STIs, it really requires laboratory testing. Laboratory tests used to diagnose trichomoniasis are microscopic examination of wet preparation, culture, rapid tests, and nucleic acid amplification tests (TAAN).[1,7,10]

Trichomonas rapid test is a point-of-care examination with an immunochromatography-based detection system using monoclonal-specific antibodies to detect TV antigen. Trichomonas rapid test results can be found within 10 to 30 minutes.[10] In a previous study in women with trichomoniasis, the sensitivity of the examination based on Trichomonas rapid test is 83%, culture 90%, and microscopic examination 56%. Based on a study which is conducted by Campbell, it is concluded that the Trichomonas rapid test has good specificity and requires fewer human resources, that is why it is recommended for screening of low prevalence patient population.[14] OSOM® Trichomonas rapid test has been recognized by the United State (US) Food and Drug Administration (FDA) since 2004.[10]

This study was therefore conducted to determine the prevalence of trichomoniasis in pregnant women in Bandung, West Java, Indonesia.

MATERIAL AND METHOD

Study Methods

The study was carried out in Rumah Sakit Khusus Ibu dan Anak (RSKIA) Bandung, West Java, Indonesia, which is the main maternity hospital in Bandung. This study was a descriptive study using cross-sectional design conducted from December 29, 2016 to January 7, 2017.

Study Participants

The study participants were pregnant women who visited for ANC in RSKIA Bandung regardless of the age of pregnancy, and willing to follow the study after being given an explanation by signing the informed consent form. The selection of the study participants was done by consecutive sampling until the samples were met. Based on the sample size formula, the study needs minimum 29 participants.

Pregnant women who has been used vaginal cleansers (vaginal douche) in the last three days, and who received metronidazole therapy in the last two weeks were excluded from the study.

Study Procedure

The study participants who had previously been examined based on RSKIA ANC procedure, then performed:
1. History and replenishment of medical records of the study.
2. Physical examination, venereological examination, and sampling of vaginal swab for Trichomonas rapid tests.

OSOM® Trichomonas rapid test uses color immunochromatographic, dipstick technology. If TV is present in the sample, it will form a complex with the primary anti-Trichomonas antibody coated on the nitrocellulose membrane. The positive result is indicated by the visible blue line along with the red control line (Figure 1).
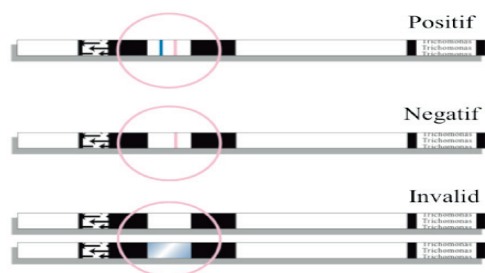
Figure 1. Trichomonas Rapid Test Dipstick: The Positive and Negative Result

Table 1. Trichomonas Rapid Test Results Based on Demographic Data of Study Participants

Variable	Total n=50	Trichomonas rapid test			
		(+)		(-)	
		Total n=1	%	Total n=49	%
Age (year old)					
>16-26	20	1	100	19	38,78
>26-45	30	0	0	30	61,22
Education					
Elementary school graduated	9	0	0	9	18,37
Junior high school graduated	13	1	100	12	24,49
Senior high school graduated	21	0	0	21	42,86
University graduated	7	0	0	7	14,29
Occupation					
Unemployee	38	1	100	37	75,51
Employee	10	0	0	10	20,41
Civil servant	1	0	0	1	2,04
Commercial sex worker	1	0	0	1	2,04
Income					
< Rp 2.600.000	20	0	0	20	40,82
Rp 2.600.000 – 4.500.000	18	0	0	18	36,73
š Rp 4.500.000	12	1	100	11	22,45
Recent gestational age					
First trimester	3	1	100	2	4,08
Second trimester	14	0	0	14	28,57
Third trimester	33	0	0	33	67,35

Table 2. Trichomonas Rapid Test Results Based on Sexual Behaviour of Study Participants

Variable	Total n=50	Trichomonas rapid test			
		(+)		(-)	
		Total n=1	%	Total n=49	%
Coitarche					
< 20 year-old	18	1	100	17	34,69
š20 year-old	32	0	0	32	65,31
Sexual partner					
1 partner	43	0	0	43	87,76
>1 partners	7	1	100	7	14,29
Sexual partner					
Stable partner					
Husband	49	1	100	48	97,96
Boyfriend	1	0	0	1	2,04
Not stable partner					
Yes	1	0	0	1	2,04
No	49	0	0	49	100
Condom used					
No	45	1	100	44	89,80
Yes:					
Routine	0	0	0	0	0
Not routine	5	0	0	5	10,20
Sexual orientation					
Heterosexual	50	1	100	49	100
Bisexual	0	0	0	0	0
Lesbian	0	0	0	0	0
Narcotic, smoking, & alcohol use					
No	45	0	0	45	91,83
Alcohol	3	1	100	2	4,08
Smoking	5	1	100	4	8,16

RESULT AND DISCUSSION

Participants of the study consisted of 50 pregnant women. Trichomonas rapid test results based on demographic data and sexual behavior characteristics of study participants are shown in table 1 and 2.

Study participant with a positive test result of Trichomonas positive was 24 years old, junior high school, unemployed (housewife), family earning above Regional Minimum Wage (RMW) of Bandung, and in first trimester of pregnancy. Based on the venereological examination, the study participant had witish yellow and thick vaginal discharge.

The youngest participant was 18 years old and the oldest was 43 years old. Almost all participants were married (98%), most were senior high school graduated (42%), unemployed (76%), had family income less than Bandung RMW (40%), and were in third trimester of pregnancy (66%).

Most (64%) of the study participants had coitarche at š20 years old. Coitarche at 15 years old was the earliest and at 30 years old was the latest. There were seven (14%) participants who had more than one sex partners in life, six of them were known to marry twice, and only one participant (who is a commercial sex worker) had multiple sexual partners.

The positive result of the Trichomonas rapid test examination of this study was obtained in one (2%) of 50 study participants. This result is similar in studies which conducted by Olowe et al.[5] in 2012 on 100 pregnant women who obtain ANC at the University of Ladoke Akintola University in Nigeria, which trichomoniasis prevalence was 2%. In that study, the diagnosis of trichomoniasis was obtained by microscopic examination of wet preparations.[5] The prevalence of trichomoniasis in pregnant women from various countries shows varying prevalence rates. The lowest prevalence has been reported for pregnant women was in South Korea in 2013, which the prevalence of trichomoniasis (based on microscopic examination of wet preparations) was 0.6%,[15] and the highest prevalence reported in the population of pregnant women in Zambia, which the prevalence of trichomoniasis (based on polymerase chain reaction/PCR) was 32.2%.[16]

The results of trichomoniasis prevalence in pregnant women in other countries are varied, ranging from 3% in South Korea in 2013 (PCR),[15] 3.3% in Iran in 2010 (microscopic and culture),[17] 7.7% in Brazil in 2009 (TAAN),[18] 8% in India in 2014 (microscopic

wet preparation),[19] 9.9% in African Republic in 1990 (culture),[20] 10.3% in Nigeria in 2013 (microscopic wet preparation),[21] to 41.4% in South Africa in 1990 (TAAN).[20] The prevalence of trichomoniasis in pregnant women is influenced primarily by demographic characteristics[22] and the participants' sexual behavior.[23] In addition, the diagnostic method used may also affect the prevalence of trichomoniasis.[13]

Some of the demographic factors significantly associated with trichomoniasis occurrence in pregnant women include active sexual age,[22,24] low levels of education,[22] as well as occupation as a prostitute.[16] High rates of trichomoniasis in the active sexual population are associated with higher sexual activity, lack awareness of STIs, as well as changes in vaginal microbiota (especially during menstrual periods).[25] In a study of pregnant women in Papua New Guinea, it was found that pregnant women aged 24 years or older had twice the risk of having trichomoniasis compared to older adults.[26]

In this study, only 40% of participants were in active sexual age population. This fact illustrated that the study population of this study was not a high risk population. Nonetheless, study participant with a positive test result of Trichomonas was a 24 years old woman. This fact showed that the result of the study was in accordance with the characteristics of pregnant women at risk of trichomoniasis.

Low levels of education are associated with a high incidence of trichomoniasis. This is due to a relation between low levels of education with unsafe sexual behavior and the number of multiple sexual partners.[26] Based on study by Allsworth et al., Romoren et al., and Miranda et al., pregnant women with education less than senior high school,[27] junior high school,[22] or only for eight years,[18] were at higher risk of TV infection.[18,22,27]

In this study, more than 50% of participants had education higher than senior high school. This fact illustrated that the study population was not a high risk population based on education level. Study participant with a positive Trichomonas rapid test result in this study was known to have junior high school education, which was consistent with the characteristics of pregnant women at risk of trichomoniasi.

Based on the results of several studies, it is known that in unemployed pregnant women were at risk of TV infection. Housewives belong to low-risk groups contracting STIs. However, transmission can be obtained by sexual partners who act as bridging populations because they are associated with commercial sexual worker/prostitute (core population).[29] Based on studies conducted by Madhivan et al.[23] in 2006 in South India, it was found that as many as 74% of women with trichomoniasis were housewives. Job that is considered to be at high risk for STIs was commercial sex worker which is associated with unsafe sexual behavior.[29] In a study performed by Crucitti et al.[16] in Zambia, it was found that commercial sex workers were more likely to be infected with TV.[16]

About 76% of this study participants were housewives. Participant with a positive test result Trichomonas positive was a housewife. There was one participant who had a job as a prostitute but no trichomoniasis was found. Further study is needed for trichomoniasis in relation with housewife work.

Trichomonas vaginalis infection can be a marker of high-risk sexual behavior.[30] Early coitarche may increase the risk of greater cumulative sexual exposure, thus increasing the risk of becoming infected with TV.[23] In a study by Madhivanan et al.[23] in 2006 in South India, two-thirds of the women who had positive trichomoniasis had coitarche when less than 19 years old.

Participants in this study were (64%) coitarche at age š19 years. This fact illustrated that the study population was largely not a risk population based on coitarche. Study participant with positive Trichomonas rapid test result had coitarche in aged 18 years. This finding was suitable with the characteristic risk of trichomoniasis.

Based on the study of Allsworth et al,[27] it was concluded that women who had 3-5 sexual partners throughout their lives had a risk of trichomoniasis nearly nine times greater. Rogers et al.[12] showed the results of his study in Baltimore in 2009 that women with trichomoniasis who had two or more previous sexual partners had a trichomoniasis risk almost three times higher than those with one sexual partner.

Most of the study participants (86%) have only one sexual partner. It also explains the low prevalence of trichomoniasis in this study. Study participant with a positive Trichomonas rapid test result in this study had a total sexual partner number of more than one person (three persons), which was fit with the characteristics of pregnant women at risk of trichomoniasis, more sexual partner–more risk of trichomoniasis.

Based on study by Miranda et al.,[18] it is known that pregnant women who have persistent sexual partners, are more at risk of being infected with TV. In this study, most of the study participants (98%) had one stable sexual partner (husband), including participant with a positive Trichomonas positive test result. The relationship between trichomoniasis and the characteristics of sexual partners needs to be further investigated.

Based on univariate analysis by Ambrozio et al.[31] in 2016 from 19 municipalities in Southern Brazil, it was found that the absence of condoms during intercourse increased the risk of being infected with TV. In a study by Swartzendruber et al.[32] of African-American women with trichomoniasis in Atlanta, it was found that there was no correlation between condom use and TV-infected risk. Most (90%) study participants never used condoms during intercourse. Participant with a positive test result of Trichomonas rapid test, was known to never use condom. Further study is needed on the use of condoms and the risk of trichomoniasis.

It was found that pregnant women who used narcotics were nearly eight times more likely to be infected with TV than those who did not.[18] Based on studies of African-

American women, it was found that smoking and alcohol consumption was associated with an increased incidence of trichomoniasis. Smoking is thought to affect the condition of the vagina to become susceptible to infection,[16] whereas alcohol consumption may increase the risk of STI infection because the effects after taking it can increase sexual desire.[30]

Most (90%) of the study participants had no history of smoking, alcohol consumption, or other drugs. This explains the participants in this study were at low risk of trichomoniasis. In this study, participant with a positive Trichomonas rapid test was known to have a history of smoking and alcohol consumption. This finding was in line with the findings of the study described before.

As known before, trichomoniasis prevalence in pregnant women is influenced by demographic characteristics,[22] the participants' sexual behavior,[23] and the diagnostic method used.[13]

In this study, almost all participants were low risk pregnant women to have trichomoniasis based on demographic characteristics and sexual behaviour. However, the study participant with a positive test result of Trichomonas rapid test was a woman with trichomoniasis risks: in active sexual age (24 years old), had low level of education (junior high school graduated), had early coitarche (at 18 years old), and had multiple sexual partners throughout her life (three partners).

The diagnostic method that used (Trichomonas rapid test) in this study was performed according to recommended procedures. This revealed that the method used in this study was probably not a factor that affects the low value of trichomoniasis prevalence.

CONFLICT OF INTEREST
We have no conflict of interest to declare.

CONCLUSION
Trichomoniasis case in low-risk population is still found. Considering trichomoniasis complications in pregnant women (premature delivery, preterm labor, and LBW) and other STIs and HIV transmission risk after trichomoniasis infection, screening and treatment of trichomoniasis are necessarily included in ANC program.

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Research Report

LEPROSY AND HUMAN IMMUNODEFICIENCY VIRUS COINFECTION: A RARE CASE

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ABSTRACT

Leprosy, or Morbus Hansen, is a chronic infectious disease which caused by Mycobacterium leprae. It is associated with inflammation that may damage the skin and peripheral nerves. Leprosy remains an important public health problem in Southeast Asia, America, and Africa. It has been speculated that, as with tuberculosis, Human Immunodeficiency Virus (HIV) infection may exacerbate leprosy lesions and/or lead to increase susceptibility to leprosy. We are reported the case of leprosy and HIV coinfection and reveals its clinical manifestation. A 34-year-old female came to outpatient clinic complaining of redness plaque on her face of 2-months duration. It was also accompanied with thick sensation without itchy or burning sensation. We found thick erythematous plaque with sharp margin and hypoesthesia on her face and body. There were no madarosis, saddle nose, lagophthalmos and sign of neuritis. The slit-skin smear revealed BI 1+ globi and MI 2%. From laboratory examination we found IgM anti PGL-1 titer was 1265 u/mL and IgG anti PGL-1 was 834 u/mL. The similar lesion of leprosy was found on her both of ear lobe and legs by using histological examination. The detection of HIV antibody was positive with CD4 count on 325 cells/μL. We treat her with multidrug treatment (MDT) for multibacillary leprosy along with anti-retroviral therapy or ART consist of Tenofovir, Lamivudine, and Efavirenz. After 6-months follow-up we are observed no progression of the lesions though the slit-skin smear become negative. M. leprae does not seem to accelerate the decline of immune function when associated with HIV infection. HIV infection does not seem to affect the clinical classification and progression of leprosy. The treatment of the HIV-leprosy coinfected patient consists of the combination of ARTs and anti-leprosy agents. Those treatment gives the good result in the bacteriological state of the patient.

Keywords: *leprosy, Hansen disease, HIV Co-infection, leprosy-HIV, MH.*

ABSTRAK

Kusta, atau Morbus Hansen, merupakan penyakit inflamasi kronik yang disebabkan oleh Mycobacterium leprae, terkait dengan inflamasi yang merusak kulit dan saraf perifer. Kusta tetap menjadi masalah kesehatan masyarakat di Asia Tenggara, Amerika, dan Afrika. Koinfeksi dengan HIV memiliki pengaruh besar terhadap perkembangan penyakit, terutama penyakit mikobakterial. Telah diduga sebelumnya, seperti halnya tuberkulosis, infeksi Human Immunodeficiency Virus (HIV) menyebabkan eksaserbasi lesi kusta dan/atau meningkatkan kerentanan terhadap kusta. Kami melaporkan kasus koinfeksi kusta dan HIV dan menunjukkan manifestasi klinisnya. Seorang wanita 34 tahun datang ke klinik rawat jalan mengeluhkan bercak kemerahan sejak 2 bulan. Hal ini disertai pula dengan sensasi tebal tetapi tanpa gatal atau rasa terbakar. Tidak disertai demam atau benjolan. Dari pemeriksaan ditemukan plak eritematosa berbatas jelas dan hipoestesia pada wajah dan badan. Tidak ditemukan madarosis, hidung pelana, lagoftalmos, atau tanda neuritis. Pemeriksaan basil tahan asam menunjukkan BI 1+ globi dan MI 2%. Pemeriksaan laboratorium darah menunjukkan dalam batas normal, titer Ig M anti PGL-1 1265 u/mL dan IgG anti PGL-1 834 u/mL. Kedua pemeriksaan histologis dari cuping telinga dan tungkai menunjukkan gambaran menyerupai kusta. Deteksi antibody HIV positif dengan hitung CD4 325 cells/μL. Pasien diterapi dengan multidrug treatment (MDT) untuk leprosy tipe multibasiler dan anti-retroviral therapy atau ART yang terdiri dari Tenofovir, Lamivudine, and Efavirenz. Setelah 6 bulan terapi dapat kami amati bahwa tidak ditemukan perkembangan bermakna dari lesi meskipun pemeriksaan basil tahan asam menjadi negatif. Kasus ini merupakan koinfeksi kusta-HIV. M. lepra tampaknya tidak

mempercepat penurunan fungsi imun terkait infeksi HIV. Infeksi HIV juga tidak mempengaruhi klasifikasi klinis dan perkembangan kusta. Terapi pasien koinfeksi kusta-HIV terdiri dari kombinasi ART dan agen anti-kusta MDT. Terapi tersebut memberikan perbaikan hasil bakteriologis yang cukup baik untuk pasien.

Kata kunci: *kusta, penyakit Hansen, koinfeksi HIV, kusta-HIV, MDT*

INTRODUCTION

Leprosy, or Hansen disease (HD), is a chronic infectious disease which caused by *Mycobacterium leprae* which is associated with inflammation that may damage the skin and peripheral nerves.[1] Despite the claim by the World Health Organization (WHO) that it would no longer be a public health problem after the year 2000, leprosy is far from being eliminated, with more than 200,000 new cases being reported yearly during the past 5 years. Leprosy remains an important public health problem in Southeast Asia, America and Africa.[2,3]

Human Immunodeficiency Virus (HIV) infection prevalence rates are high in many countries where leprosy is still endemic.[2,4,5] In 2008, 121 countries were reported a total of 249,007 new leprosy cases to WHO. Most endemic countries for leprosy also have a high HIV prevalence, increasing the possibility of HIV-leprosy coinfection. Although the number of coinfected patients has not been estimated yet, the increasing geographic overlap of these two diseases will result in increasing number of person being dually infected.[6]

Meanwhile, there are few number of case reports of leprosy that have association with HIV infection.[4,6] A few studies have tried to evaluate reasons for this rare co-existence. Tissue cell-mediated immune response against *M. leprae* is known to be preserved even though the peripheral blood lymphocyte count was reduced in concurrent leprosy and HIV-infected patients.[6] Thus probably, there are less reports of leprosy in association with HIV.

The present case of leprosy in an HIV-infected person is herewith reported for its rarity. This case report is aimed to describe the different manifestation of leprosy and HIV coinfection. The understanding about the existence of coinfection should be remember and it bring also the obligation to follow standardized guideline treatment.

CASE

A 34-year-old female came to the dermatology outpatient clinic of Dr. Soetomo General Hospital Surabaya on December 13th 2017. She came with chief complaint of redness plaque on her face. She is complained about it since about 2 months before admission. Firstly, she got this lesion on her face with small size, by the time then this lesion became larger. This symptom was also accompanied with thick sensation over the red area but without itchy or burning sensation. She had no fever before. She went to several general practitioners and was diagnosed with atopic dermatitis. She got some medications but there were no significant differences before and after taking those

treatments. After several weeks back then, there were some erythematous and blackish macule that was spread on her extremities. Because of feeling afraid of this condition, she sought any help to dermatology and venereology outpatient clinic of Dr. Soetomo General Hospital and was diagnosed as leprosy.

About one month after she got treatment from there, she had a chronic diarrhea and had a low fluid intake until she became severe dehydration. Because of this condition she was hospitalized in other hospital and did some general examination which is one of those examination panel was HIV rapid testing. Those laboratory data revealed that she got HIV infection. She was started on antiretroviral therapy (ART) one month later.

The patient was married with a man since about 10 years ago. She is refused to have a sexual intercourse before she was married. She is claimed that her husband was the one and only sexual partner of her. Her husband was a worker on the building construction project. The history of sexual activity of her husband was unknown. The patient is denied of the same disease before and her husband was not having the same symptoms. There was no history of consuming drugs before the lesions appeared, drug hypersensitivity, blood transfusion, injection drug user, or drug abuser. History of fever, headache, malaise, and weight loss were denied.

The physical examination of general state was all within normal limit. Blood pressure was 100/70 mmHg, pulse rate was 84 times per minute, respiratory rate was 18 times per minute and body temperature was $36,4^0$ C. From head and neck, there were no signs of anemia, cyanosis, icterus, or dyspneu. From thorax examination, heart and lungs were normal. From abdomen, liver and spleen were not palpable. From her upper and lower extremities there were no edema and warm on palpation. There was no enlargement of the cervical, axillar, inguinal and genital lymph nodes.

Dermatological examination on her right face especially on the periorbital region discovered the thick erythematous plaques with sharp margin, some are covered with white fine scales and hypoaesthetic (Fig. 1 A). No madarosis of the eyebrows or eyelashes was observed. There were no saddle nose or diffuse infiltrate on the face, and lagophthalmos. There was also multiple erythematous macule that sharply marginated accompanied with erythematous papules that varied in size about 0,5-1 cm on her trunk, upper, and lower extremities (Fig. 1 B-E). There was no thickened peripheral nerves on the left and right ulnar nerves and did not accompanied with tenderness on palpation. In addition, peripheral neurological symptoms, including motoric, sensory and autonomic nerve disturbance were not detected based on a neurological assessment that included light

Figure 1. The Clinical Manifestation of the Patient on The First Examination on Right Facial Region, There Was Erythematous Plaque with Sharp Margin (Picture A); The Other Manifestation of The Patient on The Trunk and Extremities Region, There Were Multiple Erythematous and Hyperpigmented Macules that Varied in Size (Picture B-E)

touch, pin–prick test, thermal sensory test, manual muscle strength test and monofilament test.

We found that acid-fast bacilli was detected by the slit – skin smear test of the ear lobes and lesion (Bacterial Index: 1+ globi; Morphological Index: 2%). The laboratory examination on December 13th 2017 was revealed: haemoglobin was 14,0 g/dL, white blood count 8.090/mm³, thrombocyte 302.000/L, and hematocrite 41,2%. Detection of HIV antibody (3 methods) on January 2018 was positive with CD4 count on 525 cells/μL. Serologic test by detecting antiphenolic glycolipid I (anti PGL-1) antibody was positive by the score of IgM = 1265 (cutt off = 605 u/mL) and IgG = 834 (cutt off = 630 u/mL).

Histological examination of the ear lobe skin was revealed atrophy and short-flattening of rete ridge on the upper epidermis, there were some group of hystiocyte or foam cell on superficial to deep dermis. No specific microorganisms were identified by Fite – Faraco staining. The conclusion of that biopsy was borderline leprosy. The picture of this examination can be clearly seen on the Figure 2A.

Because of our suspicion on several diagnosis of the lesions on her trunk and extremities, we did the biopsy examination on that location too. The skin biopsy on extremity was revealed atropy and short-flatening of rete ridges on epidermis, some epitheloid cells which form granuloma, some lymphocyte and eosinophil infiltration on the dermis. There was no bacteria were observed on Fite–Faraco staining. The conclusion was similar to the lesion of borderline tuberculoid leprosy. In those two examination we did not see any differences in the manifestation of the disease according to the histologic examination. The picture of this examination can also be clearly seen on the Figure 2B.
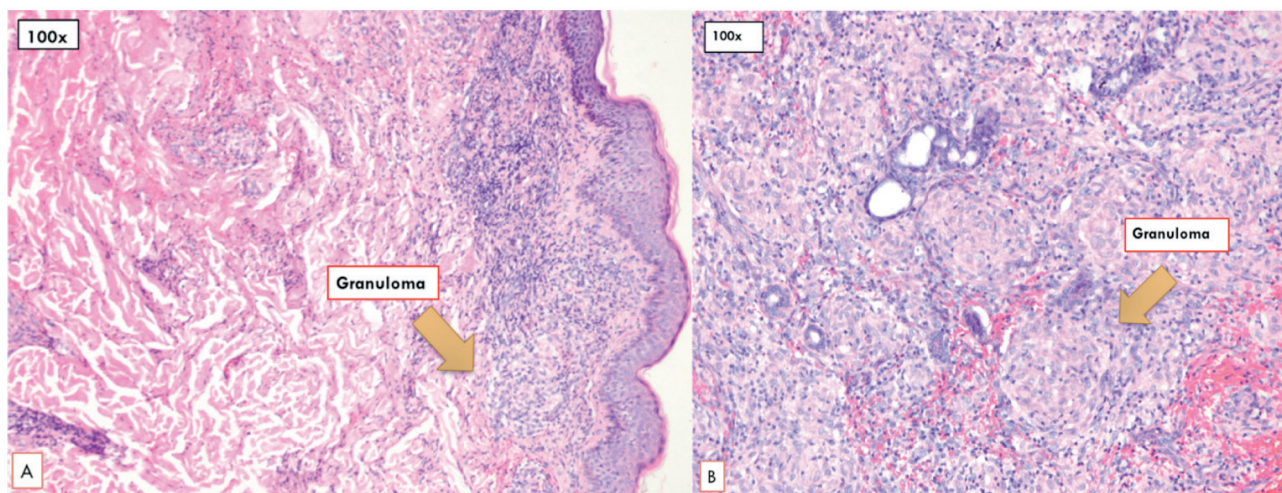
Figure 2. The Histologic Examination of Ear Lobe (Picture A) Revealed Atrophy of Epidermis with Short-Flattening of Rete Ridges, We Found Group of Histiocyte Or Foam Cell On Superficial To Deep Dermis and Datia Langhans Cell, There Were No Bacteria Observed and The Conclusion Was Borderline Leprosy; The Other Histologic Examination On The Extremity (Picture B) Revealed A Slight Different That We Found Epithelioid Cells That Form Granuloma and Some Lymphocyte and Eosinophil Infiltration, We Conclude The Result As Borderline Tuberculoid Leprosy

Figure 3. The Skin Lesion on Her Face at The First Come (Picture A), 3rd Month of MDT (Picture B); and after 6th Month of MDT (Picture C). We Found No Progression of The Lesions.

Figure 4. The Skin Lesion on Her Forearms at The First Come (Picture A), 3rd Month of MDT (Picture B); and After 6th Month of MDT (Picture C). We Still Found No Progression of The Lesions.

Based on these findings, from physical and laboratory examination, the diagnosis of multibacillary, borderline lepromatous (BL) leprosy with HIV coinfection was established. There was no sign of the leprosy reaction at this time. The patient were observed for the period of time to observe the amendment of her condition.

According to the World Health Organization (WHO) classification, she was classified as having Multibacillary Leprosy and got Multidrug Treatment of Leprosy (MDT). Those regiment consisted of Rifampicin 600 mg monthly, Clofazimine 300 mg once a month and 50 mg daily, and Dapsone 100 mg daily for 12 months which is the WHO recommended for multibacillary leprosy. She was also initiated on first-line antiretroviral therapy (ART) regimen including Tenofovir, Lamivudine, and Efavirenz.

Six months since initiating MDT for leprosy, the patient remained stable without new lesions or neurological deficits. However, there were no progression of the lesions even though she has been treated for 6 months. The progression of the disease was clearly described in Table 1 and the lesions can be seen on Figure 3 to 5

DISCUSSION

Leprosy is one of a deliberately progresssive infectious disease caused by *Mycobacterium leprae*. It is a disease which primarily affects the skin and peripheral nerve, and in highly bacillated state, any internal organ except central nervous system can be affected too. The damage to peripheral nerves results in sensory and motor impairment which characterized by dreadful abnormalities and debilities.[7-9]

Talhari *et al*. were proposed the classification for leprosy associated with HIV infection. This classification recognizes true leprosy-HIV coinfection, opportunistic leprosy disease, and leprosy related to ART.[8] Recently, it was suggested that even though leprosy–HIV coinfection does not manifest homogenously across affected populations, immunological features seem to be shared by certain subgroups. In this context, a clinical classification of *M. leprae* and HIV/
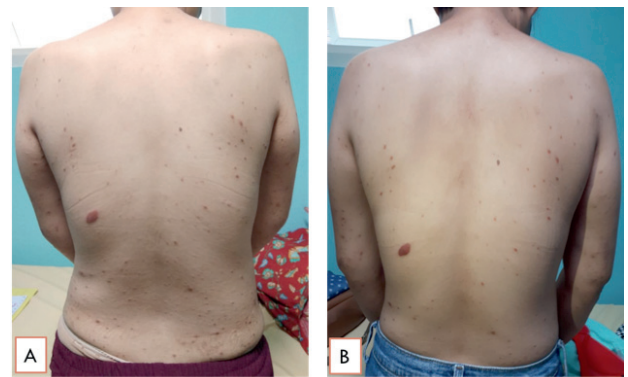
Figure 5. The Lesions on Her Back Were The Same between Her First Come (Picture A) and After 6th Month of MDT (Picture B)

Table 1. Progression of The Disease

	Dec 13, 2017	Jan 2, 2018	Feb 14, 2018	April 30, 2018
SUBJECTIVE				
1. Redness	+	+	+	+
OBJECTIVE				
1. Erythematous plaque	+	+	+	+
2. Scale	+	+	-	-
3. Patches on extremities	+	+	+	+
ASSESSMENT				
Borderline lepromatous leprosy + HIV	+	+	+	+
PLANNING OF DIAGNOSIS				
1. Slit skin smear	+	-	-	-
2. Punch biopsy	+	-	-	-
3. HIV antibody test	+	-	-	-
PLANNING OF THERAPY				
1. MDT-MB	+	+	+	+
2. HAART	+	+	+	+
3. Thiamin tablet	+	+	+	+
4. Vitamin B complex tablet	+	+	+	+
5. Urea 10%	+	+	+	+

AIDS-coinfected patients including in the following criterias.[2] The first criteria are *M. leprae*–HIV true coinfection. This group consists of HIV positive individuals who do not fulfill AIDS criteria and are not under HAART. The patients have similarity to immunocompetent subjects.[2] The next criteria are opportunistic leprosy disease. This criteria consist of AIDS patients who do not

receive HAART, presenting usually with multibacillary leprosy. This group would include individuals manifesting leprosy as an opportunistic mycobacteriosis, as expected in immunosuppressed individuals.[2] The last criteria are HAART-related leprosy. This criteria include AIDS patients presenting all clinical forms of leprosy related or not to IRIS. Combined HAART and MDT may cause upgrading shift within the leprosy clinical spectrum, as may be revealed by long-term follow-up.[2]

According to those criterias, we could define the leprosy and HIV in this case as *M. leprae*–HIV true coinfection. This case illustrates clinical manifestations of leprosy that was not worsen by HIV infection, although it slowly progressed during the follow-up.

It is a well-known fact that in tuberculosis (TB) and HIV coinfected patients, TB and HIV infection itself contributes to the progression of each other.[10-12] Active TB infection in HIV-infected patients is associated with increased immunodeficiency and mortality in those patients.[13-15] It has been hypothesized that HIV infection may exacerbate leprosy lesions and/or lead to increased susceptibility to leprosy. This condition was thought to be like in TB and HIV infection. However, there is less evidence to support this hypothesis. In the contrary, there were several studies that have found that in leprosy and HIV coinfected patients, each disease progresses independently.[6,16]

A few studies were performed to evaluate the reasons for this rare co-existence. Tissue cell-mediated immune response against *M. leprae* is known to be preserved even though the peripheral blood lymphocyte count was reduced in concurrent leprosy and HIV-infected patients.[6] The deficiency in cell-mediated immunity (CMI) is specific to the *M. leprae* antigens and has nothing to do with the decreased peripheral CD4 count of HIV.[17] Thus probably, there are less reports of leprosy in association with HIV.

Mycobacterium leprae does not seem to accelerate the decline of immune function when associated with HIV infection. This condition was different with the fact which often happens in tuberculosis coinfection.[18,19] Reactional states may occur more frequently in individuals with HIV coinfection. However, there are still many conflicting data regarding increased reaction frequency in this group.[16]

As noted in this patient, HIV infection did not seem to affect the clinical classification and progression of leprosy. As we found in a study by Pereira et al. that the clinical, immunologic, histopathology, and virology features among 22 HIV-leprosy coinfected Brazilian patients indicate that each disease is progressed as in single infection.[20] Despite overall HIV-associated immunosuppression, cell-mediated immune responses to *M. leprae* are well preserved at the site of the disease.[20,21] Based on our experience as we found in our patient, the disease was progressed slowly, and the lesions did not alter morphologically over a period of 6 months follow up. This suggests that the pathogenesis of leprosy in this patient was unaffected by her immunodeficiency. This finding was similar to the result of the study that was mentioned above.[20]

Initiation of HAART has been associated with Immune Reconstitution and Inflammatory Syndrome (IRIS) in various situations. IRIS in leprosy may trigger potential adverse effects, such as leprosy acute inflammatory episodes.[7,10,22] This usually leads to a worsening of the initial lesion characterized by erythema and tenderness in the setting of rising CD4 count and falling viral load. These reactions are more common in patients with low CD4 counts especially during the initial 3 months of initiation of ART. Typically, as the immune system further recovers, the lesions become tuberculoid or paucibacillary as opposed to lepromatous.

In our patient, there was no change in the appearance of the skin lesions after starting HAART with no evident virological suppression and immune reconstitution with the latter. More so, there were no neurological deficits noted even after 6-months therapy of MDT and HAART.

The follow-up of this case after 6 months of MDT for leprosy combined with HAART was revealed negativity of skin slit smear. Although it has no significant different in the clinical manifestation, but the progression in skin slit smear indicates the cure of leprosy in this patient. Moreover, we still continue the MDT regiment for 12 months for multibacillary leprosy based on the WHO's recommended treatment regimens for multibacillary leprosy.

The therapy for leprosy with HIV coinfection is still the same with leprosy without coinfection. HIV infection might affect the efficacy of multidrug therapy for leprosy. The HIV positive patients are potentially taking longer to be treated or experiencing a higher relapse rate of leprosy. But some published data were suggested that leprosy-HIV coinfected patients respond equally well to multidrug therapy without the need for prolonged treatment.[16] Relapses are rare after multidrug therapy. It counts about 1 per 1000 person-years for tuberculoid patients and 0-20.4 per 1000 person-years for multibacillary patients.[16,23]

CONCLUSION

Based on the available data, we can conclude that leprosy and HIV coinfection has three different criteria. One of them is the true coinfection, such in this case, is the diseases that progress independently. In general, the therapy for this patient is the same as the disease was separately. Those treatment includes standard WHO-MDT in conjunction with HAART according to the patient's clinical state. The influence of HIV infection on cell-mediated immune responses to *M. leprae* in the HIV- infected patient needs more exploration. Leprosy and HIV coinfection is an evolving situation with ongoing discoveries and further research needs.

of Dr. Soetomo General Hospital Surabaya and patient who participated in this study.

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Research Report

EFFECT OF AFRICAN LEAF (VERNONIA AMYGDALINA) TO IL-6 AND IL-10 LEVEL ON STAPHYLOCOCCUS AUREUS INFECTION

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ABSTRACT

Currently, infectious disease is increase in world wide. The African leaf (Vernonia amygdalina) – VA is used to antimicrobial treatment. It may protect the host against microbial attack in several ways. This plant has attracted the interest of researchers in recent decades because of the constituents have important roles in modulating immune system in bacteria infection. The aim of study is to analyze the prophylactic activity of VA's ethanol extract in modulating the levels of IL-6 and IL-10 as well as the number of bacteria in male Wistar rats that were (Staphylococcus aureus) – SA – infected. There were as many as 30 rats were divided into 5 treatment groups: negative control (NC) was treated by CMC Na 2% (w/v); positive control (PC) was treated by 9mg/200g body weight (BW) of cephadroxil; T1; T2; and T3 were respectively treated with ethanol extract of VA of doses 20mg/200g BW; 40mg/200g BW and 80mg/200g BW. After the oral treatment was administered, all the rats were infected with 0.25mL ($3x10^8$cfu) SA via intra peritoneal route. Their blood was drawn in order to identify the IL-6 and IL-10 levels by ELISA. Furthermore, their peritoneal fluid was also taken to count the number of survived bacteria by pour plate method. The results are showed median of IL-6 and IL-10 levels as well as bacterial number respectively in NC 370.530pg/mL; 67.044pg/mL; $7.4x10^3$cfu/mL; in PC 234.556pg/mL; 42.839pg/mL; $6.8x10^3$cfu/mL,; in T1 164.019pg/mL; 17.240pg/mL; $1.1x10^4$cfu/mL; in T2 49.291pg/mL; 2.961 pg/mL; $6.3x10^3$cfu/mL and in T3 43.342pg/mL; 13.235pg/mL; $7.1x10^3$cfu/mL. These results are implied that VA's ethanol extract is effective as a prophylactic agent to suppress the bacterial invasion at dose of 40mg/200g BW in Wistar rat particularly shown by the decrease level of IL-6 and the number of bacteria.

Keywords: Vernonia amygdalina, IL-6, IL-10, bacterial number, Staphylococcus aureus.

ABSTRAK

Saat ini, penyakit infeksi meningkat di dunia. Daun Afrika (Vernonia amygdalina) – VA digunakan sebagi terapi antimikroba. VA dapat melindungi host terhadap serangan mikroba melalui berbagai macam cara. Tanaman ini menarik perhatian para peneliti pada dekade terakhir karena VA memiliki berbagai kandungan yang berperan penting dalam memodulasi sistem imun pada infeksi bakteri. Pada penelitian ini, dilakukan analisa terhadap kemampuan profilaksis dari ekstrak etanol VA dalam memodulasi kadar IL-6 dan IL-10 serta jumlah bakteri pada tikus Wistar jantan yang terinfeksi (Staphylococcus aureus) – SA. Tikus Wistar sebanyak 30 ekor dibagi ke dalam 5 kelompok uji yaitu kontrol negatif (NC) yang diterapi dengan CMC Na 2% (b/v), kontrol positif (PC) yang diterapi sefadroksil 9mg/200g BB, kelompok T1, T2 dan T3 yang diterapi dengan ekstrak etanol VA dengan dosis: masing-masing 20mg/200g BB; 40mg/200g BB; dan 80mg/200g BB, secara berurutan. Setelah diberikan terapi, tikus diinfeksi dengan bakteri SA sebanyak 0,25mL ($3x10^8$cfu) secara intra peritoneal. Darah tikus dikoleksi untuk dianalisa kadar IL-6 dan IL-10 menggunakan ELISA dan dikoleksi pula cairan peritoneal untuk dihitung jumlah bakteri yang bertahan hidup menggunakan metode tuang. Hasil median dari kadar IL-6, IL-10 dan jumlah bakteri secara berurutan adalah sebagai berikut: pada kontrol negatif (NC) 370,530pg/mL; 67,044pg/mL; $7,4x10^3$cfu/mL; pada kontrol positif (PC) 234,556pg/mL; 42,839pg/mL; $6,8x10^3$cfu/mL; pada T1 164,019pg/mL; 17,240pg/mL; $1,1x10^4$cfu/mL; pada T2 49,291pg/mL; 2,961 pg/mL; $6,3x10^3$cfu/mL; pada 43,342pg/mL; 13,235pg/mL; $7,1x10^3$cfu/mL. Hasil penelitian ini mengimplikasikan

bahwa ekstrak etanol VA efektif sebagai senyawa profilaksis yang mencegah invasi bakteri, khususnya pada dosis 40mg/200g BB tikus karena pada dosis tersebut terjadi penurunan kadar IL-6 dan jumlah bakteri.

Kata kunci: *Vernonia amygdalina, kadar IL-6, kadar IL-10, jumlah bakteri, Staphylococcus aureus.*

INTRODUCTION

Globally, there is an increase in infectious disease especially in bacteria infection. It was caused of host body defense mechanism can't control the immune system.[1] Therefor, it is an evident from human history that we have to some new product to modulate immune system against bacteria infection. One of which is medicinal plants that it have been utilized as therapeutic agents in variety of disease including infection diseases. Medicinal plants are through to be mediated through inhibition and modulating cell-signaling pathways in immune system.[2] The immunomodulating characteristic of medicinal plants is safety, effectiveness, minor side effect and cultural acceptability.[3]

Vernonia amygdalina (*VA*) is one of medicinal plants[4] and a member of the Asteraceae family. This plant is a small tree in 2-5 m of size. *Vernonia amygdalina* leaf is ellipse in form and about 6 mm of diameter. The green leaves are showing bitter taste and odor characteristics.[5] This bitter taste serves as a protection of animals attack such as insects and microbes.[6] Bitter taste is due to the flavonoids and sesquiterpenes lactones contained in *VA*.[7] *Vernonia amygdalina* contains many other constituents such as: tannins,[8] saponins, alkaloids, terpenoids, stigmastane-type steroid glycosides, coumarin, phenolic acids, lignans, xanthones, anthraquinones and edotides.[9] Several constituents of *VA* that have been reported to function as antimicrobial agent and modulate the immune system is known as luteolin[5] and myricetin; andrographolide;[9] and chlorogenic acid.[10]

Previous studies were illustrated the antimicrobial effects of *VA* in ethanol extract. This ethanol extract is more effective to exhibit antimicrobial effects than water extract.[4,11-13] Previous in vitro studies is proved that ethanol extracts of *VA* showed high potency and effectivy against *Staphylococcus aureus* (*SA*).[11,13]

Staphylococcus aureus is a pathogenic bacterium[14] that belong to gram-positive bacteria with coccal (round) form and also known as a facultative anaerobes. It has a complex cell wall consisting of murein, teichoic acids and surface proteins.[15]

In the human body, lipoteichoic acid (LTA) on the surface of *SA*'s cell wall was known as Pathogen Associated Molecular Patterns (PAMPs) which generally be recognized by Pattern Recognition Receptors (PRRs) such as Toll Like Receptors (TLRs)-1/2 or TLRs-2/6. The PRRs is the property of the immune cells such as macrophages, dendritic cells, endothelial cells, mast cells, eosinophils and B cells.[16,17] PRR will exhibit a signal that may attract the Nuclear Factor κappa B (NFκB) transcription factor to enter the nucleus and synthesize pro-inflammatory cytokines such as interlukin (IL)-1β, Tumor Necrosis Factor (TNF)-α, IL-6,[18] IL-12 and IL-8 or CXCL8.[19]

IL-6 is known as a pleitropic cytokines found in each organ system. It is synthesized by mononuclear phagocytes, dendritic cells, vascular endothelial cells, fibroblasts and other cells in response to PAMPs and IL-1 and TNF stimulation.[17] IL-6 production immediately increases in the acute inflammatory condition that occurs due to infection, injury, trauma and other stress conditions. These cytokines retain extracellular and intracellular growth from SA but excessive production can lead to systemic inflammation with damaging effects rather than protection of the host.[18] In order to balance the IL-6 effects, another cytokine, IL-10 is produced and is served as an anti-inflammatory agent. This cytokine is produced by immune cells including macrophages and active dendritic cells, T regulators (Tregs), T Helper (T_H)-1, and T_H2 cells. IL-10 is also produced by several B lymphocytes which show immune suppression function, called regulatory B cells. IL-10 is also an important cytokine that regulate the immune responses in infection diseases.[20]

In infection caused by *SA*, generally, immune cells are stimulated to promote pro-inflammatory cytokine (such as: IL-6) dan anti-inflammatory cytokine, such as IL-10.[21] However, until recently, there is no study identify ethanol extract of *VA* activity in modulating these cytokines in microbial infections. Therefore, this study was conducted in vivo to study the *VA*'s ethanol extract activities in immune system in Wistar rats which infected by *SA*. Further, this study is aimed to analyze prophylactic activity of *VA's* ethanol extract in modulating levels of IL-6, IL-10 as well as the decrease of the number of bacteria in male wistar rats that were *SA*-infected.

MATERIAL AND METHOD

I.   Collection of Plant Materials

Fresh leaves of *Vernonia amygdalina* were obtained from Jember, East Java, Indonesia. The leaves were dried in an oven at 60°C and then blended into powder.

II.   Preparation of Ethanol Extract

Vernonia amygdalina powder was macerated with 96% ethanol (1: 5) for 72 hours was followed by filtration with Whatman No.1 filter paper and was evaporated in a rotary evaporator, the thick extract was yielded. The thick extract dissolved in CMC Na 2%.

III.   Standardization and Phytochemistry Screening Standardization of the Extract

Organoleptic

Organoleptic examination includes examination of color, odor, and taste.[22]

Total ash content

Thickened extracts were weighed 2 to 3 grams and were placed into the incandescented and were tared silicate crucible and were heated in furnace at 600°C for 3 hours. Then, it was cooled and weighed. If charcoal stays, hot water was added, then was stirred and was filtered with ash-free filter paper. The filtering residue and filter paper were applied to the same crucible. The filtrate was added to the crucible, evaporated and incandescented until the weight was fixed, then weighed. Total ash content was calculated against the weight of thick extract expressed in% b/b.[23]

Acid insoluble ash content

The ash was boiled with 25mL of diluted sulfuric acid for 5 minutes, was collected parts which were not soluble in acid, filtered through glassy crust or ash-free filter paper, was washed with hot water, incandescented until the weight remained, then was weighed. Then the ash content which is insoluble in acid against thick extract was calculated in % b/b.[23]

Loss on drying

The dried-shrinkage method was determined as follows: Carefully weigh 1 to 2 grams of substances which have previously been heated at 105°C for 30 minutes and have been tared. Before weighing, the extract was flattened in a bottle and then put into a drying chamber and dried at 105°C to a fixed weight. Let the bottle be closed and cooled in the desiccator to room temperature. Then note the fixed weight obtained to calculate the percentage of drying loss.[23]

Phytochemistry

The phytochemical screening of dried simplicia and ethanol extract of *VA*: alkaloids, flavonoids, saponins, tannins was analyzed using the standard methods as described by Soetarno (2008);[24] steroids and terpenoids, antraquinones by Kristanti et al (2008);[25] phenol and glycoside by Harbourne (2008).[26]

IV.   Collection of Staphylococcus aureus

Isolate of *Staphylococcus aureus* ATCC 25923 from Department of Microbiology, Widya Mandala Catholic University. These bacteria were rejuvenated in nutrient broth medium and were incubated at 37°C for 24 hours. Then the isolate was mixed in 0.9% NaCl and standardized to McFarland IV (1.2x10^9 cfu/mL).

V.   *Determination of Antimicrobial effect*

Thirty male wistar rats were divided into 5 groups :
Negative Control (NC): rats were treated CMC Na 2%
Positive Control (PC): rats were treated 9mg/200g BW of cefadroxyl antibiotics
T1: rats were treated 20mg/200g BW of *Vernonia amygdalina* ethanol extract
T2: rats were treated 40mg/200g BW of *Vernonia amygdalina* ethanol extract
T3: rats were treated 80mg/200g BW of *Vernonia amygdalina* ethanol extract
The rat in both NC, T1, T2 and T3 groups were treated with 1 mL VA ethanol extract orally 3 times a day. The next day, the PC group was administered 1mL of cefadroxyl orally. Then all rats were injected with 0.25mL *SA* suspension in 0.9% NaCl (3x10^8cfu) intraperitoneally. After 24 hours of bacteria injection, all rats were sacrificed and blood was withdrawn intracardially to measure IL-6 and IL-10 levels. The peritoneal fluid was collected to count the number of survived bacteria.

VI.   ELISA

Elabscience Rat IL-6 ELISA KIT (Catalog No. E-EL-R0015) and Elabscience Rat IL-10 ELISA KIT (Catalog No. E-EL-R0016) were used to quantify the IL-6 and IL-10.

VII.   Bacterial Counting

The fluid from peritoneal rat was identified by pour plate method. The 0.1mL peritoneal fluid was withdrawed and was placed in a tube containing 9.9mL of sterile aquadest (tube 1). Then, 1mL from the tube 1 was taken and it into 9mL sterile aquadest (tube 2) and so on until tube 3 (twice replication was performed). After that, from each tube, 1mL was taken and placed in a petri dish, which subsequently was added 10mL of nutrients sterile agar (at 50°C) and was rotated in order to obtain to mixture of bacteria. All petri dish were incubated at 37°C for 24 hours. Afterwards, the number of colonies was calculated.[27]

RESULT AND DISCUSSION

The standardization results were showed that *VA's* ethanol extract (Table 1) have a dark green color, charateristic odor and bitter taste. The total ash content shows high mineral content such as calcium, chlorine, chromium, copper, iron, potassium, magnesium, manganese, nickel, phosphorus, potassium, sodium in this plant[28,29] while the acid insoluble ash content was showed the contamination of fine particles from sand and soil from the environment.[30] Furthermore, 88.36% of the compounds lost during the drying process.[22]

Phytochemical screening was indicated that both simplicia and ethanol extract of *VA* contained flavonoids, saponins, tannins, steroids, terpenoids, phenols and

glycosides, while alkaloids and anthraquinones was not detected (Table 2). This might be due to geographical differences where the plants grow.

In this study, it was observed the effectiveness of *VA* in modulating immune system in Wistar rat that was infected by *SA*. Generally, in infectious condition induced by bacteria, the body generates a defence mechanism in reaction to the encountered microbes or their products. The defence is preceded by the presence of immune cells such as macrophages, dendritic cells, neutrophils, natural killer cells, and limfoid cells. They were resolved the microbes through two main actions: the first is recruiting phagocytes and other leukocytes to destroy the microbes (indicates inflammatory reaction) and secondly by limiting microbial replication or killing microbial-infected cells without inflammatory reaction.[17]

During inflammation process, immune cells recognized the molecular structure produced by *SA* through a binding between TLRs-1/2 or TLRs-2/6 with lipoteichoic acid (LTA). This binding was activated the transcription factor, NFκB, to produce high amounts of IL-6 levels.[16-18] This cytokine was needed when inflammation occurs to increase the formation of neutrophils in bone marrow and recruitment of neutrophils to the site of infection to replace the leukocyte cells that died during inflammation.[17] However, high levels of IL-6 also was stimulated a negative impact which is correlated with disease progression[31] and is contributed in exacerbating inflammation so it triggered to autoimmune diseases.[17] Therefore, the effectivity of the *VA's* ethanol extract in reducing production of IL-6 levels (Figure 1) and may be potential to reduce inflammation.

The *VA's* constituents that are playing role as the IL-6 reducing agents are luteolin and myricetin, they are belongs to the flavonoid groups. Luteolin inhibits NF-κB activation by blocking the degradation of IκBα and phosphorylation of p65.[18] Whereas, myricetin works by inhibiting the activation of p38 and extracellular signals from TLR2/6 and also blocking the degradation of IκB. Myricetin which given as prophylactic agents can significantly reduce IκB degradation,[32] furthermore, Viljoen et al. (2016) were reported that myricetin also inhibits the activation of ERK-1/2, AKT and p38 induced by LTA,[33] thus these mechanisms were blocked the production of pro-inflammatory cytokines, IL-6.[32]

The other constituents of VA ethanol extract may exhibit molecular function in regards of IL-6 reduction such as andrographolide that belongs to terpenoids groups. Andrographolide also plays a role in decreasing IL-6 by inhibiting NFκB activation, suppressing iNOS, and preventing oxygen radicals produced by neutrophils.[34] Furthermore, tannin, that is one of VA's constituents has ability to reduce intracellular kinase phosphorylation and inhibit NFκB at p65 and its catalytic activity, therefore those processes may decrease the IL-6 levels.[35]

Moreover, chlorogenic acid in VA's ethanol extract belongs to phenol group suppresses the expression of the NFκB signaling pathway inhibits the activation of this signaling pathway and reducing inflammatory cytokines production. A previous study was confirmed that taking chlorogenic acid can reduce levels of NFκB p50 and IKKα/β.[36]

On the other hands, in presence of microbial infections, immune cells were also produced anti-inflammatory cytokines such as IL-10 to reduce inflammation.[18] In

Table 1. Standardization Results of *Vernonia Amygdalina*

Determination	Ethanol extract
Organoleptics	Color: dark green
	Odor: characteristic
	Taste: bitter
Total ash content	16.18 ± 0.48%
Acid insoluble ash content	0.822 ± 0.18%
Loss on drying	88.36 ± 0.74%

Table 2. Phytochemical Screening of *Vernonia amygdalina*

Determination	Simplicia	Ethanol extract
Alkaloids	-	-
Flavonoids	+	+
Saponin	+	+
Tannin	+	+
Steroid	+	+
Terpenoids	+	+
Antraquinone	-	-
Phenol	+	+
Glycoside	+	+

Description: (+) = Identified and (-) = not identified
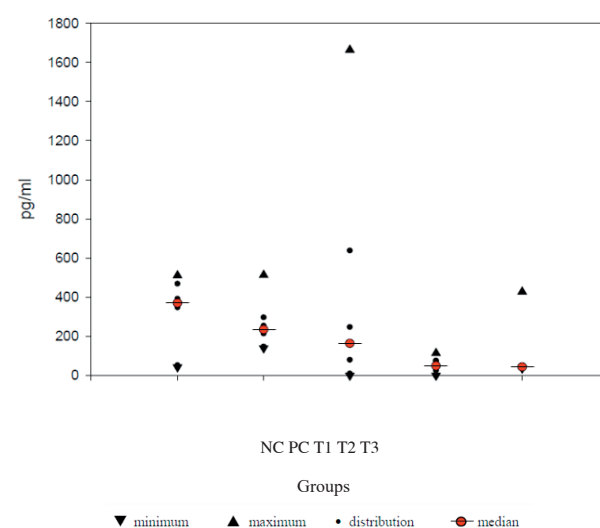
Figure 1. Graphic of IL-6 Levels Distribution. NC: Given CMC Na 2%; PC: Given 9mg/200g BW of Cefadroxyl Antibiotics; T1: Given 0mg/200g BW of *Vernonia Amygdalina* Ethanol Extract; T2: Given 40mg/200g BW of *Vernonia Amygdalina* Ethanol Extract; T3: Given 80mg/200g BW of *Vernonia Amygdalina* Ethanol Extract.

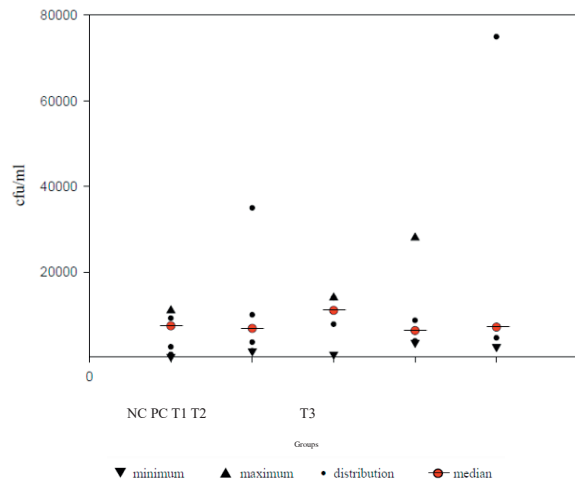contrast, this study was found that IL-10 levels decreased after *VA*'s ethanol extract administration (Figure 2). This might be occured due to the myricetin and chlorogenic acid which were suppressed the expression of JAK/STAT signaling pathways[34] and thus were inhibited the production of IL-10 in Wistar rats's immune cells.[17]

Cheng and Iyer (2012) were reported that the majority of intracellular infections were better controlled or were cleaned quickly in a no IL-10 state. Decreasing IL-10 signaling leads to increase host survival after infection and to increase adaptive immune response, including CD4^{+} T cells that produce Interferon (IFN)-γ.[37] Similar to Cheng and Iyer's report, Riley et al. (2008) were observed that IL-10 is an important regulator component in almost all infections.[20] This statement is clarified through a research conducted by McLoughlin et al. (2017), that during systemic acute infection was induced by *SA*, IL-10 was regulated local and systemic proinflammatory responses that prevented the host from immunopathology condition caused by bacteria spreading.[38] In infections which were caused by *SA*, the decreased of IL-10 levels can increase IFN-γ,[39] IL-17, IL-22 and CXCL1. Therefore it was stimulated the increasement of T_H1 cells as well as activated the phagocytes to clear the bacteria.[38] In addition, the IL-10 decreased levels may increase the expression of costimulators and Major Histocompatibility Complex (MHC) II molecules and IL-12 production in macrophages and dendritic cells. IL-12 is the main cytokine that stimulate adaptive immune response, T_H1 cells, which will secrete IFN-γ. IFN-γ plays an important role in the reaction of innate and adaptive immune cells against intracellular microbes.[17] Therefore, IL-10 deficiency in intracellular infections can reduce the
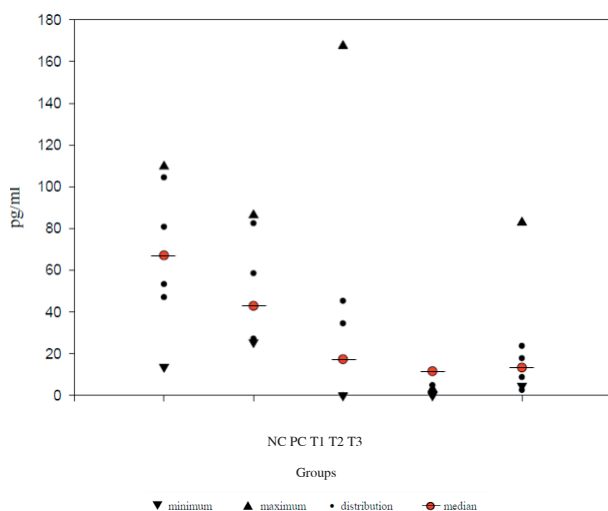
Figure 3. Graphic of Bacterial Numbers Distribution. NC: Given CMC Na 2%; PC: Given 9mg/200g BW of Cefadroxyl Antibiotics; T1: Given 0mg/200g BW of Vernonia Amygdalina Ethanol Extract; T2: Given 40mg/200g BW of Vernonia Amygdalina Ethanol Extract; T3: Given 80mg/200g BW of Vernonia Amygdalina Ethanol Extract. The Maximum Value in T1 group is 210000 and The Maximum Value in T4 Group is 1300000

number of microbes by activating the adaptive immunity to kill bacteria.[38]

Moreover, Figure 3 shown that *VA's* ethanol extract also plays a role in reducing the number of bacteria. This extract occurred due to the content of andrographolide and luteolin. Andrographolide had a bacteriostatic effect. Andrographolide will weaken DNA synthesis of *SA* so it will produced inhibition on biosynthesis pathway of intracellular DNA in *Staphylococcus aureus*.[40] In addition, luteolin also had antibacterial effects by inhibiting the activity of *Staphylococcus aureus* bacteria in DNA topoisomerase I and II which will result in a decrease in nucleic acid and protein synthesis.[41]

CONLUSION

This study was indicated that the optimum dose of VA's ethanol extract in exhibiting IL-6 and Il-10 modulation in Wistar rats is 40mg/200g BW. This dose can decreased IL-6 levels and bacterial numbers which tent to will decrease the inflammation. It may imply the effectivity of this plant as a prophylactic agent to prevent SA infection.

Figure 2. Graphic of IL-10 Levels Distribution. NC: given CMC Na 2%; PC: Given 9mg/200g BW of Cefadroxyl Antibiotics; T1: Given 0mg/200g BW of *Vernonia Amygdalina* Ethanol Extract; T2: Given 40mg/200g BW of Vernonia Amygdalina Ethanol Extract; T3: Given 80mg/200g BW of *Vernonia Amygdalina* Ethanol Extract.

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Research Report

EXPRESSION OF FOUR CYTOKINE/CHEMOKINE GENES IN PERIPHERAL BLOOD MONONUCLEAR CELLS INFECTED WITH DENGUE VIRUS

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ABSTRACT

Overproduction of numerous pro-inflammatory cytokines, during dengue virus (DENV) infection, has been related to plasma leakage in the vascular endothelium and studied elsewhere with conflicting results. The current study objective is to evaluate the expression of four cytokine/chemokine genes following DENV-2 infection within peripheral blood mononuclear cells (PBMC) isolated from a healthy donor. Venous blood was drawn, and PBMCs were isolated using Ficoll density gradient centrifugation. Cells were maintained in culture medium and infected with Indonesian isolate of DENV-2. Cells were harvested and followed by total RNA extraction and reverse-transcription into cDNA using oligo d(T) primers and Reverse Transcriptase enzyme system. The SYBR Green-based quantitative qRT-PCR was used to calculate the relative expression of IL-6, IL-8, IP-10 and MIP-1ß- encoding genes during infection time points, compared to uninfected cell controls. The observation of the cytokine was on the 6 and 18 hours post-infection. The different expression profiles of cytokines/chemokines were observed. The up-regulation of gene expression was observed for IL-8 and IP-10. In contrast, the down-regulatory of IL-6 and MIP-1ß genes expression was documented during the infection period. The cytokine IL-8 and IP-10 are potent chemoattractants in the recruitment of neutrophil, basophil, and lymphocytes in response to an infection. The highlight of this study is on the up-regulation of IL-8 and IP-10 genes expression which may confirm the roles of these chemokines in the pathogenesis of dengue infection.

Keywords: *dengue, gene expression, cytokine, chemokine, PBMC*

ABSTRAK

Produksi sitokin pro-inflamasi berlebihan pada infeksi virus dengue yang dihubungkan dengan terjadinya kebocoran plasma pada endotel vaskular telah diteliti dengan hasil yang bervariasi. Penelitian ini bertujuan untuk mendeteksi ekspresi empat gen pengkode sitokin atau kemokin pada sel mononuklear darah tepi yang di-infeksi virus dengue serotipe-2. Sel mononuklear darah tepi (PBMC) diisolasi dari donor sehat dengan menggunakan metode sentrifugasi gradient Ficoll. Ekstraksi RNA dilakukan terhadap sel mononuklear darah tepi, kemudian sintesis cDNA dilakukan dengan menggunakan primer oligo d(T) dan sistem enzim reverse transcriptase. Dengan menggunakan quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) berbasis SYBR-Green, ekspresi gen penyandi IL-6, IL-8, IL-10 dan MIP-1ß dibandingkan antara sel yang terinfeksi dan tidak terinfeksi virus dengue serotipe-2. Observasi dilakukan pada waktu pengamatan jam ke-6 dan waktu pengamatan ke-18 dari infeksi. Ekspresi gen penyandi IL-8 dan IP-10 ditemukan lebih tinggi, sebaliknya ekspresi gen penyandi sitokin IL-6 dan MIP-1ß lebih rendah pada sel mononuklear darah tepi yang diinfeksi virus dengue serotipe-2 dibandingkan dengan kontrol Interleukin-8 dan IP-10 adalah sitokin yang bersifat sebagai kemotaktik untuk memicu kemotaksis dari sel netrofil, basophil dan limfosit sebagai respon dari suatu inflamasi. Hasil penelitian ini menunjukkan bahwa ekspresi kedua gen penyandi kemokin yang meningkat setelah infeksi virus dengue serotipe-2 mungkin berperan pada patogenesis terjadinya kebocoran plasma pada infeksi virus dengue.

Kata kunci: *dengue, ekspresi gen, sitokin, kemokin, PBMC*

INTRODUCTION

Infection caused by dengue virus is still the major cause of acute febrile illness in the world, particularly in the tropical and subtropical area, including Southeast Asian countries.[1] The widely-spread dengue virus (DENV) and the *Aedes* mosquitoes vector have now become a major problem, and more than 125 countries are known to be dengue-endemic regions, including Indonesia.[2] The high DENV expansion is related to climate change, globalization effect, traveling communities, socioeconomics, settlement and viral evolution.[2] Dengue is a complex disease, caused by an RNA virus, entailing of four antigenically similar but with immunologically distinct serotypes (DENV-1 to DENV-4).[3,4] A serotype-specific DENV infection confers life-long immunity with only partial protective immunity for the other serotypes hence people in endemic countries can be infected up to four times by different DENVs.[5] The clinical manifestation varies from asymptomatic to the severe, life-threatening manifestation.[6] The majority of dengue clinical manifestation is mild, asymptomatic dengue or mild fever. In the lesser incidence, the more severe form of dengue is Dengue Hemorrhagic Fever (DHF) with various degrees and Dengue Shock Syndrome (DSS), in which the fatality rate may exceed more than 5% in special populations.[4,5] Expanded dengue syndrome or unusual dengue syndrome with a high mortality rate can be appeared without any sign of plasma leakage, the hallmark of severe dengue.[7]

The detailed pathogenesis of dengue is not yet entirely understood. The existence of cytokine storm-induced endothelial dysfunction in DENV infection has been published over the past decades.[8] The excessive release of various cytokines recognized as cytokine storm has been regarded as the underlying mechanism of plasma leakage in DENV infection.[9] The role of diverse cytokines and chemokines were observed during the more severe manifestation of dengue might be considered to be correlated with the infecting DENV serotype.[10] At the different phase of illness, cytokines/chemokines profiles were found to be increased in patients at the febrile phase.[11] Several studies were also reported the increased expression of cytokines/chemokines within *in vitro* in human monocytes[12] or epithelial[13] cells or *in vivo* in dengue patients' serum.[14] The dual role of both innate and inflammatory pathways were activated during dengue disease and revealed the involvement of immune mediators.[14] Utilizing dengue human cell line infection model, the up-regulated cytokines/chemokines gene expression profiles during DENV serotypes infection have been described and among them were IL-6, IL-8, and IP-10.[13] Other report is highlights the induction of MIP-1β by dengue virus.[15] In this study, we are reported the expression profiles of four genes encoding cytokines and chemokines in the peripheral blood mononuclear cells (PBMCs), infected with Indonesian isolate of DENV-2.

MATERIAL AND METHOD

Ethical Considerations

The ethical considerations of this study have been reviewed and approved by the Institutional Review Board of Udayana University, Bali, Indonesia (Document No. 2072/UN.14.2/KEP/2017).

Blood Collection and Peripheral Blood Mononuclear Cells (PBMCS) Isolation

Thirty mL of venous blood was drawn from a healthy donor and subjected to PBMC isolation using Ficoll Histopaque-1077 (Sigma-Aldrich, St. Louis, MO) density gradient centrifugation. Isolated PBMCs were maintained in 1 RPMI medium supplemented with 10% of fetal bovine serum (FBS), 1% of antibiotic/antimycotic, and 2 mM of L-glutamine (all from Gibco-Thermo Fisher Scientific, Carlsbad, CA). Cells were seeded at 1×10^6 cells per well of 24-well plates (Corning, NY). The seeded cells were allowed to rest during overnight incubation at 37°C incubator with 5% CO_2 supplementation.

DENV Infection

The DENV-2 virus strain SMG-SE001 was isolated from a severe dengue patient from Semarang in 2012[16] (Eijkman's collection). Cells were infected with DENV-2 using the multiplicity of infection (MOI) of 1 (theoretical calculation of one virus PFU per cell), prepared in 1 RPMI medium-2% FBS, performed in duplicate. To achieve the MOI of 1 (theoretically one virus particle infecting one cell), a number of 10^6 Plaque Forming Unit (PFU) of DENV-2 was inoculated into 10^6 PBMC cells. The DENV PFU titre was measured using a standard plaque assay method, as described elsewhere,[16] meanwhile uninfected cells controls were inoculated using the addition of medium only. Plates were then incubated for 1 hour at 37°C, 5% CO_2 to facilitate virus infection. The incubation period was continued and cells were harvested at 0, 6, and 18 hours post-infection to represent the early phase of dengue illness.

Total RNA Extraction and qRT-PCR

Infected cells were harvested at the designated time points and subjected to the total RNA extraction using miRCURY RNA Isolation kit – Cell and Plant (Exiqon, Vedbaek, Denmark), as described in the manufacturer's instructions. Total RNA quantity was measured using Qubit 3.0 fluorometer and Qubit RNA BR Assay Kit (Life Technologies-Thermo Fisher Scientific, Eugene, OR). An amount of 100 ng of total RNA was used in cDNA synthesis performed using oligo d(T) primer and GoScript Reverse Transcription System (Promega, Madison, WI). The resulting cDNA was then amplified by quantitative real-time PCR using GoTaq qPCR Master Mix (Promega) and primers as listed in Table 1. The relative gene expression analysis was performed using the equation of $2^{-\delta\delta Ct}$ of normalized Ct value to human β-actin.

Table 1. Primers Used in Cytokine/Chemokine Relative Gene-Expression Analysis Using qRT-PCR.

Target gene	Direction	Sequence (5'-3')
IL-6	Sense	GAGGATACCACTCCCAACAGACC
	Antisense	AAGTGCATCATCGTTGTTCATACA
IL-8	Sense	TGCCAAGGAGTGCTAAAG
	Antisense	CTCCACAACCCTCTGCAC
IP-10	Sense	TTCAAGGAGTACCTCTCTCTAG
	Antisense	CTGGATTCAGACATCTCTTCTC
MIP-1β	Sense	CTGTGCTGATCCCAGTGAATC
	Antisense	TCAGTTCAGTTCCAGGTCATACA
β-Actin	Sense	CATCTCTTGCTCGAAGTCCA
	Antisense	ATCATGTTTGAGACCTTCAACA

RESULT AND DISCUSSION

The increased expression of IL-8 and IP-10 was observed during infection of DENV-2, with the highest level seen at 18 hours post-infection (Figure 1). By contrast, the relative expression of IL-6 and MIP-1β genes was relatively decreased along the infection time. The up-regulation of IL-8 and IP-10 was reaching more than two-fold at 18 hours post-infection, relative to the uninfected
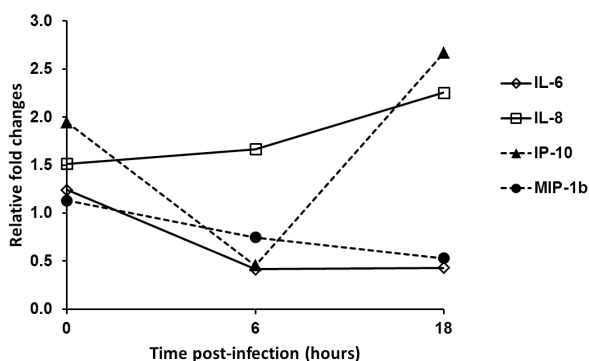
Figure 1. The Relative Cytokine/Chemokine Genes Expression of DENV-2 infected-PMBCs Compared to Uninfected Controls During Three Infection Time Points.

control. The reduction of IL-6 and MIP-1β genes expression to the level of nearly half-fold was apparent at the same time points.

We observed the up-regulation of IL-8 and IP-10 in PBMCs infected with DENV-2, relative to the uninfected controls. The IL-8 is the pro-inflammatory cytokines, a member of CXCL chemokine family and the factor of a neutrophil chemotactic factor, which have been widely investigated and found to increase at the protein level.[17] The cytokine IL-8 is a neutrophil chemoattractant produced by macrophages and other cell types, such as endothelial cells, fibroblasts, and synovial cells.[17] It has been reported that increased levels of IL-8 cytokine in the sera of dengue patients were correlated with the severe form of dengue.[18] The level of IL-8 was significantly higher in samples from severe dengue cases and lower in cases of dengue without warning signs than in healthy controls. Samples that were positive for anti-DV IgG antibody had higher levels of IL-8.[18]

The IP-10 is a member of CC-motif chemokine in response to interferon-γ in infectious diseases.[18] The previous study result on the induced expression of IP-10 chemokine has been well-documented in dendritic cells and other primary cell lineages in response to *in vitro* dengue infection[19] as well as in PBMCs of dengue patients.[20] The level of IP-10 has been found to be increased in serum of dengue-infected patients in studies in Venezuela[19] and Singapore.[21] Moreover, the increased level of IL-8 and IP-10 cytokines/chemokine was also observed in A549 human lung epithelial cells infected with DENV.[13]

We observed the reduction trend in both IL-6 and MIP-1β gene expressions during DENV-2 infection of PBMCs. The increased level of IL-6 has been reported in dengue patients.[22] In addition, the increased level of IL-6 has been correlated to disease severity with higher level observed in the more severe form of dengue manifestation, through the up-regulation of inflammatory responses in macrophages and induction of B cell maturation.[23] Dengue NS1 protein was found to significantly increase the production of IL-6 thoroughly activation of TLR-2 and TLR-6 in PBMC infected with DENV.[24] The higher expression of MIP-1β was reported in PBMC from DENV-infected patients.[11] However, in this study we did not found the elevated level of gene expressions for IL-6 and MIP-1β within 18 hours of DENV infection. A systematic review on markers of dengue disease severity revealed that increased level of IL-6 and MIP-1β was observed in samples taken more than

48 hours after onset of fever.25 The results from this study may be related to the difference in the experimental setup of genes expressions analysis and the designated sample collection period to represent the early phase of dengue disease development during 18 hours of virus infection. However, the decreasing levels of IL-6 and MIP-1β have been observed in dengue with warning signs during disease progression from febrile to convalescence phase.11 The current study result concordant to another study result where increased serum levels of IL-6 and IL-8 were detected in patients with dengue haemorrhagic fever but not dengue fever.26 More in-depth research is needed to study the kinetics of these cytokines during dengue infection.

CONCLUSION

It has been reported here the dynamic of four cytokines related to an early phase of DENV infection. The up-regulation of IL-8 and IP-10, chemokines with profiles that highly correlated with dengue than other febrile diseases and have the potential to be used as a biomarker of dengue, since high expression of these cytokines at the earlier phase of observation in this study.

ACKNOWLEDGEMENT

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Research Report

PREVALENCE AND RISK FACTORS OF DIABETES MELLITUS IN TUBERCULOSIS PATIENT AT PATRANG DISTRICT INDONESIA

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ABSTRACT

Tuberculosis (TB) is one of cause of death in infectious disease domain. The control of TB is complicated because the inclination of case numbers people with Diabetes Mellitus. Diabetes Mellitus (DM) is an important risk factor for TB development, with prove that more than ten percent of TB patient is DM patient. People with DM have risk three times more likely to suffer from TB than people without DM. The results of TB treatment with comorbid DM will be easier to be failed. Puskesmas Patrang have the highest bacteriologically confirmed BTA TB cases and DM cases in Jember during 2014 until 2016. The aim of this research is to know the DM prevalence in TB patients and to analyze the correlation between DM risk factors in TB patient to TB-DM incidence at Puskesmas Patrang Jember in 2017. The research uses observasional analytic with cross sectional approach. The sampling technique uses simple random sampling with 47 samples. The independent variables include respondent characteristics (age, sex, type of TB, medication category, and family history of DM), central obesity and smoking behavior. While the dependent variable is the DM status. The result shows that the prevelance of DM in TB patients at Puskesmas Patrang Jember regency is 23.4%. Factors associated with TB-DM are age (p-value = 0.012), family history of DM (p-value = 0.003), and smoking status (p-value = 0.035). Factors that do not associated with TB-DM are sex (p-value = 0.731), type of TB (p-value = 0.170), treatment category of TB (p-value = 0.560), central obesity (p-value = 0.435), the number of cigarette (p-value = 1.000) and smoking duration (p-value = 1.000). The most important factor of TB-DM is family history of DM that 10,850 times higher of getting TB-DM than patients without family history of DM.

Keywords: *TB, DM, risk factors of DM*

ABSTRAK

Tuberkulosis (TB) adalah salah satu penyebab kematian dalam kelompok penyakit menular. Pengendalian TB dipersulit karena kecenderungan kasus penderita Diabetes Melitus. Diabetes Melitus (DM) merupakan faktor risiko penting untuk perkembangan TB, dengan bukti bahwa lebih dari sepuluh persen penderita TB merupakan penderita DM. Penderita DM berisiko 3 kali lebih tinggi untuk menderita TB dibandingkan dengan orang tanpa DM. Hasil pengobatan TB dengan komorbid DM akan lebih mudah mengalami kegagalan. Puskesmas Patrang memiliki kasus TB BTA terkonfirmasi bakteriologis dan DM tertinggi di Kabupaten Jember selama tahun 2014 sampai 2016. Penelitian ini bertujuan untuk mengetahui prevalensi DM pada pasien TB dan mengetahui hubungan faktor risiko DM pada pasien TB terhadap kejadian TB-DM di Puskesmas Patrang Kabupaten Jember tahun 2017. Penelitian ini menggunakan metode penelitian analitik observasional dengan pendekatan cross sectional. Teknik pengambilan sampel menggunakan metode simple random sampling dengan jumlah sampel sebanyak 47 orang. Variabel bebas penelitian meliputi karakteristik responden (usia, jenis kelamin, jenis TB, kategori pengobatan, dan riwayat DM pada keluarga), obesitas sentral dan perilaku merokok. Sedangkan varibel terikat penelitian yaitu status DM. Hasil penelitian menunjukkan bahwa prevalensi DM pada pasien TB di Puskesmas Patrang Kabupaten Jember yaitu sebesar 23,4%. Faktor yang berhubungan secara signifikan dengan kejadian TB-DM yaitu usia (p-value = 0,012), riwayat DM pada keluarga (p-value = 0,003), dan status merokok (p-value = 0,035). Sedangkan faktor yang tidak berhubungan secara signifikan dengan kejadian TB-DM yaitu jenis kelamin (p-value = 0,731), jenis TB (p-value = 0,170), kategori pengobatan TB (p-value = 0,560), obesitas sentral (p-value = 0,435), jumlah rokok yang dihisap (p-value = 1,000) dan lama merokok (p-value =

1,000). Faktor yang paling berhubungan terhadap kejadian TB-DM adalah riwayat DM pada keluarga dengan risiko sebesar 10.850 kali untuk menderita TB-DM dibandingkan pasien tanpa riwayat DM pada keluarga.

Kata kunci*: TB, DM, faktor risiko DM*

INTRODUCTION

Tuberculosis (TB) is infectious disease that caused by *Mycobacterium tuberculosis* (*M.tb*) which can attack various organs especially the lungs.[1] TB is one of 10 causes of death in the world and the leading cause of death in the world in infectious disease domain.[2] Diabetes Mellitus (DM) is a metabolic disorder disease characterized by elevated blood sugar levels due to decreased insulin secretion by pancreatic beta cells and or insulin function i.e. insulin resistance. Diabetics are three times more likely to have TB than people without DM.[3] TB patients with DM were at risk for the failure of sputum conversion after undergoing TB treatment, 1.69 times higher risk for treatment failure, 1.24 times higher for anti tuberculosis drug resistance, 3.89 times higher for relapse, and 4.95 times higher risk for death during treatment than those without DM.[4] Previous TB-DM studies have found that age, sex, pulmonary TB, and family history of DM are associated with TB-DM incidence.[5] Other studies were found that age, family history of DM, alcohol consumption, and central obesity were associated factors with TB-DM incidence.[6,7]

TB-DM is a problem in TB endemic countries with lower middle income because 70% of DM patients live in TB endemic countries and 80% of DM patients live in lower middle income countries.[8,9] Indonesia is in 4th highest TB cases below India, China and South Africa with 48,000 cases.[10] East Java is in second place of highest bacteriologically confirmed BTA TB cases after West Java and is included in the top 10 of DM patients in Indonesia.[11,12] Jember has the second largest number of bacteriologically confirmed BTA TB cases after Surabaya and has DM patients of 8619 people by 2012.[13,14] Puskesmas Patrang has the highest cases of TB BTA confirmed bacteriology and DM cases with 111 cases of TB BTA confirmed bacteriology and 371 DM cases by 2016.

Studies on the effect of DM on TB epidemiology in 13 high TB burden countries were showed that 6 million TB cases and 1.1 million TB deaths can be avoided through DM control.[8] Previous studies have suggested that risk factors for DM incidence in TB patients include race, age, sex, type of TB, treatment category, family history of DM, central obesity, smoking, and alcohol. Race is thought to be due to genetic factors that contribute to the incidence of insulin resistance. People who have black skin are more susceptible to DM than people with white skin. TB-DM patients are generally occur in old age especially after the age of 40 years. The aging process causes the ability of pancreatic beta cells in producing insulin reduced.[15,16] TB-DM is more common in men than women as a result of risk factors such as smoking and alcohol consumption.[17]

Based on type of TB, DM is more common in pulmonary TB patients than extrapulmonary TB.[18] The prevalence of recurrent TB is higher in TB-DM patients than TB without DM. TB-DM patients are 3.9 times more likely to get TB relapse than TB without DM.[19] DM tends to be inherited so the family members of patients with DM have the possibility of 2-6 times suffering DM compared with family members who do not suffer from DM.[20] Another factor is central obesity which can lead to insulin resistance due to excess fat that makes insulin work difficult so that blood sugar levels rise.[15] Based on the previous research, majority of TB-DM patients had ever use tobacco (78,3%). The effect of nicotine causes a decrease in insulin release due to catecholamine hormone activation, a negative effect on insulin action, impairment of pancreatic β cells and leads to insulin resistance.[21] Alcohol can affect the endocrine glands by releasing epinephrine that leads to transient hyperglicemia and hyperlipidemia so that alcohol consumption is contraindicated with diabetes.[22]

The problem of TB-DM in Jember especially in Puskesmas Patrang tends to be in the top three highest cases of bacteriologically confirmed BTA TB and DM during 2014-2016. A bacteriologically confirmed BTA TB cases for three consecutive years i.e 94, 107 and 111 and DM cases for three consecutive years i.e 2049, 1011 and 371 cases. The declining of DM cases may be caused by undiagnosed patients. DM patients are usually asymptomatic but it is still a risk of TB-DM incidence. Moreover, TB-DM problem in Jember has not been studied more deeply, therefore we need to know the risk factors of DM incidence in TB patient at Puskesmas Patrang Jember in 2017 for preventing and controlling DM incidence in TB patient considering its impact if TB and DM occur together. The purpose of this research is to know the risk factors of DM incidence in TB patients at Puskesmas Patrang Jember in 2017.

MATERIAL AND METHOD

This research uses observational analytic method with cross sectional approach. The research was conducted at Puskesmas Patrang Jember in August 2017. The number of samples were 47 TB patients using simple random sampling technique.

The variables of this research consist of independent and dependent variables. The independent variables include respondent characteristics (age, sex, type of TB, medication category, and family history of DM), central obesity and smoking behavior. While the dependent variable is the DM status.

Table 1. Prevalence of Diabetes Mellitus in Tuberculosis Patient at Puskesmas Patrang in 2017

DM Status	Frequency (people)	Percentage (%)
TB-DM	11	23.4
TB	36	76.6

Sources of research data consist of primary data and secondary data. Primary data was obtained from interview and measurement of abdominal circumference in TB patients. Secondary data was obtained from the data of quarterly reports of new cases of TB BTA confirmed bacteriologic finding in Health Office Jember district during 2014-2016, DM report data at Health Office Jember district during 2014-2016 and form TB 01 at Puskesmas Patrang Jember district in 2017.

Data collection techniques are conducted through interview, measurement and observation. Instrument of data collection was determined using questionnaire and meter measuring tool. Data analysis techniques used univariate, bivariate analysis using chi-quare, and multivariate using logistic regression.

RESULT AND DISCUSSION

Prevalence of Diabetes Mellitus in Tuberculosis Patient at Puskesmas Patrang Jember in 2017

Patients with TB as respondents in the study were 47 respondents. The respondents were divided into TB-DM patients and TB patients. TB-DM patients were TB patients who also were diagnosed by health professionals suffering from DM or taking DM drug while TB patients were those who only suffer TB. Prevalence of DM in TB patient can be seen in Table 1.

In Table 1, the prevalence of DM in TB patients is 23.4%. This figure exceeds the prevalence of DM only in the population in Indonesia that is 3.2%. In addition, Alisjahbana found that DM is more common in patients with TB than in control subjects. Of 454 TB patients, 60 patients (13.2%) have DM rather than of 556 non TB patients, 18 patients (3.2%) have DM.[23] Guptan and Shah (2000) were stated that impaired glucose tolerance (IGT) in TB is much higher than overt DM. According to the National Diabetes Data Group of NIH, one to five per cent of patients with IGT may progress to overt DM. In 1990, Fong Y et al was stated that glucose intolerance due to TB occurs because of increased cytokines IL-1 and TNF alpha which can stimulate the anti insulin hormones. In addition, in 1993, Sinurova et al was stated that cortisol which controls blood sugar levels also decrease. Even, TB germs are able to release toxins through the bloodstream or TB germs directly attack the pancreas that causes the production of amyloid (abnormal protein) in the pancreas which further inflict DM.[24,25] The mechanism is the reason why DM is more prevalent in people with TB than in general population. The exceeds of the DM prevalence in

Table 2. Distribution of Tuberculosis Patient Characteristics by Age, Gender, Type of Tb, Treatment Category, and Family History of DM in Puskesmas Patrang Jember in 2017

Characteristics	Frequency (people)	Percentage (%)
Age		
≥ 45 years old	18	38,3
< 45 years old	29	61,7
Sex		
Male	26	55,3
Female	21	44,7
Type of TB		
Pulmonary	39	83,0
Extrapulmonary	8	17,0
Treatment Category		
Category 2	3	6,4
Category 1	44	93,6
Family History of DM		
Yes	12	25,5
No	35	74,5

TB patient rather than in general population also occurs in countries where TB and DM cases are most prevalent.[8] Another research which conducted by Raghuraman *et al* in India also found higher prevalence of DM in TB patients that is 29% than the prevalence of DM in the population. The population is 7% because India is included in high load TB cases that have a growing number of DM cases.[6]

Relationship between Respondent Characteristics with TB-DM Incidence in Tuberculosis Patients at Puskesmas Patrang Jember in 2017. Characteristics are the attributes or things possessed by an element. Characteristics of respondents in this study include age, sex, type of TB, treatment category, and family history of DM. The distribution of TB patient characteristics can be seen in Table 2. Based on Table 2, out of 47 patients were enrolled for this research, most TB patients are < 45 years old (61,7%). Indonesia's Health Profile (2017) was also stated that the highest proportion of TB cases are found in the < 45 years old. During 2012-2016, the proportion is 61.48%, 60.96%, 60.26%, 60.31%, and 59.91%.[26] TB can attack anyone, especially the productive age (15-50 years old). People with productive age are easy to interact with others, high mobility makes it possible to transmit bacteria to others. Infant or younger than 4 years old also have greater risk of experiencing the progression of TB infection because the T-cell mediated immune response of young children is not fully developed (immature) so it works inefficiently to antigen challenge. [27-29]

This research was also found that most TB patients are males (55,3%). Males are at higher risk of developing TB than females because they have smoking habit that makes lung function impaired, damages the macrophages as cells that can eat bacteria, making it easier to get TB.[28,30] Based on National Health Indicator Survey (SIRKESNAS) in 2016, the prevalence of smoking in male is 59% and female is 1.6%.[26]

Table 2 also was showed that most of respondents had pulmonary tuberculosis (83%). According to the definition, TB is a contagious infectious disease caused by *Mycobacterium tuberculosis* that can attack various organs especially the lungs. TB transmission occurs through sputum sparks containing TB bacteria that are transmitted when people with TB cough or sneeze, so that pulmonary TB is the most common clinical manifestation compared to other organs.[1,31]

Most TB patients in this research are in undergoing category 1 treatment (93,6%) especially TB BTA confirmed bacteriologically. The number of TB BTA confirmed bacteriology cases can be used as an indicator of TB patient discovery to achieve National indicators. National indicators are indicator of TB programs that can measure the success of a program.[32] It means more getting TB BTA confirmed bacteriologically patients among suspected sputum examination, getting treated faster.

The result was showed that the majority of TB patients have no family history of DM. In this study, family history of DM was not associated with the incidence of TB. Patient who has family history of DM is 2-6 times higher to get DM. DM is characterized by hyperglycaemia due to insulin secretion defect, insulin work or both. Hyperglycaemia is capable of causing immune disorders i.e decreased gamma interferon function (IFN γ) which serves to stimulate macrophages to kill M.tuberculosis through the mechanism of endocytosis into the macrophages. This indicates that in this study there are other risk factors that cause TB such as

Table 3. Bivariate Analysis of The Relation between Respondent Characteristics and Tb-DM Incidence in Tuberculosis Patients at Puskesmas Patrang Jember in 2017

Characteristics	Status DM TB-DM	Status DM TB	*p-value*	OR (95% CI)
	n	N		
Age				
≥ 45	8	10	0,012*	6,933 (1,525-31,515)
< 45	3	26		1
Sex				
Male	7	19	0,731	1,566 (0,389-6,298)
Female	4	17		1
Type of TB				
Pulmonary	11	28	0,170	
EP	0	8		-
Treatment Category				
Category 2	1	2	0,560	1,700 (0,139-20,749)
Category 1	10	34		1
Family History				
Yes	7	5	0,003*	10,850 (2,304-51,101)
No	4	31		1

EP = extrapulmonary

*Significant ≤ α (0,05)

close contact with untreated TB patients, lack of nutritional status, low immunity, and smoking habits.[28,33]

The relation between the characteristics of respondents with TB-DM incidence could be identified through bivariate analysis. The related factors of TB-DM incidence were age and family history of DM. The unrelated factors of TB-DM incidence were sex, type of TB, and treatment category. Table 3 gives the bivariate analysis of the relation between respondent characteristics and TB-DM incidence at Puskesmas Patrang Jember in 2017.

Table 3 was showed that TB-DM was associated with older age. This result is in line with research by Raghuraman et al (2014) that found the median age of TB-DM patients that was 49.5 ± 11.8.[6] While Mansuri et al (2015) found the average age of TB-DM patients is 47 ± 16.[7] TB-DM tends to be experienced by people with old age as a result of DM cases often found in old age.[8,34] The risk of developing DM usually appears after 45 years old. At that age there is a physiological decline in humans that affects the reduced ability of pancreatic beta cells in the production of insulin.[15,16]

Family history of DM was also significantly related to TB-DM incidence. Most patients with family history of DM tend to suffer from TB-DM (14.9%) than suffering TB (10.6%). These results are in line with other research that the family history of DM is associated with TB-DM incidence.[6] DM tends to be inherited rather than transmitted. Family members of patients with diabetes (diabetics) are 2 to 6 times more likely to suffer DM compared to family members who do not have DM.[20]

This research has not found any significant association between sex and TB-DM. Similar results were reported by researches by Raghuraman *et al* and Mansuri *et al*.[6,7] But the other study by Suwanpimolkul *et al* was stated that men was associated with TB-DM. Men are 1.66 times more likely to have TB-DM than women.[35] In this research, most of the men (40.4%) and women (36.2%) only had TB.

Type of TB was found to be statistically insignificant. Previous research also was found that the type of TB was not significantly related to TB-DM incidence.[7] Different results were found by Workneh *et al* (2016) that pulmonary TB was significantly associated with TB-DM incidence. The prevalence of pulmonary TB among TB-DM patient was higher (64.2%) than TB patient (58.1%).[5] While in this research, patients with pulmonary TB tend to suffer from TB (59.6%) than TB-DM (23.4%). It is also known that all extrapulmonary TB patients suffer from TB with percentage of 17%.

Treatment category was also found to be statistically insignificant. There are two treatment category i.e category 1 that consists of TB BTA confirmed bacteriology, pulmonary TB patients diagnosed clinically, and extrapulmonary TB and category 2 that consists of relapse, drop out, and fail treatment.[32] This result is in line with other researchers that also found that treatment category 2 was not significantly associated with TB-DM.[6,7] But, research which was conducted by Mi et al (2013) found that category 2

Table 4. Bivariate Analysis of The Relation Central Obesity and TB-DM Incidence in Tuberculosis Patients at Puskesmas Patrang Jember in 2017

Central Obesity	DM Status		*p-value*	OR (95% CI)
	TB-DM	TB		
	n	N		
Yes	4	8	0,435	2,000 (0,465-8,597)
No	7	28		1

*Significant ≤ α (0,05)

significantly associated with TB-DM. They found a higher prevalence of treatment category 2 in patients with TB-DM (13.8%) than TB patients (7.9%). They also were stated that the risk of patient with category 2 treatment was 1.7 times more likely suffering TB-DM than patient with category 1 treatment.[19] In this research, the prevalence of patient with treatment category 2 among TB group (4.3%) is higher than TB-DM group (2.1%). Furthermore, the prevalence of patient with treatment category 1 among TB group (72.3%) is higher than TB-DM group (21.3%).

Relationship between Central Obesity and TB-DM Incidence in Tuberculosis Patient at Puskesmas Patrang Jember in 2017 Cental obesity is fat accumulation in the abdominal area that exceeds normal value. Respondents were classified as central obesity if the abdominal circumference is š90 cm for men and š80 cm for women. The relation between central obesity and TB-DM incidence is shown in Table 4.

In Table 4, central obesity is not significantly related to TB-DM incidence (p-value = 0.435). This result is in line with a study by Raghuraman et al (2014) who found central obesity was not statistically related to TB-DM incidence.[6]

In central obesity occurs fat accumulation in the body exceeds the normal value in the abdominal area.[36] Futhermore, fat in the stomach causes fat cells to secrete proinflammatory chemicals that make the body less insensitive to insulin by disrupting the function of insulin responsive cells and the ability of cells to respond insulin. The more fat deposits in the stomach, the more difficult the insulin works so that blood sugar rises.[15,37] But, in this research, majority of respondent or 74.5% are not categorized to central obesity that respectively is 59,6% for TB patients and 14,9% for TB-DM patients. Research by Raghuraman *et al* was also found that majority of respondent were not categorized to central obesity (90,3%).[6] Relationship between Smoking Behaviour with TB-DM Incidence in Tuberculosis Patients at Puskesmas Patrang Jember in 2017.

TB-DM risk factors also were influenced by smoking behaviour variables. Smoking behavior variables in this study include smoking status, number of cigarettes, and smoking duration. The results of the bivariate analysis between smoking behaviour and TB-DM incidence are listed in Table 5. In Table 5, only smoking status was associated with the incidence of TB-DM (p-value = 0.035).

Table 5. Bivariate Analysis of Smoking Behaviour with TB-DM Incidence in Tuberculosis Patients at Puskesmas Patrang Jember in 2017

Smoking Behaviour	DM Status		*p-value*	OR (95% CI)
	TB-DM	TB		
	n	n		
Smoking Status				
Yes	3	1	0,035*	13,125 (1,203-143,233)
No	8	35		1
The Number of Cigarettes				
> 10	1	1	1,000	
Ý 10	2	0		-
Smoking Duration				
> 20 years	1	1	1,000	
Ý 20 years	2	0		-

*Significant ≤ α (0,05)

While the number of cigarettes (p-value = 1,000) and duration of smoking (p-value = 1,000) are not significantly related to TB-DM incidence.

Research by Viswanathan *et al* was stated that smoking was risk factor for TB-DM. People that smoking is at risk 1.92 times higher than that of non-smokers.[17] Smoking causes inflammation and oxidative stress in the cells of the body thus increasing the risk of developing DM. Nicotine content in cigarettes can lead to decreased insulin secretion, impaired function in pancreatic beta cells, increased beta cell apoptosis and risk of insulin resistance by binding and activating nicotinic acetylcholine receptors (nAChRs) which are part of the transmembrane group of protein chanel ions in the central nervous system and the peripheral nervous system. Activation of nAChRs results in the increase and release of catecholamines in the bloodstream which can result in increased blood pressure, free fatty acid, and blood sugar mobilization/in contrast to the effects of insulin. Exposure to nicotine in a short time (more than 1 μmol/L) and within 48 hours can inhibit the release of insulin. The risk of people who smoke is 30-40% higher for DM than nonsmokers.[21,38.] Most Associated Factor with TB-DM in Tuberculosis Patients at Puskesmas Patrang Jember in 2017

The most associated factor with TB-DM in TB patients in Patrang Health Center in Jember Regency in 2017 could be known through multivariate analysis. Based on bivariate analysis, variables that could be analyzed using multivariate analysis were age, family history of DM, and smoking status. The result of multivariate analysis aims to get the most dominant independent variable related to the dependent variable by looking at the biggest OR value. The analysis can be seen in Table 6.

Based on Table 6, it can be seen that the history of DM in the family was the most associated factor with TB-DM incidence. TB patients who had a family history of DM is at risk 18.250 times higher for getting TB-DM rather than TB patients with no family history of DM.

Table 6. Analysis of Most Associated Factor with TB-DM Incidence in Tuberculosis Patient at Puskesmas Patrang Jember in 2017

Variable	Coefficient	*p-value*	OR	*Confidence interval 95%*
Age				
≥ 45	2,875	0,016*	17,717	1,710-183,506
< 45			1	
Family History				
Yes	2,904	0,014*	18,250	1,813-183,708
No			1	
Smoking Status				
Yes	1,841	0,174	6,303	0,444-89,439
No			1	

*Significant ≤ α (0,05)

This result is similar with research by Sirait *et al* that the risk of DM was 2.4 times greater in those whose parents had a history of DM compared with those whose parents had no history of DM.[39] The magnitude of family history is possible because of a genetic defect of pancreatic β cells and is possible due to genetic abnormalities in the action of insulin in the presence of abnormalities in insulin receptors.[40]

CONCLUSION

The conclusion of this research is the prevalence of DM in TB patients in Puskesmas Patrang higher than prevalence of DM in general population in Indonesia. In Indonesia, East Java is in the second place of highest TB cases under West Java. Futher the second city of highest TB cases in East Java is found in Jember especially in Puskesmas Patrang which not only tend to be in the top three highest cases of TB but also tend to be in the top three highest cases of DM. Factors associated with TB-DM incidence are age, family history of DM and smoking status. Factors unassociated with TB-DM incidence were gender, type of TB, treatment category, central obesity, number of smoked cigarettes and duration of smoking. Factors most associated with TB-DM incidence is family history of DM.

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Research Report

PREVALENCE OF SOIL TRANSMITTED HELMINTHIASIS AMONG ELEMENTARY CHILDREN IN SORONG DISTRICT, WEST PAPUA

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ABSTRACT

Soil transmitted helminthiasis are common in the world and cause illness, especially in developing countries. It can cause infection in humans by contact with parasitic eggs or larvae that live in moist and warm soil. Soil-transmitted helminthiasis is often caused by Ascaris lumbricoides, Trichuris trichiura, Ancylostoma duodenale, and Necator americanus. In Indonesia, Soil transmitted helminthiasis prevalence is still high in some places. The tropical climate and high humidity support for the development of worms like in Sorong District, but there was no data. The purpose of this study is to identify the presece of Soil transmitted helminthiasis in primary school children in Sorong District. A cross-sectional study was conducted in two elementary schools located in Sorong District, West Papua, Indonesia. The two elementary schools are SDN 22 in Klain village and SD Inpres 24 in sub-district Mayamuk. Once collected, the pot that has contained stool is given formalin 10%. Stool examinattion using direct smear method to determine the presence of soil transmitted helminthiasis. Researchers get the subject as many as 147 children. The proportion of elementary school children by sex consists of 72 boys (49%) and 75 girls (51%). The prevalence of Soil transmitted helminthiasis as a whole was 30.6% (45/147) with 40.1% (18/45) single infections and 59.9% (27/45) mixed infections. The single infection that most frequent is Trichuris trichiura, then followed by Ascaris lumbricoides. Soil-transmitted helminthiasis mostly found in girl than boy and mostly found in 6-9 years age group. The worm species that infect elementary school children in the district is Ascaris lumbricoides, Trichuris trichiura, Hookworm, and Strongyloides stercoralis. This is probably related with the climate and low sanitation level. To eliminate soil transmitted helminthiasis among elementary school children, in addition to routine treatment also needs intensive counseling about the importance of maintaining personal hygiene and the environment.

Keywords: *prevalence, soil transmitted helminthiasis, direct smear method, elementary school, Sorong District*

ABSTRAK

Soil-transmitted helminthiasis sering terjadi di dunia dan penyebab kesakitan khususnya di negara berkembang. Hal ini dapat menyebabkan infeksi pada manusia melalui kontak dengan telur parasit atau larva yang tinggal di tanah lembab dan hangat. Soil-transmitted helminthiasis sering disebabkan oleh Ascaris lumbricoides, Trichuris trichiura, Ancylostoma duodenale dan Necator americanus. Di Indonesia, prevalensi soil transmitted helminthiasis masih tinggi di beberapa tempat. Iklim tropis dan kelembaban yang tinggi mendukung untuk perkembangan cacing seperti di Kabupaten Sorong, tetapi belum ada data. Tujuan dari studi ini adalah untuk mengidentifikasi keberadaan Soil-transmitted helminthiasis pada anak sekolah dasar di Kabupaten Sorong. Studi cross-sectional dilakukan di dua sekolah dasar yang terletak di kabupaten Sorong, Papua Barat, Indonesia. Dua sekolah dasar tersebut adalah SDN 22 berada di desa Klain dan dan SD Inpress 24 berada di sub-distrik Mayamuk. Setelah terkumpul, pot yang berisi tinja kemudian diberi formalin 10%. Pemeriksaan feses menggunakan metode pemeriksaan langsung direct smear untuk mengetahui adanya soil transmitted helminthiasis Peneliti mendapatkan subjek penelitian sebanyak 147 anak. Proporsi anak-anak sekolah dasar berdasarkan jenis kelamin terdiri dari 72 laki-laki (49%) dan 75 perempuan (51%). Prevalensi soil transmitted helminthiasis secara keseluruhan adalah 30,6%

(45/147) dengan infeksi tunggal 40,1% (18/45) dan 59,9% (27/45) infeksi campuran. Infeksi tunggal yang sering ditemukan adalah Trichuris trichiura diikuti dengan Ascaris lumbricoides. Soil-transmitted helminthiasis sebagian besar ditemukan pada perempuan daripada laki-laki dan lebih banyak ditemukan pada kelompok usia 6-9 tahun. Spesies cacing yang menginfeksi anak sekolah dasar di kabupaten tersebut adalah Ascaris lumbricoides, Trichuris trichiura, cacing tambang dan Strongyloides stercoralis. Hal ini mungkin terkait dengan iklim dan tingkat sanitasi yang rendah. Untuk mencegah kejadian soil transmitted helminthiasis, selain perawatan rutin juga perlu dilakukan konseling intensif tentang pentingnya menjaga kebersihan diri dan lingkungan.

Kata kunci: *prevalensi, soil-transmitted helminthiasis, pemeriksaan tinja mikroskopis cara langsung, Kabupaten Sorong*

INTRODUCTION

The worms parasite have infected humans in the world more than 1 billion. WHO estimates that more than 1.5 billion people worldwide or 24% of the world's population are infected with worms and widely distributed in tropical and sub-tropical areas, mostly in sub-Saharan Africa, the Americas, China and East Asia. More than 270 million pre-school children and over 600 million school-aged children live in endemic areas and require preventive therapy and intervention[1]. In 2011, Indonesian Health ministers said about 195 million Indonesians live in worms endemic areas, including 13 million pre-school children and 37 million school-aged children[2]. Soil transmitted helminthiasis is often caused by *Ascaris lumbricoides, Trichuris trichiura, Ancylostoma duodenale* and *Necator americanus*(1).

Soil transmitted helminthiasis is transmitted through eggs collected with the patient's stool. Female worms live in the human intestine and produce thousands of eggs daily. In areas with low sanitation levels, the eggs will contaminate the soil. Things that may cause Ascaris and Trichuris eggs to be swallowed human beings include eating egg contaminated vegetables, while the vegetables are not cooked and washed properly, eggs contaminate drinking water and eggs are swallowed by children who play the soil and do not wash their hands thoroughly. Hookworm and Strongyloides stercoralis hatch in the soil, releasing filariform larvae that can infect humans through penetration of the larvae on human skin. Penetration usually occurs through the skin of the foot that is not covered by footwear(3).

Worms produce eggs or larvae in very large quantities and have high reproductive capacity, which can lead to high incidence of infection in humans when the condition of the host is conducive to infections such as in the marginal areas in tropical countries(4).

MATERIAL AND METHOD

Subject of Research

The research is conducted by cross sectional study on two elementary schools in Sorong District, West Papua, Indonesia. The two elementary schools are SDN 22 in Klain village, and SD Inpres 24 in sub-district Mayamuk (see Figure 1).

Sorong district lies in the coordinates of 000 33 '42' '- 010 35' 29 '' South Latitude and 1300 40 '49' '- 1320 13' 48 '' East Longitude with an area of 12,159.42 km², which consists of land area of width 11,644.77 km² and the sea area of 514.65 km². Sorong district consists of 19 sub-districts with 18 urban villages and 135 villages. One of the sub-districts is Mayamuk. The research conducted in sub-district Mayamuk.

Large sample calculations using Lemeshow formula with unknown population. From the calculation, the sample size is at least 96, but to anticipate the error result, the researcher adds 10% of the minimum sample size.
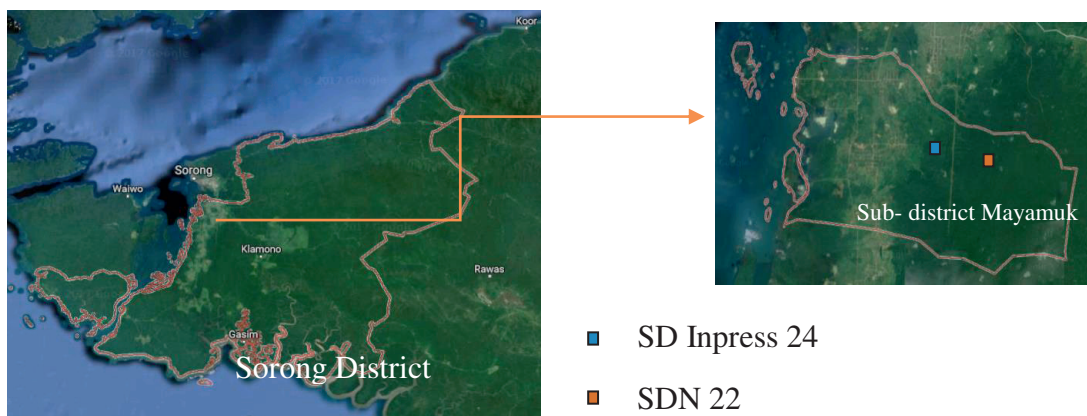
Figure 1. Map of Sub-District Mayamuk and Klain Village in Sorong District, Showing The Location of SDN 22 and SD Inpres 24.

Data Collection

Samples are collected on August 2017. Students are given an explanation of how to collect and store stool samples. Once collected, the pot that has contained stool is given formalin 10%. Stool samples were taken by researchers to be examined at the Department of Parasitology Faculty of Medicine, Universitas Airlangga, Surabaya.

Stool examination using direct smear/wet mounting method to determine the presence of soil transmitted helminthiasis. Pots containing stools, stirred using sticks to be homogeneous, then stool taken and mixed on a object glass that has been given a drop of lugol 1% and then leveled. The rest of the food and crude fiber are removed with a stick and then closed with a cover glass and check under microscope systematically. The preparations are examined under a microscope with magnification 10x then 40x.

Data Analysis

Results of direct-smear data then analyzed descriptively to determine the prevalence of infection and the type of worm infection.

RESULT AND DISCUSSION

Researchers get the subject of research as many as 147 children. The distribution of male and female students in both primary schools is almost the same, with the total of male students being 72 students while the total female students are 75 students. By age group, the highest group was age 6–7 years (44.9%) while the lowest was 11-12 years old (2.7%) (see Table 1).

The results of microscopic examination showed 45 (30.6%) students from 147 students infected, with soil

Table 1. Demographic Characteristics of Research Subjects at SDN 22 and SD Inpres 24 in Sorong District.

	SDN 22 (n=55) n (%)	SD Inpres 24 (n=92) n (%)	N=147 N (%)
Age (years)			
6-7*	23 (41,8)	43 (46,7)	66 (44,9)
8-9*	22 (40)	43 (46,7)	65 (44,2)
10-11*	8 (14,5)	4 (4,4)	12 (8,2)
11-12	2 (3,7)	2 (2,2)	4 (2,7)
Grade Level			
I	16 (29,1)	40 (43,5)	56 (38,1)
II	13 (23,6)	23 (25)	36 (24,5)
III	6 (10,9)	27 (29,3)	33 (22,4)
IV	14 (25,5)	2 (2,2)	16 (10,9)
V	6 (10,9)	0	6 (4,1)
Sex			
Boy	29 (40,3)	43 (59,7)	72 (49)
Girl	26 (34,7)	49 (65,3)	75 (51)

* Some subjects are unknown of date of birth so the age of the subject is classified according to grade level.

Table 2. Characteristic of Soil Transmitted Helminthiasis Cases by Sex in Children of SDN 22 and SD Inpres 24 in Sorong District.

	SDN 22 (n=24) n(%)	SD Inpres 24 (n=21) n (%)	N (45) N (%)
Sex			
Boy	13 (59,1)	9 (40,9)	22 (48,9)
Girl	11 (47,8)	12 (52,2)	23 (51,1)
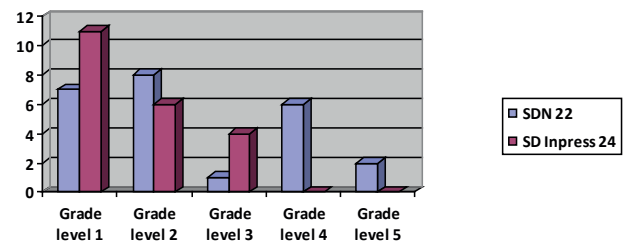
Figure 2. Soil-Transmitted Helminthiasis Based on Grade Level.

transmitted helminthiasis. Prevalence in girls is higher than boys (see Table 2). The highest prevalence is in level grade 1 (see Figure 2).
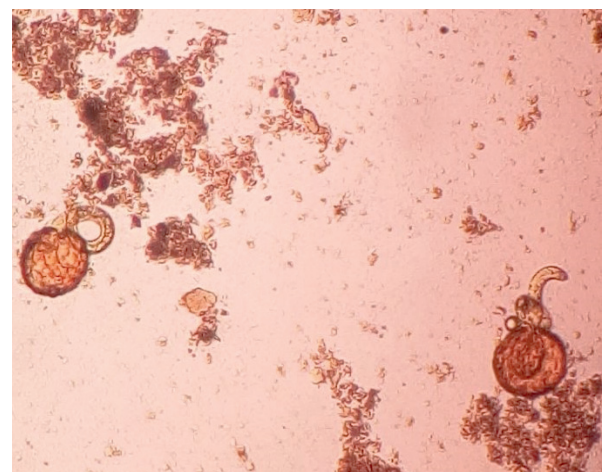
Mixed soil transmitted helminthiasis was more common than single infection with 62.2% (28/45) prevalence compared to 37.8% (17/45). The single infection that most frequent is *Trichuris trichiura* infection, followed by *Ascaris lumbricoides*. The most common mixed infection are *Ascaris lumbricoides* and *Trichuris trichiura* also *Ascaris lumbricoides, Trichuris trichiura* and Hookworm.

In one microscopic stool examination sample found many mature *Ascaris lumbricoides* eggs and *Ascaris*

Figure 3. *Ascaris Lumbricodes* Larvae Goes Out The Egg (Arrow), 40 x obj
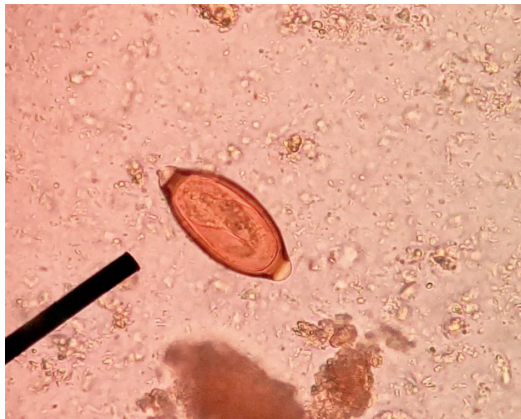
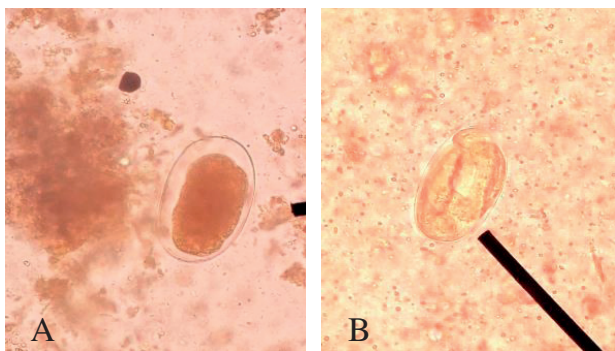**Figure 4.** *Trichuris Trichiura* Egg, 40x Obj

Figure 5. (A) Hookworm Egg. (B) an Advance Stage of Hookworm Egg, 40 X Obj.

lumbricoides larvae that came out of the egg (see Figure 3). In this sample also found *Trichuris trichiura* eggs containing embryos or in an infective stage (see Figure 4).

In this study hookworm eggs mostly found in stadium that containing 2-8 cell embryos, but there are also an advanced stage that containing larvae (see Figure 5).

Figure 6. *Strongyloides Stercoralis* Rhabditiform Larvae, 40 X Obj.

Strongyloides stercoralis infection is not found in a single infection but there was in mixed infection (see Figure 6). Students with mixed infection of *Strongiloides*

Table 3. Results of Stool Examination of Children of SDN 22 and SD Inpres 24 in Sorong District.

STH Infection	SDN 22 (n=55) n (%)	SD Inpres 24 (n=92) n (%)	N = 147 N (%)
Single infection			
Ascaris lumbricoides (**AL**)	0	5 (5,4)	5 (3,4)
Trichuris trichiura (**TT**)	6 (10,9)	2 (2,2)	8 (5,4)
Hookworm (**HW**)	4 (7,3)	0	4 (2,7)
Strongyloides stercoralis (**SS**)	0	0	0
Mixed Infection			
AL – TT	1 (1,8)	6 (6,5)	7 (4,8)
AL – HW	1 (1,8)	0	1 (0,7)
AL – TT – HW	3 (5,5)	4 (4,3)	7 (4,8)
AL – TT – SS	1 (1,8)	0	1 (0,7)
AL – TT – HW – SS	1 (1,8)	0	1 (0,7)
TT – HW	5 (9,1)	2 (2,2)	7 (4,8)
TT – HW – SS	2 (3,6)	2 (2,2)	4 (2,7)
Not Infected	31 (56,4)	71 (77,2)	102 (69,3)

stercoralis were 6 students, 4 of whom were from SDN 22. The description of stool examination result of the sample was explained on Table 3.

From Table 2 it can be concluded that the prevalence of soil transmitted helminthiasis is more common in girls than in boys. This may be due to female hygiene in this area is less good compared with male. Bestari(5) in 2015 doing research in Surakarta city also found the same results.

Most of the infected subjects were 6-9 years old. The prevalence of high soil transmitted helminthiasis at 6-10 years of age can be attributed to habit factors of play. Generally children at that age play more outside home and contact with the ground which is a medium of worm transmissions. Transmission can occur among school students through holding hands while playing with students who often play outside the house and contact with the ground(6).

The types of worms found in stool examinations vary, from *Ascaris lumbricoides*, *Trichuris trichiura*, Hookworm to *Strongyloides stercoralis*. Several surveys in Indonesia show that often high prevalence of *Ascaris* is accompanied by a high prevalence of *Trichuris* as well(6,7). In this study, it was found similar but the prevalence of *Trichuris trichiura* was higher than *Ascaris lumbricoides*. Sorong district areas include tropical areas that have a hot and humid climate(8). This becomes one of the risk factors because *Trichuris trichiura* spread mainly in hot and humid areas(9,10). Tropical climate with high air humidity as well as fertile soil are the optimal environment for worm life. These two types of worms often lead to mixed infection because their habitats and life cycles equally require soil media(9,11). The number of found mixed infection indicates the level of

hygiene and sanitation in the children's environment is very bad(6). The main factors that cause the occurence of soil transmitted helminthiasis is a behaviour factor that reflects low personal hygiene such as not washing hands with soap before eating and after defecation, cleanliness of the nails that are not maintained, eating foods which cleanliness is questioned, fingernail biting, not wearing footwear during outdoor activities, defecate in the open area[12–14]. The spread of soil transmitted helminthiasis is strongly influenced by the occurence of faeces contamination on the soil and water, so the defecation habits will be very decisive.

In this study, hookworm infection is also commonly found. Generally, the prevalence of hookworm is more common in adults. Hookworm infections often occur in areas where human faeces are used as fertilizer or where defecation onto soil happens[15]. This may explained higher prevalence is found in plantation areas as well as in mining[16,17]. Most of the Sorong district community has major jobs in agriculture, plantation and forestry[18]. There was possibility of an infected adult defecating outside (near bush, in a garden, or field) then a mature hookworm egg and hatch, releasing larvae (immature worms)[15]. The larvae became mature into a form that can penetrate childern's skin. Many elementary school children do not wear footwear. This can increase the risk of getting infected with hookworm and *Strongyloides stercoralis*[19–21].

Strongyloides stercoralis infection was found in 6 students with mixed infection. This is probably related with the climate[22,23]. Sorong's climate is humid, the himidity ranges between 81-87%[8]. This tropical climate and high humidity support the development of *Strongyloides stercoralis*.

Knowing the prevalence of worms can be useful for worm management strategies and can be used for basic data on allergic correlation research with worm infection. The prevalence of allergies is increasing dramatically in the world, both in developing and developed countries, especially in low- and middle-income countries. It is estimated that 30-40% of the world's population is exposed to one or more allergic conditions. This increase is especially true in children.

There is much debate about the interaction between helminths and allergic disease. Some epidemiologic studies suggest that helminth infections induce or increase the severity of atopic diseases. Other studies report children with soil transmitted helminthiasis have lower prevalence and milder atopic symptoms. Although there have been many recent studies, the relationships between allergic and helminth infections remains controversial. The "Hygiene Hypothesis" is a very popular concept among scientists. The so-called "hygiene hypothesis" which posits that allergic phenomena arise from the sanitized living conditions of the developed world has been one of the major theories to account for this remarkable difference in prevalence of allergy. Multiple mechanisms may account for the hygiene hypothesis, but there is considerable evidence to suggest that helminth infection plays a central role[24].

To eliminate soil-transmitted helminthiasis among elementary school children, in addition to routine treatment or deworming through mass drug administration also needs intensive counseling about the importance of maintaining personal hygiene and the environment. Interventions Water, sanitation and hygiene (WASH) and health education could sustain the benefits of antihelmintic therapy[25,26]. They play an extremely important role in breaking the cylce of transmission and preventing infection. There was a study in Sri Lanka showed that even after 10 years of antihelmintic therapy, prevalence can be restored after discontinuation of preventive deworming, if the initial force of transmission is strong and other long-term control measures are not concurrently implemented[25].

CONCLUSION

Prevalence of Soil-transmitted helminthiasis among elementary school children in Sorong district is 30,6% (45/147) with 40.1% (18/45) single infections and 59.9% (27/45) mixed infections. The single infection that most frequent is *Trichuris trichiura*, then followed by *Ascaris lumbricoides.* The worm species that infect elementary school children in the Sorong district is *Ascaris lumbricoides, Trichuris trichiura,* Hookworm and *Strongyloides stercoralis.* Soil-transmitted helminthiasis mostly found in girl than boy and mostly found in 6-9 years age group.

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Guide for Author

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equation number. The use of Microsoft Equation is allowed.

$$c^2 = a^2 + b^2. \tag{1}$$

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