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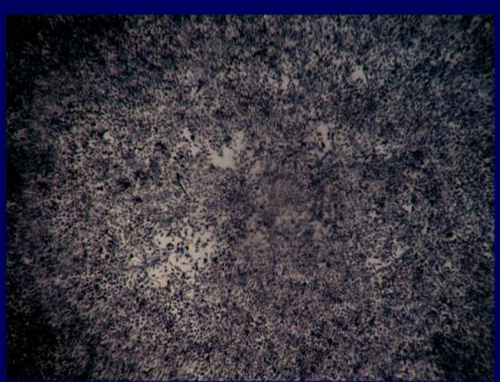

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Research Report

Identification of SCC MEC Methicillin-Resistant Staphylococcus Aureus (MRSA) From Hospitals' Clinical Samples in Jambi using Polymerase Chain Reaction (PCR)

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ABSTRACT

Staphylococcal cassette chromosome mec (SCCmec) is one of the mobile genetic elements of Methicillin-Resistant Staphylococcus aureus (MRSA) that carries many resistance genes and allows SCCmec to move from one bacterium to another. Twelve types of SCCmec have been identified throughout the world. Identification of SCCmec type is needed to determine the pattern of MRSA resistance in a particular region. This study aimed to identify the type of SCCmec MRSA from clinical samples. Specifically, this study was conducted at the Biomolecular Laboratory of the Faculty of Medicine and Health Sciences of Jambi University in June 2018-February 2019. Culture was carried out on 100 clinical specimens of festering wound swabs from inpatients at hopitals in Jambi City. A total of 32 samples of Staphytect plus test positive were tested using Cefoxitin disc diffusion method and MecA Polymerase Chain Reaction (PCR). There were 14 samples identified as MRSA isolates, namely twelve samples (85.72%) of SCCmec type III, one sample (7.14%) of SCCmec type II, and one sample (7.14%) of SCCmec type IVb. The results were different from previous studies where all MRSA isolates (100%) in Indonesia were SCCmec type III, although most SCCmec types were still dominated by SCCmec type III. This study concludes that there has been a shift in the content of SCCmec in MRSA isolate originating from hospitals in Jambi city.

Keywords: *MRSA, MecA, SCCMec, genetic, resistance*

ABSTRAK

Staphylococcal cassette chromosome mec (SCCmec) merupakan salah satu elemen genetik yang mobile pada Methicillin Resistant Staphylococcus aureus (MRSA) yang membawa beberapa gen resistensi dan memungkinkan SCCmec berpindah dari satu bakteri ke bakteri lainnya. Terdapat dua belas tipe SCCmec yang telah teridentifi kasi di seluruh dunia. Identifi kasi tipe SCCmec sangat diperlukan untuk mengetahui pola resistensi MRSA di suatu wilayah tertentu. Penelitian ini bertujuan untuk mengidentifi kasi tipe SCCmec MRSA dari sampel klinik. Penelitian ini dilakukan di Laboratorium Biomolekuler Fakultas Kedokteran dan Ilmu Kesehatan Universitas Jambi pada bulan Juni 2018-Februari 2019. Kultur dilakukan terhadap 100 spesimen klinik berupa swab luka yang bernanah pada pasien yang dirawat inap di Rumah Sakit di Kota Jambi. Sebanyak 32 sampel yang positif pada Uji Staphytect plus diuji dengan Cefoxitin Disk Difusion Metode dan Polymerase Chain Reaction (PCR) MecA. Terdapat 14 sampel yang teridentifi kasi sebagai isolat MRSA. Sebanyak 12 sampel (85,72%) merupakan SCCmec tipe III, satu sampel (7,14%) SCCmec tipe II dan satu sampel (7,14%) SCCmec tipe IVb. Hasil penelitian ini berbeda dengan penelitian sebelumnya dimana seluruh (100%) isolat MRSA di Indonesia merupakan SCCmec tipe III, meskipun tipe SCCmec terbanyak masih didominasi oleh SCCmec tipe III. Kesimpulan dari penelitian ini adalah mulai ditemukannya perubahan kandungan SCCmec pada isolat MRSA yang berasal dari rumah sakit di Kota Jambi.

Kata kunci: *MRSA, MecA, SCCmec, genetic, resistensi*

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INTRODUCTION

S. aureus is a common bacterial pathogen that causes minor to serious disease in human. *S. aureus* can be treated with methicillin (MSSA) and resistant to methicillin (MRSA). Infection of MRSA becomes an important concern throughout the world and associated with infection in both Hospital-acquired Methicillin-Resistant *Staphylococcus aureus* (HA-MRSA) and Community-acquired Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA).[1,2,3] Infection caused by MRSA keeps increasing year to year. According to research in Indonesia, the prevalence of MRSA is approximately 30–40%. The prevalence of MRSA in Cipto Mangunkusumo Hospital on 2010 and Abdul Moeloek Hospital Lampung on 2013 were 32% and 38%, respectively.[4,5]

The resistance of MRSA against beta-lactam antibiotic is encoded by the mecA gene. MecA gene is a part of the conserved MRSA genetic elements of the Staphylococcal cassette chromosome mec (SCCmec), encoding PBP2a or PBP2 mutants.[6,7] MecA gene is located in a genetic element called the Staphylococcal Cassette Chromosome (SCCmec). SCCmec is integrated into the chromosome of *S. aureus* at a unique site located near the *S. aureus* origin of replication. SCCmec is a mobile genetic element that carries many resistance genes and allows SCCmec to move from one bacterium to another.[8] Thirteen types of SCCmec have been identified throughout the world.[9]

The components of SCCmec are recombinase genes (ccr complexes), mec complex genes, additional resistant genes, and insertion sequences (IS).[8,10] Differences between SCCmec are determined by variations in the ccr complex and the mec complex. SCCmec type I about 39 kb, in the 1960s era, has a composition of type 1 ccr complex and class B mec complex. SCCmec type II about 52 kb, dominant in the 1980s era, has a composition of type 2 ccr complex and the class A mec complex. SCCmec type III about 67 kb, dominant in the 1980s, has the composition of the type 3 ccr complex and the class A mec complex. SCCmec type IV (a and b) about 20.9–24,3 kb, found in 2002, has a composition of type 2 ccr complex and class B mec complex.[4,5,6]

Various findings of MRSA patterns in the last decade have shown the changes in distribution, sensitivity to various antibiotics, and possible changes in the SCCmec type.[11,12] Identification of SCCmec type is needed to determine the pattern of MRSA resistance in a particular region. Based on the previous description, it is important to identify the type of SCCmec MRSA from clinical samples.

MATERIALS AND METHODS

This study was a cross-sectional study. This study was conducted in the Biomolecular Laboratory of the Faculty of Medicine and Health Sciences in Jambi University from June 2018 to February 2019.

A hundred samples of swabs from festering wound were collected from three secondary referral hospitals in Jambi (Raden Mattaher hospital, dr. Bratanata hospital, and Kambang hospital). The swabs were incubated at 30 ºC on Mannitol Salt Agar (MSA) for 18-24 hours, the yellowish colony would be confirmed by Gram staining. Gram-positive coccus bacteria were tested using Staphytect plus Test DR 850 M (Oxoid) to detect clumping factor, protein A and type 5 and 8 capsules of polysaccharide.

Positive samples were tested for resistance to cefoxitin antibiotics by using the disc diff usion method in Mueller Hinton (MH) Agar. The susceptibility testing was conducted as a standard of CLSI 2011.[13] Identification of *MecA* gene and the type of SCCmec were using Polymerase Chain Reaction (PCR). Primers used are shown in Table 1.

Preparation of Bacterial DNA Samples, PCR Mec A and PCR SCCmec

DNA samples 5 µl of bacterial suspension (0.5 Mc Farland) from yellowish colonies were incubated at 30°C 18-24 hours on MSA. PCR was performed in a final volume of 25 µl consisting of 5 µl of DNA samples, 10 µl of 2x GoTaq green master mix (Promega), 2 µl 1mM forward primer (Mec A1), 2 µl 1mM reverse primer (Mec A2) and 6 µl of nuclease-free water. Positive control and negative control were S. aureus ATCC 43300 and S. aureus ATCC 25923. The mixture was denatured at 94°C for 5 minutes followed by 30 cycles, 94°C for 45 seconds, 72°C for 90 seconds, and 72°C for 10 minutes. DNA was amplified with a thermocycler (Thermo scientific, USA).

Multiplex PCR SCCmec was carried out on positive samples of MecA gene to detect SCCmec chromosomes. Primers used are shown in Table 1. PCR was performed in a final volume of 25 µl consisting of 5 µl of DNA samples, 12.5 µl of 2x GoTaq green master mix (Promega), 0.5 µl 1 mM of forward primer, 0.5 µl 1 mM of reverse primer (SCC mec primers type I, II, III, IVa, and IVb) and 2.5 µl nuclease-free water. PCR to identify the type of SCCmec began with an initial denaturation at 94°C for 5 minutes followed by 10 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds, extension at 72°C for 90 seconds, then continued with 25 cycles of denaturation at 94°C for 45 seconds, annealing at 50°C for 45 seconds, extension at 72°C for 90 seconds, and final extension 72°C 10 minutes. The amplicons were visualized in 0.8% agarose stained using Sybr safe DNA (Invitrogen), and images were obtained using a gel documentation system.

RESULTS AND DISCUSSION

A total of 100 festering wound swab samples were obtained from hospitalized patients in Raden Mattaher hospital, dr. Bratanata hospital, and Kambang hospital. Thirty-two samples were positive *S. aureus* through staphytect plus test. There were 14 isolates of MRSA based on cefoxitin resistance in disc diffusion method and PCR mecA positive (Figure 1).

Multiplex PCR was performed on 14 MRSA isolates to identify the type of SCCmec in the samples. There were 12 samples (85.72%) of SCCmec type III, 1 sample (7.14%) of SCCmec type II, and 1 sample (7.14%) of SCCmec type IVb (Figure 2).

The SCC*mec* types distribution were depended on geographical manner. Most MRSA isolates from Eastern and Middle Eastern countries hospitals contain SCC*mec* type III.[15] This SCC*mec* type is common in some South East Asia countries hospitals such as Thailand, Singapore, Indonesia and Malaysia.[16] Different with some South East Asian countries, MRSA isolates from

Table 1. Sequence of oligonucleotide primers.14

Target Gene	Primer	Nucleotide sequence (5'-3')	Amplicon (bp)
MecA gene	MecA1	GTA GAA ATG ACT GAA CGT CCG ATA A	310
	MecA2	CCA ATT CCA CAT TGT TTC GGT CTA A	
SCCmec I	I-F	GCT TTA AAG AGT GTC GTT ACA GG	613
	I-R	GTTCTCTCATAGTATGACGTCC	
SCCmec II	II-F	CGTTGAAGATGATGAAGCG	398
	II-R	CGAAATCAATGGTTAATGGACC	
SCCmec III	III-F	CCATATTGTGTACGATGCG	280
	III-R	CCTTAGTTGTCGTAACAGATCG	
SCCmec IVa	IVa-F	GCCTTATTCGAAGAAACCG	776
	IVa-R	CTACTCTTCTGAAAAGCGTCG	
SCCmec IVb	IVb-F	TCTGGAATTACTTCAGCTGC	493
	IVb-R	AAACAATATTGCTCTCCCTC	

Figure 1. Agarose gel electrophoresis of PCR product amplified from MecA gene (310 bp). M is DNA marker; K(+) is positive control, Lane 1-14 are MecA fragments.

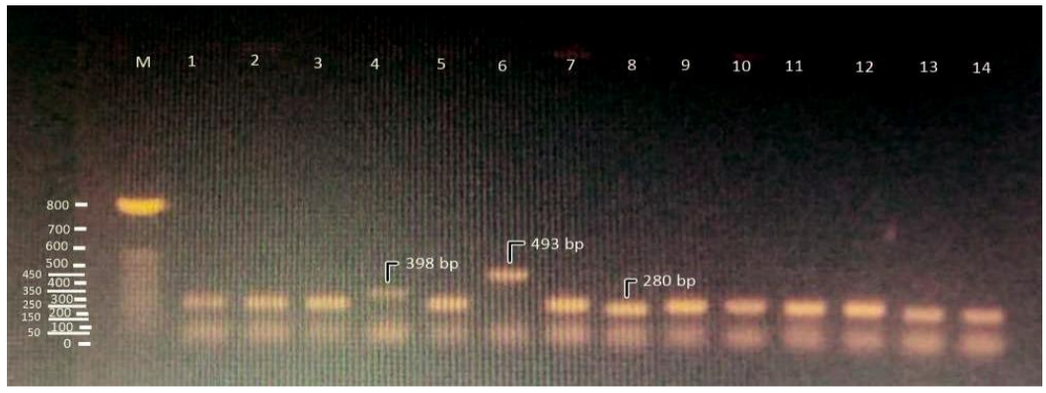

Figure 2. Agarose gel electrophoresis of PCR product amplified from SCCmec type. M is DNA marker; Lane 1-3,5,7-14 are SCC*mec* type III fragments (280 bp). Lane 4 is SCC*mec* type II fragment (398 bp). Lane 6 is SCC*mec* type IVb fragment (493 bp).

Korea and Japan predominantly contain SCC*mec* type II.[16] While some European countries MRSA isolates contain SCC*mec* type IV.[17]

In this study, the majority of SCCmec types was type III (85.72%). These results were consistent with studies conducted in seven countries in Asia including Indonesia and studies conducted in Iran where SCCmec type III was the most common in MRSA isolates.[16,18,19]

In addition to SCCmec type III, this study also found a small proportion of MRSA isolates contained SCCmec type II and type IVb. SCCmec type I, II, and III were the commonly found types in hospitals (HA-MRSA), while SCCmec type IV and V were the commonly found types in communities (CA-MRSA).[20,21,22]

SCCmec type II also found in Jakarta, a study mentioned that the majority of MRSA isolates in hospitals were SCCmec type II.[23] While SCCmec type IV also found in Denpasar (12.5%) and Malaysia (3.18%) among MRSA isolates in hospitals.[24,25] This means that there has been a shift in the content of SCCmec in MRSA isolates in Indonesia. The discovery of SCCmec type IV in the Hospital raises concerns because this type is more mobile, generally causes more severe clinical symptoms, and is more difficult in the selection of suitable antibiotics.[21,24] In comparison to other SCCmec elements, SCCmec IV is small in size and more variable, which has possibly enabled it to spread easily within *S. aureus*.

CONCLUSIONS

Based on the results revealed in this study, there has been a change in the type of SCCmec in MRSA isolates from hospitals. Therefore, it is

recommended to conduct further research with a larger sample size, both from hospitals and communities to identify the SCCmec type and its relationship to patterns of sensitivity to antibiotics. Keeping in view, the finding of SCCmec type IV in Jambi should be investigated, whether it is a circulator or a persisting invader. Further molecular analysis of these MRSA isolates by pulsed-field gel electrophoresis or MLST (Multi Locus Sequence Typing) may provide much useful information regarding the origin and the epidemiology of local isolates.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Alrabiah K, Al Alola S, Al Banyan E, Al Shaalan M, Al Johani S. Characteristics and risk factors of hospital acquired e Methicillin-resistant Staphylococcus aureus (HA-MRSA) infection of pediatric patients in a tertiary care hospital in Riyadh, Saudi Arabia. Int J Pediatr Adolesc Med. 2016; 3(2): 71–7.
2. Thomas R, Ferguson J, Coombs G, Gibson PG. Community-acquired methicillin-resistant Staphylococcus aureus pneumonia: A clinical audit. Asian Pacific Soc Respirol. 2011; 16: 926–31.
3. Bukharie HA. A review of community-acquired methicillin-resistant Staphylococcus aureus for primary care physicians. J Fam Community Med. 2010; 17(3): 117–20.
4. Mahmudah R, Soleha TU, Ekowati C. Identifikasi Methicillin Resistant Staphylococcus aureus (MRSA) pada Tenaga Medis dan Paramedis di Ruang Intesive Care Unit (ICU) dan Ruang Perawatan Bedah Rumah Sakit Umum Daerah Abdoel Moeloek. Med J Lampung Univ. 2013; 2(4): 70–8.
5. Liana P. Gambaran Kuman Methicillin-Resistant Staphylococcus Aureus (MRSA) di Laboratorium Mikrobiologi Departemen Patologi Klinik Rumah Sakit Dr. Cipto Mangunkusumo (RSCM) Periode Januari-Desember 2010. MKS. 2014; 46(3): 171–5.
6. Hill-cawthorne GA, Hudson LO, Fouad M, El A, Piepenburg O, *et al*. Recombinations in Staphylococcal Cassette Chromosome mec Elements Compromise the Molecular Detection of Methicillin Resistance in Staphylococcus aureus. PLoS One. 2014; 9(6).
7. Paterson GK, Harrison EM, Holmes MA. The emergence of mecC methicillin-resistant Staphylococcus aureus. Trends Microbiol. 2014; 22(1): 42–7.
8. Ito T, Hiramatsu K, Oliveira DC, De Lencastre H, Zhang K, Westh H, et al. Classification of staphylococcal cassette chromosome mec (SCCmec): Guidelines for reporting novel SCCmec elements. Antimicrob Agents Chemother. 2009; 53(12): 4961–7.
9. Kaya H, Hasman H, Larsen J, Stegger M, Johannesen B. SCCmecFinder, a Web-Based Tool for Typing of Staphylococcal Cassette Chromosome mec in Staphylococcus aureus Using Whole-Genome Sequence Data. Am Soc Microbiol. 2018; 3(1): 1–9.
10. Nitschke H, Pfohl K, Monecke S, Jatzwauk L, Mu E, *et al*. Diversity of SCC mec Elements in Staphylococcus aureus as Observed in South-Eastern Germany. PLoS One. 2016; 11(9): 1–24.
11. Yuwono, Sunarjati S, Masria S, Supardi I. Staphylococcus aureus dengan Polymerase Chain Reaction Identification of Staphylococcal Cassette Chromosome Mec Methicillin Resistant Staphylococcus aureus Using Polymerase Chain Reaction. Maj Kedokt Bandung. 2009; 43(2): 60–5.
12. Sudigdoadi S. Analisis Tipe Staphylococcal Cassette Chromosome mec (SCCmec) Isolat Methicillin Resistant Staphylococcus aureus (MRSA). Maj Kedokt Bandung. 2014; 42(4): 149–54.
13. Cockerill FR, Wikler MA, Bush K, Craig WA, Dudley MN, Eliopoulos GM, et al. Performance Standards for Antimicrobial Susceptibility Testing ; Twenty-First Informational Supplement. Vol. 31, CLSI document. 2011. M100-S21 p.
14. McClure-Warnier J-A, Conly JM, Zhang K. Multiplex PCR Assay for Typing of Staphylococcal Cassette Chromosome Mec Types I to V in Methicillin-resistant *Staphylococcus aureus*. J Vis Exp. 2013; (79).
15. Holden MTG, Hsu L, Kurt K, Weinert LA, Mather AE. A genomic portrait of the emergence, evolution, and global spread of a methicillin-resistant Staphylococcus aureus pandemic. Cold Spring Harb Lab Press. 2013; 23: 653–64.
16. Asghar AH. Molecular characterization of methicillin-resistant Staphylococcus aureus isolated from tertiary care hospitals. Pak J Med Sci. 2014; 30(4): 698–702.
17. Kinnevey PM, Shore AC, Brennan GI, Sullivan DJ. Extensive Genetic Diversity Identified among Sporadic Methicillin-Resistant Staphylococcus aureus Isolates Recovered in Irish Hospitals between 2000 and 2012. Antimicrob Agents Chemother. 2014; 58(4): 1907– 17.

18. Ghanbari F, Saberianpour S, Ghanbari N. Staphylococcal Cassette Chromosome mec (SCC mec) Typing of Methicillin-Resistant Staphylococcus aureus Strains Isolated from Community-and Hospital-Acquired Infections. Avicenna J Clin Microb Infec. 2017; 4(2).
19. Peters B, Liu J, Chen D, Peters BM, Li L, *et al*. Staphylococcal chromosomal cassettes mec (SCCmec): A mobile genetic element in methicillin-resistant Staphylococcus aureus Microbial Pathogenesis Staphylococcal chromosomal cassettes mec (SCCmec): A mobile genetic element in methicillin-resistant Sta. Microb Pathog. 2016; 101 (July 2018): 56–67.
20. Ahmad N, Ruzan IN, Kamel M, Ghani A, Hussin A, Nawi S, et al. Characteristics of community- and hospital- acquired meticillin-resistant Staphylococcus aureus strains carrying SCC mec type IV isolated in Malaysia. J Med Microbiol. 2009; 58: 1213–8.
21. Ouchenane Z, Smati F, Rolain J, Raoult D. Molecular characterization of methicillin-resistant Staphylococcus aureus isolates in Algeria. Pathol Biol. 2011; 59: e129–32.
22. Monecke S, Schwarz S, Hotzel H, Ehricht R. Rapid Microarray-Based Identification of Diff erent mecA Alleles in. Antimicrob Agents Chemother. 2012; 56(11): 5547–54.
23. Sabir M, Dwiyanti R, Hatta M, Buntaran L, Sultan AR. Sccmec type II gene is common among clinical isolates of methicillin-resistant Staphylococcus aureus in Jakarta, Indonesia. BMC Res Notes. 2013; 6(1): 110.
24. Santosaningsih D, Santoso S, Setijowati N, Rasyid HA, Budayanti NS, *et al*. Prevalence and characterisation of *Staphylococcus aureus* causing community-acquired skin and soft tissue infections on Java and Bali, Indonesia. Tropical Medicine and International Health. 2018; 23(1): 34–44.
25. Hannan A, Javed F, Saleem S, Tahira K, Jahan S. Frequency of Staphylococcal Cassette Chromosome mec Type IV and Type V in Clinical Isolates of Methicillin Resistant Staphylococcus aureus. Open J Med Microbiol. 2015; 5(June): 69–75.

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Original Article

First Line Anti-Tuberculosis Drug Resistance Pattern in Multidrug-Resistant Pulmonary Tuberculosis Patients Correlate with Acid-Fast Bacilli Microscopy Grading

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ABSTRACT

Multidrug-resistant tuberculosis (MDR-TB) is a global public health crisis. Acid-fast bacilli (AFB) gradation in sputum examination is an important component in Pulmonary Tuberculosis (PTB) diagnosis and treatment outcome monitoring. Previously treated pulmonary TB patients with a higher AFB smear gradation may have higher rates of acquired resistance. Patients with a higher AFB grade indicate a higher bacillary load and had higher rates of acquired resistance. This study aims to evaluate the correlation between AFB gradation and first-line anti-TB drug resistance patterns in MDR pulmonary TB patients. This was a retrospective study conducted from August 2009 to April 2018 in Dr. Soetomo Hospital. Sputum samples were taken from MDR PTB patients. Sputum smear examination was done using Ziehl–Neelsen staining and gradation was measured according to IUATLD criteria. Samples with positive smear were evaluated for resistance patterns based on culture and resistance tests using the MGIT 960 BACTEC System. There were 433 sputum samples with AFB positive collected from MDR PTB patients. Resistance to RHES was found in 22 (14%) AFB +1, 19 (15%) AFB +2, and 29 (20%) AFB +3. Resistance to RHS was found in 22 (14%) AFB +1, 12 (9%) AFB +2, and 13 (9%) AFB +3. Resistance to RHE was found in 39 (25%) AFB +1, 38 (29%) AFB +2, and 35 (24%) AFB +3. Resistance to RH was found in 74 (47%) AFB +1, 61 (47%) AFB +2, and 69 (47%) AFB +3. Statistic analysis by Spearman test showed that there was no significant correlation between AFB gradation and first-line anti-TB drug resistance patterns. Acquired resistance to RHES can also found in lower bacillary load AFB +1.

Keywords: *MDR pulmonary TB, AFB grading, first line anti-TB drug resistance pattern*

ABSTRAK

Tuberkulosis multidrug-resistant (TB-MDR) merupakan salah satu masalah kesehatan utama di dunia. Pemeriksaan basil tahan asam (BTA) pada sampel dahak merupakan komponen yang penting dalam diagnosis dan pemantauan hasil pengobatan pasien TB paru. Pasien TB paru dengan jumlah BTA yang lebih tinggi memiliki potensi tinggi terjadi resistensi obat. Pasien dengan jumlah BTA yang lebih tinggi menunjukkan jumlah basil yang lebih banyak dan memiliki potensi terjadi resistensi yang lebih tinggi. Penelitian ini bertujuan untuk mengevaluasi hubungan antara gradasi BTA dan pola resistensi obat anti-TB lini pertama pada pasien TB paru MDR. Studi ini merupakan studi retrospektif yang dilakukan di Rumah Sakit Dr. Soetomo pada bulan Agustus 2009 hingga bulan April 2018. Sampel dahak diambil dari pasien TB paru MDR. Pemeriksaan dahak dilakukan menggunakan pewarnaan Ziehl-Neelsen dan jumlah BTA diukur sesuai dengan kriteria IUATLD. Sampel BTA positif dilakukan evaluasi pola resistensi obat anti-TB lini pertama berdasarkan uji kultur dan resistensi dengan Sistem BACTEC MGIT 960. Terdapat 433 sampel dahak dengan BTA positif dari pasien TB paru MDR. Resistensi terhadap RHES ditemukan pada 22 (14%) BTA +1, 12 (9%) BTA +2, dan 13 (9%) BTA +3. Resistensi terhadap RHE ditemukan pada 39 (25%) BTA +1, 38 (29%) BTA +2, dan 35 (24%) BTA +3. Resistensi terhadap RH ditemukan pada 74 (47%) BTA +1, 61 (47%) BTA +2, dan 69 (47%) BTA +3. Analisis statistic dengan uji Spearman menunjukkan bahwa tidak terdapat

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hubungan yang signifikan antara gradasi BTA dan pola resistensi obat anti-TB lini pertama. Pola resistensi RHES juga dapat ditemukan pada jumlah basil yang lebih rendah BTA +1.

Kata kunci: *TB paru MDR, gradasi BTA, pola resistensi obat anti-TB lini pertama*

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INTRODUCTION

Drug-resistant tuberculosis (DR TB) continues to be a public health crisis. In 2017, around 558,000 people in the world developed rifampicin-resistant TB (RR-TB) and 82% had multidrug-resistant TB (MDR-TB).[1] MDR-TB is defined as TB which caused by strain *Mycobacterium tuberculosis* resistant at least to isoniazid (H) and rifampicin (R), two of the main first-line anti-TB drugs.[2] First-line anti-TB drugs consist of isoniazid (H), rifampicin (R), pyrazinamide (Z), ethambutol (E), and streptomycin (S). Globally, Indonesia is the 7th rank in the estimated incidence of RR-TB cases in 2017 is 23.000 people with MDR percentage among RR-TB cases was 91%.[1]

From all of TB cases, 2.4% of new TB cases and 13% of previously treated cases had MDR/ RR-TB. This means the miss management of TB cases is still dominant as the cause of DR TB. Drug resistance occurs when drug-susceptible TB (DS TB) patients receive inadequately or interrupted therapy which leads to the selection of drug-resistant bacteria and 'acquired' drug resistance. Infectious patients who are infected by resistant strain *Mycobacterium tuberculosis* could spread through airborne droplets as transmitted drug resistance.[3]

Acid-fast bacilli (AFB) microscopy examination is a common simple tool for the diagnosis and treatment outcome monitoring of pulmonary TB.[4] Patients with higher AFB grade indicates higher bacillary load and increasing baseline drug resistance had higher rates of acquired resistance.[5] The recent dogma stated that the level of resistance to INH and RIF (required for MDR-TB) was caused by the individual mutation rates for INH and RIF; that is, in the order of 10^{-6}. For the evolution of MDR strains, a total population of at least 10^6 bacilli must be present in each infected person.[6] The possibility that a single drug-resistant mutant may arise earlier after infection, and could replicate to a large enough population from which the possibility of a second drug-resistance mutation will not be too slow.[7] The potential drug-resistant mutation is varied in each drug, ranging from around 1 in 10^8 bacilli for rifampicin, to about 1 in 10^6 bacilli for isoniazid, streptomycin, and ethambutol. Besides, *Mycobacterium tuberculosis* consists of various phylogenetic lineages,[8] that could have some intrinsic drug resistance character in the bacilli population of the PTB patients. On the other hand, MDR-PTB cases with several an active disease process with AFB bacilli production in sputum with many population characteristics of anti-TB resistance that related to multi factors.

Some clinicians assume that more amount of AFB can cause acquired more drug resistance. This study aims to determine the drug resistance pattern of all positive smear in MDR PTB patients and evaluate its correlation with AFB microscopy grading.

MATERIALS AND METHODS

Study Definition

Patients were divided by a history of previous TB treatment according to WHO guideline[9]

1. New cases: who have never been treated for TB or have taken anti-TB drugs for less than 1 month.
2. Previously treated patients have received 1 month or more of anti-TB drugs in the past. They are further classified by the outcome of their most recent course of treatment:

a. Relapse patients have previously been treated for TB, were declared cured or treatment completed at the end of their most recent course of treatment, and are now diagnosed with a recurrent episode of TB (either a true relapse or a new episode of TB caused by re-infection).
b. Treatment after failure: patients are those who have previously been treated for TB and whose treatment failed at the end of their most recent course of treatment (WHO category I regimen or WHO category II regimen).
   - WHO category I regimen: 2 (HRZE)/ 4(HR)3 or 4(HR)
   - WHO category II regimen: 2 (HRZE)S/ (HRZE)/ 5(HR) 3E3 or 5(HR)E
c. Treatment after loss to follow-up: patients have previously been treated for TB and were declared lost to follow-up at the end of their most recent course of treatment (these were previously known as a treatment after default patients).
d. Other previously treated patients are those who have previously been treated for TB but whose outcome after their most recent course of treatment is unknown or undocumented.

Study Subjects and Design

This was a retrospective study. Samples were collected from all MDR pulmonary TB (MDR PTB) patients who are treated from August 2009 to April 2018 in Dr. Soetomo Hospital. The medical records of enrolled patients were reviewed to obtain their microbiological examinations. Sputum samples were taken from new and previously treated MDR PTB patients. Sputum smear examination was done using Ziehl–Neelsen staining. Direct smears were made from each sputum sample and were stained with Ziehl-Neelsen (ZN) stain according to the WHO recommendation. AFBs identified were graded according to the International Union against Tuberculosis and Lung Disease (IUATLD) and the WHO smear grading scale. Findings were scored as follows: 1–9 AFB/100 fields (1+); 1–9 AFB/10 fields (2+); and 1–9 AFB/ field (3+). Each slide was examined by three independent readers to ascertain the presence of AFB and grade positive smears. The slide readers were blinded on the clinical and laboratory diagnoses of the participants whose samples were studied. Samples with positive smear were evaluated for resistance pattern based on culture method using MGIT 960 BACTEC System for determinate the sensitivity to Rifampicin (R), Isoniazid (H), Ethambutol (E), and Streptomycin (S). Examination of microscopic sputum smears, culture method for identification and drug sensitivity test were carried out at the Surabaya Health Laboratory Center which has been certified by WHO. Statistic analysis using the Spearman test was used to analyze the significance of AFB grading and resistance pattern.

RESULTS AND DISCUSSION

There were 433 MDR-TB patients with positive smear, 253 (58.4%) men and 180 (41.6%) women in MDR-TB clinic care of Dr. Soetomo Hospital from August 2009 to April 2018. The number of MDR PTB patients were higher in men than women in this study with 253 (58.4%) and 180 (41.6%) women, respectively. Another study also found that the MDR/RR TB strains were three times more common in men than women.[10] Being a man or woman can be a factor to develop drug resistance; however, the findings vary on the subject. A global prevalence study did not find sex to be a risk factor for MDR-TB.[11]

The average age of MDR PTB patients was 43.82 years old and most MDR TB patients were productive with age range 15-49 year-old with a count of 291 (67.2%). Globally, there were cases in all countries and age groups but overall 90% were adults (aged ≥15 years).[1] A study in Switzerland reported that age <35 years old increased risk of resistance to first-line drugs (OR=1.5; 95% CI 1.0–2.3).[12]

Based on TB treatment history, MDR PTB patients were divided into new cases and previously treated cases (relapse, return after

default, failure of the WHO category I, failure of the WHO category II, and other cases such as unstandardized treatment). Most of MDR PTB patients were ones with previously treated with 426 (98%). Relapse cases were dominant with 160 (36.9%), followed by failures of the WHO Category I regimen with 110 (25.4%), and return after default with 91 (21%). This result was shown in Table 1.

There were 426 (98%) of MDR-TB patients were coming from patients with the previous history of TB treatment in this study. Previously treated TB patients were a risk factor for MDR-TB.[13] Previous anti-TB treatment was by far a solid predictor of drug resistance.[14] Previously treated TB patients had a higher chance as many as 8.1 times to develop an MDR-TB infection compared to newly diagnosed TB patients.[15] In this study, relapse cases were the most common with 160 cases (36.9%), followed by failures of the WHO Category I regimen with 110 cases (25.4%). Relapse cases were dominant among patients with MDR-TB in this study. The previous study reported that most of drug-resistant TB were relapse cases with 123/290 patients (42.4%), followed by treatment failures with 123/290 (34.8%).[16] The dominance of relapse cases among MDR-TB patients may caused by inadequate treatment and less compliance of patient during previous treatment resulted dormant MDR-TB. Subsequently, the survival of dormant MDR-TB increased the risk of TB relapse.[17] The dominance of relapse cases also happened because TB recurrence resulted from either relapse or reinfection was remained defined as relapse according to the WHO guideline. To defined relapse or reinfection cases, the examination of *Mycobacterium tuberculosis* strain was needed to know whether it was relapse of an original infection or exogenous reinfection with a new *Mycobacterium tuberculosis* strain. In the previous study, 51.4% of relapse happened in ≤2 years and 48.6% of relapse happened in >2 years, while 57.1% of reinfection happened in >2 years and 42.9% reinfection happened in ≤2 years.[17]

Although new TB diagnosing technologies have been improved, the use of AFB microscopy still the main of the diagnostic[18] and patients with positive AFB are often considered as MDR-TB due to greater AFB leads the bacterial mutation. Patients with higher bacterial load are more potential for drug-resistant mutations and have a greater risk of developing MDR-TB.[19] Initial AFB sputum smear ≥3+ was correlated with acquired drug resistance.[5] Of the 433 sputum samples with AFB positive collected from MDR PTB patients, resistance to RHES was 14% in AFB +1, 15% in AFB +2, and 20% in AFB +3. Resistance to RHS was 14% in AFB +1, 9% in AFB +2, and 9% in AFB +3. Resistance to RHE was 25% in AFB +1, 29% in AFB +2, and 24% in AFB +3. Resistance to RH was 47% in AFB +1, 47% in AFB +2, and 47% in AFB +3. Based on statistic analysis by Spearman test, there was no significant correlation between AFB gradation and resistance pattern with p-value 0.786 as presented in Table 2.

The results in Table 2 showed that resistance to more drugs was also happened by the lower AFB grading (AFB +1) and indicated that the grade of AFB might not represented the number of *Mycobacterium tuberculosis.* AFB-positive smears may be because of the presence of

Table 1. History of TB treatment profile of MDR TB patients in Dr. Soetomo Hospital.

Variable	R+H	R+H+E	R+H+S	R+H+E+S	Total
New cases	3 (43%)	3 (43%)	0 (0%)	1 (14%)	7
Previously treated cases	201 (47%)	109 (26%)	47 (11%)	69 (16%)	426
• Failure treatment with WHO Category II regimen	19 (34.5%)	16 (29%)	8 (14.5%)	12 (22%)	55
• Failure treatment with WHO Category I regimen	53 (48%)	29 (26%)	8 (7%)	20 (18%)	110
• Relapse	84 (52.5%)	39 (24%)	17 (11%)	20 (12.5%)	160
• Return after default	43 (47%)	22 (24%)	14 (15%)	12 (13%)	91
• Other case	2 (20%)	3 (30%)	0 (0%)	5 (50%)	10

Table 2. Analysis of correlation between AFB grading and the first line anti-TB drug resistance pattern.

AFB Grading	Resistance Pattern				Total	P Value
	R+H+E+S	R+H+S	R+H+E	R+H		
+++	29 (20%)	13 (9%)	35 (24%)	69 (47%)	**146 (34%)**	0.786
++	19 (15%)	12 (9%)	38 (29%)	61 (47%)	**130 (30%)**	
+	22 (14%)	22 (14%)	39 (25%)	74 (47%)	157 (36%)	
Total	70 (16%)	47 (11%)	112 (26%)	204 (47%)	433 (100%)	

*P value based on Spearman Test. Correlation coefficient (0.013).

Table 3. Correlation between AFB grading vs. every treated group.

History of TB treatment	AFB			P-value
	+	++	+++	
New cases (n=7)	2(28.5%)	2(28.5%)	3(43%)	0.895
Failure treatment with WHO Category II regimen (n=55)	18(32.7%)	18(32.7%)	19(34.6%)	
Failure treatment with WHO Category I regimen (n=110)	43(39%)	31(28%)	36(33%)	
Relapse (n=160)	53(33%)	49(31%)	58(36%)	
Return after default (n=91)	37(40%)	27(30%)	27(30%)	
Other case (n=10)	4(40%)	3(30%)	3(30%)	

nonviable *Mycobacterium tuberculosis* bacilli or nontuberculous mycobacteria (NTM).[20]

Our study found that the AFB grading did not represent the resistance pattern of first-line anti-TB drugs. AFB +1, which was the lower bacillary load, also showed resistance to RHES. Based on statistical analysis using the Spearman test, AFB grading was not correlated with the resistance pattern of MDR TB patients with *p* 0.786. This result showed that the bacillary load did not affect the resistance to some TB drugs. A different result was shown by another study that reported higher smear grade (+2 and +3) has a higher rate of MDR-TB/ RIF resistance with 76/256 (29.7%) compared with smear grades of +1, scanty positive and negative with 61/301 (20.3%) (p-value = 0.01).[10] There was no reveal the correlation of the first-line anti-TB drug resistance pattern with AFB grading in this study. Resistance to more drugs (RHES) also found in patients with AFB +1.

Analysis of correlation between AFB grading and every treated group showed that there was not a significant difference with a p-value of 0.895 as presented in Table 3. The definition of each group has been described in the methodology.

The results in Table 3 showed that the AFB grading was not affected by the history of TB treatment. Actually, AFB smear can be used to assess TB treatment outcome, but careful examination of microbiologic status, including culture and drug susceptibility testing were also needed to confirm the AFB smear examination.[4]

Greater AFB grading is often considered associated with the incidence of drug resistance. A higher AFB grading represented higher bacilli and it possible to acquired drug resistance. Acquired resistance to rifampicin was estimated by mutation of 10^8 bacilli and acquired resistance to isoniazid, streptomycin, and ethambutol by mutation of 10^6 bacilli.[21] This rate might also be affected by the drug concentration in the medium, the drug resistance profile of the strain and its genetic background.[22] Drug resistance-associated genes were *kat*G and *inh*A in isoniazid, rpoB in rifampicin, *rps*L in streptomycin, and *emb*B in ethambutol.[23] Previous studies reported that there were varies drug resistance patterns among sputum-smears positive; MDR-TB, non-MDR two drug resistance, and resistance to any one of the first line of drugs (isoniazid, ethambutol, and rifampicin).[24]

Acquired resistance to more drugs may correlate with *Mycobacterium tuberculosis* strain in MDR pulmonary TB patients. Different strain of *Mycobacterium tuberculosis* also represented diff erent frequencies of genes which played role in drug resistance. The prevalence of specific drug resistance-associated mutations also varies within the lineage, such as the frequencies of the *rpo*B S531L and *kat*G S315T mutations are greater in the modern (typical) Beijing strains than in ancient (atypical) ones. There was a significant variation in the mutation rates of strains, the study also showed that strains from Lineage 2 of *Mycobacterium tuberculosis* (includes Beijing family of strains) acquire drug resistance *in vitro* rapidly than strains from Lineage 4.[22,25]

CONCLUSIONS

There was no significant correlation between the first-line anti-TB resistance pattern of MDR PTB strain with AFB microscopy grading. Acquired resistance to RHES can also found in lower bacillary load AFB +1.

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CONFLICT OF INTEREST

There is no conflict of interest of this paper.

REFERENCES

1. World Health Organization. Global Tuberculosis Report 2018.Geneva: WHO; 2018.
2. World Health Organization. Multidrug and extensively drug-resistant TB (M/XDR-TB): Global report on surveillance and response. Geneva: WHO; 2010.
3. Fox GJ, Schaaf HS, Mandalakas A, Chiappini E, Zumla A, Marais BJ. Preventing the spread of multidrug-resistant tuberculosis and protecting contacts of infectious cases. Clin Microbiol and Infect. 2017; 23: 147–53.
4. Kang HK, Jeong BH, Lee H, Park HY, Jeon K, Huh HJ, et al. Clinical significance of smear positivity for acid-fast bacilli after ≥5 months of treatment in patients with drug-susceptible pulmonary tuberculosis. Medicine. 2016; 95(31): e4540.
5. Kempker RR, Kipiani M, Mirtskhulava V, Tukvadze N, Magee MJ, Blumberg HM. Acquired Drug Resistance in *Mycobacterium tuberculosis* and Poor Outcomes among Patients with Multidrug-Resistant Tuberculosis. Emerging Infect Dis. 2015; 21(6): 992–1001.
6. McGrath M, van Pittius NCG, van Helden PD, Warren RM, Warner DF. Mutation rate and the emergence of drug resistance in *Mycobacterium tuberculosis*. J Antimicrob Chemother. 2014; 69: 292–302.
7. Colijn C, Cohen T, Ganesh A, Murray M. Spontaneous emergence of multiple drug resistance in tuberculosis before and during therapy. PLoS One. 2011; 6: e18327.
8. Dominguez J, Boettger EC, Cirillo D, Cobelens F, Eisenach KD, Gagneux S, et al. Clinical implications of molecular drug resistance testing for *Mycobacterium tuberculosis*: a TBNET/RESIST-TB consensus statement. Int J Tuberc Lung Dis. 2016; 20(1): 24– 42.
9. World Health Organization. Definitions and reporting framework for tuberculosis – 2013 revision (updated December 2014). Geneva: WHO; 2013.
10. Singhal R, Arora J, Sah GC, Bhalla M, Sarin R, Myneedu VP. Frequency of multi-drug resistance and mutations in *Mycobacterium tuberculosis* isolates from Punjab state in India. J Epidemiol Glob Health. 2017; 7: 175–80.
11. Zignol M, van Gemert W, Falzon D, Sismanidis C, Glaziou P, Floyd K, et al. Surveillance of anti-tuberculosis drug resistance in the world: an updated analysis, 2007e2010. Bulletin of the World Health Organization 2012; 90: 111De9D.
12. Lomtadze N, Aspindzelashvili R, Janjgava M, Mirtskhulava V, Wright A, Blumberg HM, et al. Prevalence and risk factors for multidrug-resistant tuberculosis in Republic of Georgia: a population based study. *Int J Tuberc Lung Dis*. 2009; 13(1): 68–73.
13. Caminero JA. Multidrug-resistant tuberculosis: epidemiology, risk factors and case finding. Int J Tuberc Lung Dis. 2010; 14(4): 382–90.
14. Hafez SA, Elhefnawy AM, Hatata EA, El Ganady AA, Ibrahiem MI. Detection of extensively drug resistant pulmonary tuberculosis. Egypt J Chest Dis Tuberc. 2013; 62(4): 635–46.
15. Eshetie S, Gizachew M, Dagnew M, Kumera G, Woldie H, Ambaw F, et al. Multidrug resistant tuberculosis in Ethiopian settings and its association with previous history of anti-tuberculosis treatment: a systematic review and meta-analysis. BMC Infectious Diseases. 2017; 17: 219.

16. Kumar P, Kumar P, Balooni V, Singh S. Genetic mutations associated with rifampicin and isoniazid in MDR-TB patients in North-West India. Int J Tuberc Lung Dis. 2015; 19(4): 434–9.
17. Zong Z, Huo F, Shi J, Jing W, Ma Y, Liang Q, et al. Relapse versus reinfection of recurrent tuberculosis patients in a National Tuberculosis Specialized Hospital in Beijing, China. Front Microbiol. 2018; 9: 1858.
18. Odubanjo MO, Dada - Adegbola H . O . The microbiological diagnosis of tuberculosis in a resource-limited setting: is acid-fast bacilli microscopy alone sufficient?. Ann. Ibd. Pg. Med. 2011; 9(1): 24–9.
19. Sander MS, Vuchas CY, Numfor HN, Nsimen AN, Abena JL, Noeske J. Sputum bacterial load predicts multidrug-resistant tuberculosis in retreatment patients: a case-control study. Int J Tuberc Lung Dis. 2016; 20(6): 793–9.
20. Chien JY, Chen YT, Shu CC, Lee JJ, Wang JY, Yu CJ. Outcome correlation of smear-positivity for acid-fast bacilli at the fifth month of treatment in non0multidrug-resistant TB. Chest. 2013; 143(6): 1725–32.
21. Dookie N, Rambaran S, Padayatchi N, Mahomed S, Naidoo K. Evolution of drug resistance in *Mycobacterium tuberculosis*: a review on the molecular determinants of resistance and implications for personalized care. J Antimicrob Chemother. 2018; 73: 1138–51.
22. Nguyen QH, Contamin L, Nguyen TV, Banuls AL. Insight into the processes that drive the evolution of drug resistance in *Mycobacterium tuberculosis.* Evol Appl. 2018; 11: 1498–1511.
23. Palomino JC, Martin A. Drug resistance mechanisms in *Mycobacterium tuberculosis.* Antibiotics. 2014; 3: 317–40.
24. Goswami A, Chakraborty U, Mahaputra T, Mahapatra S, Mukherjee T, Das S, et al. Correlates on treatment outcomes and drug resistance among pulmonary tuberculosis patients attending tertiary care hospitals of Kolkata, India. PLoS ONE. 2014; 9(10): e109563.
25. Ford CB, Shah RR, Maeda MK, Gagneux S, Murray MB, Cohen T, et al. *Mycobacterium tuberculosis* mutation rate estimates from different lineages predict substantial diff erences in the emergence of drug resistant tuberculosis. Nat Genet. 2013; 45(7); 784–90.

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Research Article

Lower Perceived-Stigmatization by Health Workers Among HIV-AIDS Patients of Key Population Backgrounds

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ABSTRACT

The stigma of people living with HIV-AIDS (PLWHA) by health workers may have a broad impact, so it is necessary to identify the factors that influence the occurrence of stigma. Identification of factors that cause a decrease in stigmatization by health workers will have an impact on improving the quality of life of people with HIV, increasing compliance with medication, and ultimately reducing the incidence of HIV infection itself. The purpose of this study was to analyze factors related to PLWHA's perception of stigma among health workers in the community health center. This research applied a cross-sectional design using interviews. Ninety-four patients from the Infectious Disease Intermediate Care of Dr. Soetomo Hospital Surabaya, a tertiary level hospital, were interviewed. The stigma perception was assessed using a questionnaire modified from the Standardized Brief Questionnaire by Health Policy Project with Cronbach's Alpha of 0.786. The data were simultaneously analyzed with binary multiple regressions on IBM SPSS Statistics 22.0 for Windows software. There were 30 out of 94 patients with key population backgrounds, and most population was injecting drug users (IDUs) and female sex workers (FSWs). PLWHA perceived most stigmatized community health workers when they drew blood, provided care, and considered they were involved in irresponsible behavior. There were relationships between age (p=0.008), marital status (p=0.013), and the history of key population (p=0.006)to people living with HIV-AIDS (PLWHA)'s perception of stigma among health workers in East Java community health center. Future research on factors influencing HIV-related stigma is needed to improve patients' quality of life.

Keywords: *Health workers, HIV-AIDS, key population, stigma*

ABSTRAK

Stigma terhadap orang dengan HIV-AIDS (ODHA) oleh tenaga kesehatan dapat berdampak luas, maka perlu dilakukan identifikasi faktor-faktor yang memengaruhi terjadinya stigma. Identifikasi faktor-faktor yang menyebabkan penurunan stigmatisasi oleh tenaga kesehatan akan berdampak terhadap peningkatan quality of life orang dengan HIV, meningkatnya kepatuhan minum obat, dan akhirnya akan mengurangi angka kejadian infeksi HIV itu sendiri. Tujuan dari penelitian ini yaitu untuk menganalisis faktor-faktor yang berhubungan terhadap persepsi orang dengan HIV-AIDS (ODHA) atas stigma oleh tenaga kesehatan puskesmas. Penelitian ini menggunakan rancangan penelitian cross-sectional dengan metode wawancara. Sembilan puluh empat pasien dari Poli Rawat Jalan Instalasi PIPI RSUD Dr. Soetomo, yang merupakan rumah sakit tersier diwawancarai. Persepsi stigma pasien dinilai menggunakan kuesioner standar oleh Health Policy Project dengan nilai Cronbachs Alpha 0,786. Data dianalisis dengan uji regresi logistic berganda dengan perangkat lunak IBM SPSS Statistics 22.0 for Windows. Didapatkan 30 dari 94 pasien yang memiliki riwayat kelompok risiko, dengan kelompok risiko terbanyak adalah Penasun dan WPS. Gambaran stigmatisasi oleh tenaga kesehatan terhadap ODHA yaitu khawatir ketika mengambil darah,

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memberikan perawatan berkualitas rendah, dan menganggap seseorang terinfeksi HIV karena mereka terlibat perilaku yang tidak bertanggung jawab. Terdapat hubungan antara usia (p=0,008), status perkawinan (p=0,013), dan ODHA beriwayat kelompok risiko (p=0,006) dengan persepsi ODHA atas stigma oleh tenaga kesehatan puskesmas. Usia yang muda, menikah, dan memiliki riwayat kelopok risiko merupakan faktor-faktor yang signifikan terhadap rendahnya persepsi ODHA atas stigma oleh tenaga kesehatan puskesmas Jawa Timur. Penelitian terkait faktor-faktor yang berhubungan dengan stigma HIV dibutuhkan untuk meningkatkan kualitas hidup ODHA.

Kata kunci: *Tenaga kesehatan, HIV-AIDS, kelompok risiko, stigma*

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INTRODUCTION

The stigma against PLWHA, which arises from the mind of an individual or society who believes that AIDS is a result of immoral behavior that cannot be accepted by society, is reflected in cynical attitudes, feelings of excessive fear, and negative experiences to PLWHA[1]. Stigma and discrimination are not only carried out by commoners who do not have enough knowledge about HIV and AIDS but can also be carried out by health workers[2]. The opinion that states AIDS is a curse because of immoral behavior also greatly affects how people comport themselves and behave towards PLWHA[3]. In 2014, UNAIDS established a program in accordance with Millennial Developmental Goals (MDGs) namely 3 Zeros, which includes Zero new infections, Zero AIDS-related deaths, and Zero stigma and discrimination[4]. This program is a human-centered HIV prevention and treatment service to end the AIDS epidemic by 2030[5]. However, this has not been in contrary to the reality in the field.

Research by Stringer involving 651 health workers found that almost 90% of health workers gave at least one stigma to PLWHA. 18.9% of health workers agreed that PLWHA had a large number of sexual partners, 33.3% agreed that PLWHA could avoid HIV infection if they wanted to, and 35.3% thought that sufferers could become infected with HIV due to irresponsible sexual behavior[6]. Research in Indonesia in 2014 also found stigma by health workers, including landfills that are differentiated and labeled HIV, feeding under the door, not changing patient's bedsheets, excessive use of protective equipment, isolation, and taking action without informed consent[7].

Stigma by health workers towards people with HIV certainly still has a strong impact. Eventually, this will impact how others perceive a person, social rejection, decreased acceptance of social interaction, increased discrimination, and adding family burden[8]. The impact of this stigma is not good and can be fatal for HIV patients, as mentioned in the study conducted by Ardani[9]. Drug-addict-PLWHA who feel stigmatized will reduce the possibility of seeking treatment, for those who have undergone treatment may choose to end the treatment. Furthermore, stigma affects the lives of PLWHA by causing depression and anxiety, sadness, guilt, and feelings of worthlessness. Besides, stigma can reduce the quality of life and limit access and use of health services[9]. Labeling and discrimination against people living with HIV-AIDS are the foremost effective barriers in preventing HIV and also in providing drugs, care, and support[10].

Because of the stigma of people with HIV can have a wide-ranging impact, it is necessary to identify the factors that influence stigma to PLWHA by health workers. Identification of factors that cause a decrease in stigmatization by primary health center workers will have an impact on improving the quality of life of people with HIV, improving medication adherence, so the incidence rate of HIV itself will be reduced.

Therefore, this study was aimed to identify the correlating factors between PLWHA and stigmatization by community health center's workers using subjects of people with HIV

in the Outpatient Care Clinic of Intermediate and Infectious Disease Care Unit (Perawatan Intermediet Penyakit Infeksi - PIPI) Dr. Soetomo Hospital Surabaya. It is hoped that the results of this study can provide input to policymakers to initiate a stigma reduction program for people with HIV that can be started from PLWHA who has the highest stigma, to make it easier for PLWHA to disclose their status and treatment. Also, it is hoped that the prevention of HIV transmission to the community will be more controlled and help improve the quality of life with HIV-AIDS (PLWHA).

MATERIALS AND METHODS

This study used an observational analytic study with cross-sectional study design. The sample of this study was 94 HIV positive patients in the Outpatient Care Clinic of Intermediate and Infectious Disease Care Unit Dr. Soetomo Hospital Surabaya from October to December 2018 who were referral patients from a community health center or had received health services at a community health center in East Java after being diagnosed with HIV. The sampling technique used was consecutive. Respondents were interviewed using a modified questionnaire by the Health Policy Project available at www.stigmaindex. com, which has been tested for reliability and validity with a Cronbach's Alpha coefficient of 0.786. The Standardized Brief Questionnaire by the Health Policy Project was developed and verified through a calculated collaborative process that involved experts from various countries. There are four areas which are pertinent to stigma and discrimination in health care environment that the experts are complied to focus on: 1) fear of HIV infection among health facility staff; 2) stereotypes and prejudice related to people living with or thought to be living with HIV; 3) observed and secondary stigma and discrimination; and 4) policy and work environment[11].

In the questionnaire by the Health Policy Project, the health workers' point of view is used as the object. What is new in this study is using the perspective of people living with HIV-AIDS. The questionnaire was about socio-demographic data and HIV-related questions that illustrate the understanding, awareness, and experience of attitudes by health center workers towards PLWHA. This questionnaire was divided into four sections. The first section was background information containing questions about sex, age, marital status, duration of HIV diagnosis, the origin of residence, occupation, and history of key population. The second section, infection control, contained questions about the stigma that has been experienced related to HIV infection control at the time of examination. The third section, Health Facilities' Environment, contained questions related to stigma in the health facility environment. The fourth section, Opinion about People Living with HIV, contained statements related to the opinion of health workers towards people living with HIV-AIDS. The choice of answers to each question was how often the stigma occurred so that it would describe which stigma is most often obtained.

RESULTS AND DISCUSSION

Sociodemographic Characteristics

The sample in this study was varies based on the gender, age, marital status, occupation, duration of patient diagnosed with HIV, HIV control/check-up, residence, and history of key population as described in Table 1.

Patients from Surabaya were grouped according to the sub-district of residence. The distribution of patients from Surabaya is shown in Table 2.

The number of females infected with HIV-AIDS was higher than males, in contrast to data released by the Ministry of Health in 2017. The higher number of infected females is because females are vulnerable to HIV due to biological factors, reduced sexual autonomy, and it is explained that women want to prevent HIV but do not have enough strength to against[12]. Prospective studies of serodiscordant couples and male contact with FSW show that women are twice as likely to be infected if exposed to HIV[13]. The age classification in Table 1 is based on the Indonesian Ministry of Health in the annual HIV-AIDS disease progress

Table 1. Sociodemographic Characteristics

Sociodemographic Characteristics	Frequency	Percentage (%)
Gender		
Male	45	47.9
Female	49	52.1
Age		
20-24 years old	2	2.1
25-49 years old	84	89.3
≥50 years old	8	8.6
Marital Status		
Married	58	61.7
Single	23	24.5
Widowed	13	13.8
Occupation		
Housewife	25	26.6
Female Sex Worker	45	47.9
Health Worker	1	1.1
Others	23	24.6
Duration of patient diagnosed with HIV		
1 year	26	27.7
2 years	7	7.4
3 years	17	18.1
4 years	9	9.6
5 years	8	8.5
6 years	8	8.5
7 years	4	4.3
8 years	2	2.1
9 years	3	3.2
≥10 years	10	10.7
HIV Control/Check-up		
Twice or more in a month	11	11.7
Once in a month	79	84
Once in three months	2	2.1
Once in 4-6 months	2	2.1
Residence		
Blitar	2	2.1
Bondowoso	1	1.1
Gresik	3	3.2
Jombang	1	1.1
Mojokerto	1	1.1
Ngawi	1	1.1
Pasuruan	3	3.2
Sidoarjo	9	9.6
Sumenep	2	2.1
Surabaya	71	74.3
Trenggalek	1	1.1
History of Key Population		
Yes	30	33.9
No	64	68.1

Table 2. Distributions of patients from Surabaya

Sub-districts	Frequency	Percentage (%)
Benowo	2	2.9
Bubutan	1	1.4
Genteng	1	1.4
Gubeng	6	8.6
Karang Pilang	1	1.4
Kenjeran	1	1.4
Krembangan	7	10
Mulyorejo	3	4.3
Pabean Cantian	2	2.9
Rungkut	2	2.9
Sawahan	10	14.3
Semampir	2	2.9
Sukolilo	3	4.3
Sukomanunggal	1	1.4
Simokerto	1	1.4
Tambaksari	12	17.1
Tegalsari	7	10
Wiyung	3	4.3
Wonocolo	1	1.4
Wonokromo	4	5.7

report, which used the same age classification so that the comparison of results is appropriate. The age of most PLWHA obtained from this study was 25-49 years because it is the age of sexually active. The same data is issued by the Indonesian Ministry of Health in the Report on the Development of HIV-AIDS & Sexually Transmitted Infectious Diseases for the First Quarter 2017, that is 69.6% is the 25-49 years age group, 17.6% is the 20-24 years age group and 6.7% is the age group of >50 years[14]. Most marital status was marriage, which could be a clue that sexual contact was the most cause. The longest HIV diagnosis was one year or less, which could be understood because Dr. Soetomo Hospital Surabaya is a third-level health facility that accepts referral cases and cannot be resolved at a first or second level health facility. ARVs were taken at the Dr. Soetomo so that many new patients immediately went to the Dr. Soetomo Hospital Surabaya to get treatment. The most times of having HIV control to health services was once in a month at Dr. Soetomo Hospital Surabaya due to the rules of taking antiretroviral drugs.

Most patients lived in Surabaya, precisely in Tambaksari District. This can be understood because it is located near to Dr. Soetomo Hospital Surabaya, which is about 2 km measured using the Google Maps application. There are four community health centers in this district, namely Pacarkeling Health Center, TambakRejo Health Center, Rangkah Health Center, and Gading Health Center. The second most was from Sawahan District. This is consistent with data from the Ministry of Health of the Republic of Indonesia, which is as many as 139 patients tested positive for HIV in the first quarter of 2017, the most after Health Center of Putat Jaya Surabaya[14]. The number of patients who did not have a history of key population was greater than those who had a history of key population, which is as much as 68.1%.

The Distribution of Key Population Background of People Living with HIV-AIDS (PLWHA)

History of key population was obtained through interviewing the patients using questionnaires. The data obtained is displayed in Table 3.

The results have been obtained that patients with the most history of key population are injected-type drug users (IDUs) and prostitute (FSW) as many as nine people. The same data issued by the Ministry of Health of the Republic of Indonesia shows the data of IDU has the highest prevalence of 41% compared to other key populations[15]. HIV prevalence in the IDU group is high because they inject drugs more than once a day and more than 60% of them using needles that are not sterilized. While risky sexual behavior that causes HIV prevalence among FSWs remains high, because of unprotected sex. MSM groups of 7 people followed this. It was reported that condom use in MSM consistently lower than FSW, despite the higher level of HIV prevention knowledge[16].

Description of PLWHA's Perceived Stigmatization by Health Center Workers

The description of stigmatization by health workers at the community health center perceived by PLWHA was obtained from interviewing the patients using questionnaires. The data obtained is displayed in Table 4, 5, 6, and 7.

Table 3. Distribution of key population background of PLWHA

Category	Frequency	Percentage (%)
Patient with History of Key Population		
Female Sex Workers (FSW)	9	9.6
Injecting Drug User	9	9.6
FSW sex partner	4	4.3
Men Who Have Sex With Men (MSM)	7	7.4
Transvestite Homosexual	1	1.1
Patient without History of Key Population		
Housewife	28	29.8
Private Sector Worker	20	21.3
Others	16	17.0

In section 2: Infection Control, was divided into two parts. Part 1 was health center workers' concern when examining people living with HIV-AIDS since part 2 was exclusive protection in treating people living with HIV-AIDS.

From 13 questions on the questionnaire that describe stigmatization by health workers at the health center, the stigmatization of health workers was taken which was often obtained from the number of subjects who have been stigmatized, the answers to that are least worried, worried, very worried in section Infection Control. Also, the answer once or twice, several times, and almost every time in section health Facilities' Environment and Health Workers Opinion about People Living with HIV-AIDS.

In section infection Control, the most stigmatization was obtained when health workers were worried when they did blood sampling. A study by Sismulyanto[17] conducted at a hospital in Banyuwangi shows that from 96 nurses, as many as 7.5% of the nurses were afraid to take laboratory samples, such as blood and urine. According to Sismulyanto[17], this is because they were afraid of contracting HIV when in direct contact with the patient's blood.

In section Health Facility's Environment, the most stigmatization was obtained when health care workers provide low-quality care to HIV

Table 4. Description of PLWHA's Perceived Stigmatization on Infection Control: Part 1

Form of Stigma	Not worried		A little worried		Worried		Very worried		Never experienced	
	n	%	n	%	N	%	n	%	n	%
Worried when touching the clothes	82	87.2	3	3.2	1	1.1	0	0	8	8.5
Worried when dressing wounds	47	50.0	21	22.3	3	3.2	1	1.1	22	23.4
Worried when drawing blood	66	70.2	19	20.2	7	7.4	0	0	2	2.1
Worried when taking the temperature	81	86.2	7	7.4	1	1.1	0	0	5	5.3

Table 5. Description of PLWHA's Perceived Stigmatization on Infection Control: Part 2

Form of Stigma	Never		Rarely		Often		Always	
	n	%	N	%	n	%	n	%
Avoid physical contact	83	88.3	9	9.6	2	2.1	0	0
Wear double gloves	87	92.6	3	3.2	2	2.1	2	2.1
Wear gloves during all treatments	78	83.0	4	4.3	4	4.3	8	8.5
Use any special infection-control that are not used while examining other patients	78	83.0	4	4.3	4	4.3	8	8.5

Table 6. Description of PLWHA's Perceived Stigmatizationon-Health Facilities' Environment

Form of Stigma	Never		Once or twice		Several times		Almost every time	
	n	%	n	%	n	%	n	%
Health workers unwilling to care for you	91	96.8	2	2.1	1	1.1	0	0
Health workers providing poorer quality of care to relative to other patients	87	92.6	4	43	2	2.1	1	1.1
Health workers talking badly about you	87	92.6	6	6.4	1	1.1	0	0
Health workers do not want to do blood sampling	92	97.9	1	1.1	1	1.1	0	0
Health workers treat in a place that is not closed	91	96.8	3	3.2	0	0	0	0
Disclose the status of HIV patients to others without consent	93	98.9	0	0	1	1.1	0	0
Using an HIV-related name when calling you when waiting in sequence number	93	98.9	0	0	1	1.1	0	0
During the examination, health workers call improperly	93	98.9	0	0	0	0	1	1.1
During examinations or other activities at the health center, health workers say that you are HIV patient with a loud tone	93	98.9	0	0	1	1.1	0	0

patients compared to other patients, including rejecting patients with HIV-AIDS because they consider HIV-AIDS patients are people who have a great risk if direct contact with patients[7]. A study in Aceh, Indonesia, shows that some doctors treat PLWHA with disrespect, push other patients away from them, and keep them away from care services[18]. It was also found that most stigmatization was obtained when health workers talk badly about HIV patients. This was due to the high stigma in the community and health workers which causes health workers to stay away from them, so they tended to provide low-quality care.

In section Health Workers' Opinions of People Living with HIV-AIDS, the most stigmatization was obtained when health care workers assume that someone who is infected with HIV because of irresponsible behavior. This was because the community thinks that "bad" behavior is seen from free sex and blames PLWHA as a source of AIDS transmission[7].

Relationship Analysis

Relationships between variables were tested using IBM SPSS Statistics 22.0. All data about age, sex, marital status, occupation, place of residence, history of risk groups, and duration of HIV diagnosis were transformed into binomial forms for analysis. The statistical test used is the binary logistic multiple regression test.

Relationship of stigmatization data by health center's workers with age, sex, marital status, occupation, residence, history of risk groups, and duration of HIV diagnosis are shown in Table 8

Table 7. Description of PLWHA's Perception of health Workers' Opinions of People Living with HIV-AIDS

Form of Stigma	Never		Once or twice		Several times		Almost every time		Not know	
	n	%	n	%	n	%	n	%	n	%
Hearing health workers say most of PLWHA do not care if they infect other people	88	93.6	2	2.1	1	1.1	1	1.1	2	2.1
Hearing health workers say HIV patients should feel ashamed of themselves	88	93.6	4	4.3	0	0	0	0	2	2.1
Hearing health workers say most HIV patients have multiple sexual partners	81	86.2	6	6.4	2	2.1	0	0	5	5.3
Hearing health workers say someone infected with HIV because they engage in irresponsible behavior	78	83.0	12	12.8	1	1.1	0	0	3	3.2
Hearing health workers say HIV is punishment for bad behavior	85	90.4	6	6.4	2	21	0	0	1	1.1

Table 8. Bivariate analysis of stigmatization variables on independent variables

Dependent Variables	Stigma				Significance (Chi-square test)
	Low Stigma		Greater Stigma		
	n	%	N	%	
Age					
≤37	25	52,1	23	47,9	P = 0.019
>37	13	28,3	33	71,7	
Gender					
Male	14	31,1	13	68,9	P = 0.078
Female	24	49	25	51	
Marital status					
Married	29	50	29	50	P = 0.016
Single	9	25	27	75	
Occupation					
Low risk	36	40	54	60	P = 0.690
High risk	2	50	2	50	
Duration of HIV diagnosis					
≥5 years	15	42,9	20	57,1	P = 0.711
< 5 years	23	39	36	61	
Residence					
Surabaya	8	34,8	15	65,2	P = 0.526
Outside of Surabaya	30	42,3	41	57,7	
History of key population					
Do not have any history	32	50	32	50	P = 0.006
Have history	6	20	24	80	

Table 9. Multivariate logistic regression analysis of stigmatization variables against independent variables

Dependent Variables	Independent Variables	P	Exp (B)	Significance
Stigma perception	Age	0.008	0.249	Significant
	Gender	0.950	1.033	Not significant
	Marital status	0.013	0.251	Significant
	Occupation	0.339	3.174	Not significant
	Duration of HIV diagnosis	0.140	0.444	Not significant
	Residence	0.092	2.713	Not significant
	History of key population	0.006	0.180	Significant

using the chi-square test and again tested using the binary logistic multiple regressions test in Table 9. The binary logistic multiple regressions test was carried out to eliminate confounding variables, find out which groups received greater stigma, and get an exponential rate of PLWHA perceptions of stigma by health center workers.

The history of key population was divided into two groups. Having a history of key population was one of the FSWs, FSW's sex partners, MSMs, transvestites, and injecting drug users (IDUs). Choices other than FSWs, FSW's sex partners, MSMs, transvestites, and IDUs were included as do not have a history of key population. The chosen cut-off for the stigma was 24. It was a high stigma if greater or equal to 24, while smaller than 24 was a low stigma. The score of 24 indicates that the respondent answered *never* or *not worried*, which is score 1, in all of the 24 questions, which means that the respondent never got any form of stigma from the health center workers. *Once* or *twice*, got 2 on the score. Score 3 for *worried*, *often*, and *several times*. If the answer was *very worried*, *always*, and *almost every time* got score 4. The score of each respondent was obtained from the sum of each question. The cut-off for age was the mean of them, which was 37.46 rounded to 37. If greater or equal to 37 years old, it was said to be old age. While it was said to be young if smaller than 37 years old. Jobs were categorized into 2, high and low-risk jobs. High-risk jobs were health workers, doctors, nurses, security, ward attendants, sex workers, and flight attendants. Meanwhile, choices other than those mentioned were low-risk jobs. The cut-off chosen residence was Surabaya, where patients from the city of Surabaya were said to live near and outside Surabaya said to be distant. The cut-off time for HIV diagnosis was its mean, which was 4.29. If greater or equal to 4.29 years, it was old patients. While it is new patients if smaller than 4.29 years.

Analysis of the relationship between age, sex, marital status, occupation, residence, history of key population, and duration of HIV diagnosis with stigmatization by health workers in East Java community health centers on patients in Outpatient Care Clinic of Intermediate and Infectious Disease Care Unit (Perawatan Intermediet Penyakit Infeksi - PIPI) provided significant results on the variables of age, marital status, and key population history. Whereas sex, occupation, residence, and duration of HIV diagnosis variables provided insignificant results.

The history of key population had Exp (B) of 0.18, which means PLWHA who have the history of key population get a stigma 0.18 times compared to those without a history of key population. So, it showed a protective factor of stigmatization by health workers. PLWHA who have the history of key population got a lower stigma than PLWHA who did not have. This was because PLWHA who have the history of key population have a psychological mentality that is accustomed to being stigmatized in the community. Pala, Villano, and Clinton[19] explained that HIV stigma is not because someone is HIV-positive but also because of other conditions of social stigmatization, such as having same-sex partners with other people, female sex workers, and her partner/s, and Injecting drug users

(IDUs). Both female sex workers (prostitute) and PLWHA face the same type of stigma, which is seen as "unclean", a danger to public health, and making decisions that are detrimental to their families and communities. For FSW living with HIV, they get these two stigmas. Sex workers living with HIV are regularly exposed to negative stereotypes about themselves and consider them 'worthy' to become HIV positive[20]. Due to the frequent exposure to negative stereotypes from the community, PLWHA's psychological state who have a history of key population is more vulnerable to stigma.

PLWHA who do not have a history of key population, have a different mentality than PLWHA who have a history of key population because they are not accustomed to experiencing stigma from the community. HIV-AIDS brings an unprecedented problem for that person, regardless of background. A person suffering from HIV-AIDS experiences severe psychological distress and feels hopeless about the future, including work, family life, health, and self-esteem[21]. Old age, above 37 years old, gets a higher stigma compared to the age below 37 years old. This is because older adults are at a significant risk of experiencing HIV stigma[22]. Research has shown that older PLWHA may experience greater stigma due to the double stigma of being HIV positive plus age discrimination, which is usually referred to as layering[23]. Emlet has stated that layering or co-occurring stigmas of ageism and HIV stigma had been experienced by about 68% of older HIV positive adults in Washington DC. Internalized stigma has a negative impact on the self-esteem and psychological well-being of older adults living with HIV[24].

PLWHA who were married got lower stigma compared to PLWHA who were not currently married, which was 0.251 times. In this case, the factor of being married is associated with social support. PLWHA who are married has higher social support compared to PLWHA who are single. Research conducted by Emlet explains that social support is associated with lower levels of HIV stigma[25]. A significant relationship had been proven found between the participation of peer groups and the improvement of the quality of life of PLWHA[26,27]. Reducing the impact of stigma and perceived behavior of PLWHA can be done by changing individual and community perceptions about HIV-AIDS by using peer support and counseling approaches[28]. It was also explained that social support affects lower levels of depression and anger[29].

Sex, occupation, residence, and duration of HIV diagnosis variables gave insignificant results related to stigmatization by health workers. Some factors that are thought to cause this result include the research method in the form of interviews so that there could be biased information. The cut-off values that do not have standard rules yet in categorizing continuous variables can affect the relationship and interpretations of the research results. Also, it will randomize the research findings[30,31]. Categorizing variable will make some information loss, so the statistical power to know the relation between variables will be lower[32]. This is well understood because if the threshold for the definition of "exposure" changes, the magnitude of the estimated effect such as the odd ratio (OR), will vary too[30].

CONCLUSIONS

Stigma against people living with HIV-AIDS (PLWHA) by health workers is still often found in the community health center in East Java. The stigma could have a wide impact, so it is necessary to identify the factors that influence the occurrence of stigma, which is expected to reduce stigmatization by health workers. Factors related to PLWHA's perception of stigma among health workers found in this research were the history of key population, age, and marital status. PLWHA who have a history of key population, got a lower stigma than PLWHA who do not have because PLWHA who have a history of key population have a psychological mentality that The score to being stigmatized in the community. Old age got higher stigma compared to the young age, because of having the double stigma of being HIV positive and age discrimination. PLWHA who were married, got lower stigma compared to PLWHA who were not currently married because they have higher social support compared to PLWHA

who are single. It is hoped that the results of this study can provide input to policymakers to initiate a stigma reduction program for people with HIV that can be started from PLWHA who has the highest stigma, to make it easier for PLWHA to disclose their status and treatment. Besides, it is hoped that the prevention of HIV transmission to the community will be more controlled and to help improve the quality of life people living with HIV-AIDS (PLWHA).

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CONFLICT OF INTEREST

There is no conflict of interest of this study.

REFERENCES

1. Maman S, dkk. A Comparison of HIV Stigma and Discrimination in Five International Sites: The Influence of Care and Treatment Resources in High Prevalence Settings. Soc Sci Med. 2009; 2271–8.
2. Paryati T, dkk. Faktor-Faktor yang Mempengaruhi Stigma dan Diskriminasi kepada ODHA (Orang Dengan HIV/AIDS) oleh Tenaga Kesehatan: Kajian Literatur. Pustaka Unpad. 2013;
3. Musthofa SB, Shaluhiyah Z, Widjarnoko B. Stigma Masyarakat terhadap Orang Dengan HIV/AIDS. J Kesehat Masy Nas. 2015; 9(4): 333–9.
4. Komisi Penanggulangan AIDS. Strategi dan Rancana Aksi Nasional 2015-2019 Penanggulangan HIV dan AIDS di Indonesia. 2015.
5. UNAIDS. Fast Track: Ending the AIDS Epidemic by 2030. 2014.
6. Stringer KL, Turan B, McCormick L, Durojaiye M, Nyblade L, Kempf MC, et al. HIV-related Stigma among Health Care Providers in the Deep South. AIDS Behav. 2016; 115–25.
7. Maharani R. Stigma dan Diskriminasi Orang Dengan HIV/AIDS (ODHA) pada Pelayanan Kesehatan di Kota Pekanbaru Tahun 2014. J Kesehat Komunitas. 2014; 2(5): 225–32.
8. Khairiyah R. Peningkatan Self Regard untuk menyikapi Stigma Masyarakat terhadap Orang dengan HIV/AIDS di Yayasan Abdi Asih Surabaya. Diligib UIN Surabaya. 2018;
9. Ardani I, Handani S. Stigma terhadap Orang Dengan HIV/AIDS (ODHA) sebagai Hambatan Pencarian Pengobatan: Studi Kasus pada Pecandu Narkoba Suntik di Jakarta Tahun 2017. Bul Penelit Kesehat. 2017; 45(2): 81–8.
10. Shisana O, Rehle T, Simbayi LC, Zuma K, Jooste S, Zungu N, et al. South African national HIV prevalence, incidence, and behaviour survey, 2012. HSRC Press. 2014;
11. Health Policy Project. Measuring HIV Stigma and Discrimination Among Health Facility Staff: Monitoring Tool for Global Indicators. 2015.
12. Sern TJ. The Knowledge, Perceptions, Attitudes, and Perceived Risk in HIV/AIDS Among Woman in Malaysia: A Cross-Sectional Study. Int J Soc Sci. 2018; 8(9): 725–34.
13. Higgins JA, Hoffman S, Dworkin SL. Rethinking Gender, Heterosexual Men, and Women's Vulnerability to HIV/AIDS. Am J Public. 2011; 435–45.
14. Kementerian Kesehatan Republik Indonesia. Laporan Perkembangan HIV/AIDS 7 Penyakit Menular Seksual (PIMS) Triwulan I Tahun 2017. Faktor-Faktor Risiko Penularan HIV/AIDS pada Laki-Laki dengan Orientasi Seks Heterose. 2017.
15. Kementerian Kesehatan Republik Indonesia. Surveilans Terpadu Biologis dan Perilaku 2011. 2011.
16. Awofala AA, Ogundele OE. REVIEW HIV: Epidemiology in Nigeria. Saudi J Biol Sci King Saudi Univ. 2018; 967–703.
17. Sismulyanto, Supriyanto S, Nursalam. Model to Reduce HIV-related Stigma among Indonesian Nurses. Int J Public Heal Sci. 2015; 4(3): 184–91.
18. Harapan H. SciVerse ScienceDirect Discriminatory Attitudes toward People Living with HIV among Health Care Workers in Aceh, Indonesia: A Visa from a Very Low HIV Caseload Region. CEGH Clin Epidemiol Glob Heal. 2013; 29–36.
19. Pala AN, Villano P, Clinton L. Attitudes of Heterosexual Men and Women Toward HIV Negative and Positive Gay Men. J Homosex. 2017; 64(13): 1778–1792.
20. NSWP. Stigma and Discrimination Experienced by Sex Workers Living with HIV. 2015.
21. Sharma P, Kirmani MN. Psychotherapy in HIV/AIDS. Int J Indian Psychol. 2015; (3): 115.
22. LeBlanc A. Aging with HIV/AIDS. In R. Settersten Jr & J. Angel (Eds.). Handb Social aging. 2011; 495–512.
23. Emlet CA. The Impact of HIV-related Stigma on Older and Younger Adults of AIDS/HIV Care. Psychol Sociomedical Asp AIDS/HIV. 2014;
24. Emlet CA. Understanding the impact of stigma on older adults with HIV. Psychology and AIDS Exchange Newsletter. 2014;
25. Emlet C, Brennan D, Brennenstuhl S, Rueda S, Hart T, Rourke S, et al. Protective and risk factors associated with stigma in a population of older adults living with HIV in Ontario Canada. AIDS Care. 2013; 1330–9.

26. Fajriyah YL, Demartoto A, Murti B. The Eff ect of Depression, Stigma, and Peer Support Group, on the Quality of Life of People Living with HIV/AIDS in Solo Plus Peer Support Group, Surakarta, Central Java. J Heal Promot Behav. 2018; 3(1): 27–36.
27. Kurniasari MA, Murti B, Demartoto A. Association Between Participation in HIV/AIDS Peer Group, Stigma, Discrimination, and Quality of Life of People Living with HIV/AIDS. J Epidemiol Public Heal. 2016; 1(2): 127–34.
28. Vyavaharkar M, Moneyham L, Murdaugh C, Tavakoli A. Factors Associated with Quality of Life among Rural Women with HIV Disease. AIDS Behav. 2012; 16(2): 295–303.
29. Whitehead N, Hearn L, Burrel L. The association between depressive symptoms, anger, and perceived support resources among underserved older HIV positive Black/African American adults. AIDS Patient Care STD's. 2014; 507–12.
30. Heavner K, Burstyn I. A Simulation Study of Categorizing Continous Exposure Variables Measured with Error in Autism Research: Small Changes with Large Eff ects. Int J Environ Res Public Health. 2015; 12: 10198–234.
31. DeCoster J, Gallucci M, Iselin A. Best Practices for Using Median Splits, Artificial Categorization, and their Continous Alternatives. J Exp Psychopathol. 2011; 2(2): 197–209.
32. Gyimesi ML, Vilsmeier JK, Voracek M, Tran US. No Evidence That Lateral Preferences Predict Individual Differences in the Tendency to Update Mental Representations: A Replication-Extension Study. Collabra Psychol. 2019; 5(1): 38.

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Original Article

Relationship Between Level of Serum Adiponectin and Frailty in Elderly Patients with Chronic Obstructive Pulmonary Disease

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ABSTRACT

Elderly are prone to the health effects of chronic obstructive pulmonary disease (COPD). Frailty is a geriatrics syndrome, adiponectin is an adipokine that regulates energy. Adiponectin is affected by age. Increased adiponectin can lead to muscle wasting which will further reduce body mass index (BMI), which indirectly increases the degree of frailty. The relationship between adiponectin with frailty degree in COPD is still unknown. The aims of this study was to investigate the relationship between plasma adiponectin levels and frailty in COPD elders. This was an observational analytic cross-sectional study. All anthropometric parameters, including weight, height, and BMI, were measured. Adiponectin was measured by ELISA methods obtained from venous blood samples. Aged more than or equal to 60 years old, the patients underwent spirometry and the degree of frailty defined by the Fried criteria. Statistic analysis used Rank Spearman. Thirty-eight male COPD patients became the subject of the study. The average age was 70-74 years, with a total of 13 robust, 12 prefrails and 13 frail patients. Level of adiponectin (mean and SD) in robust, prefrail, and frail were 6.84+ 2.66 , 6.58 + 4.27, and 11.62 + 4.90 respectively, p=0.015. Further analysis showed that the level of adiponectin rose progressively with an increasing number of components of frailty. The degree of obstruction mostly with mild (42.1%), and no subjects with very severe. There was an increase in serum adiponectin levels in all subjects. In conclusion, the level of adiponectin serum correlates positively with the degree of frailty.

Keywords: *adiponectin, COPD, frailty*

ABSTRAK

Lansia sangat rentan terhadap efek kesehatan yang merugikan dari penyakit paru obstruktif kronik (PPOK). Frailty adalah sindrom geriatrik yang penting, sedangkan adiponektin adalah adipokin yang mengatur homeostasis energi. Adiponektin dipengaruhi oleh usia. Peningkatan adiponektin dapat menyebabkan pengecilan otot yang selanjutnya akan mengurangi indeks massa tubuh (IMT), yang secara tidak langsung meningkatkan derajat frailty. Hubungan antara adiponektin dengan derajat frailty pada PPOK usia lanjut masih belum diketahui. Tujuan penelitian ini adalah untuk menentukan hubungan antara kadar adiponektin plasma dan frailty pada lansia dengan PPOK. Penelitian ini adalah penelitian cross-sectional analitik observasional. Semua parameter antropometrik, termasuk berat badan, tinggi badan, dan IMT, diukur. Adiponektin diukur pada sampel darah vena dengan metode ELISA. Pasien yang berusia lebih dari atau sama dengan 60 tahun menjalani spirometri dan derajat frailty menurut kriteria Fried. Analisis statistik menggunakan Rank Spearman. Tiga puluh delapan pasien PPOK laki-laki menjadi subjek penelitian. Usia rata-rata adalah 70-74 tahun, dengan total 13 pasien robust, 12 prefrail dan 13 frail. Kadar adiponektin (rerata dan SD) pada kelompok robust, prefrail, dan frail masing-masing adalah 6,84 + 2,66, 6,58 + 4,27, dan 11,62+ 4,90, p=0,015. Analisis lebih lanjut menunjukkan bahwa kadar adiponektin meningkat secara progresif seiring peningkatan jumlah komponen frailty. Derajat obstruksi sebagian besar ringan (42,1%), dan tidak ada subjek dengan obstruksi berat. Terdapat peningkatan

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kadar adiponektin serum pada semua subjek. Sebagai simpulan, kadar serum adiponektin berkorelasi positif dengan derajat frailty.

Kata kunci: *adiponectin, PPOK, frailty*

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INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a typical disease of aging with a prevalence of around 12% in the age group >64 years.[1,2,3,4] In elderly who suff er from COPD, the process of this disease can also increase the level of adiponectin through inspiratory muscle mechanism that is exercised continuously (chronic exercise), thereby increasing the REE (resting energy expenditure). As a result, an increase in fatty tissue activity will release adipokine and cause an increase in plasma adiponectin levels.[5,6,7,8]

Increased severity and shortness of breath result in the inactivity of COPD patients, which in turn results in loss of muscle strength, leading to mobility problems, which contribute to the high frequency of frailty in those patients.[9,10,11] Underweight patients have an increase in REE compared to overweight and normal-weight patients, which is associated with decreased serum and adipose tissue leptin. Increased serum adiponectin also occurs, demonstrating the role of adipokines in cachexia-related energy imbalances in COPD.[12,13,14,15,16]

This study was conducted to identify the relationship between serum adiponectin levels and the degree of frailty measured using the Cardiovascular Health Study (CHS) scoring system[17], a scoring system that is most widely used and has the broadest validity to determine the degree of frailty in the population of COPD elderly patients in Surabaya.

MATERIALS AND METHODS

This study was a cross-sectional analytic study to analyze diff erences in serum adiponectin levels between degrees of frailty in elderly COPD patients. This study was conducted at the Pulmonary and Geriatric Outpatient Unit, dr. Soetomo Hospital, Surabaya, Indonesia. The study samples were subjects aged ≥ 60 years at the Outpatient Unit, dr. Soetomo Hospital, Surabaya, who fulfilled the inclusion criteria, ie aged over or equal to 60 years old, a Mini Mental State Examination (MMSE) score of ≥ 18, and was willing to follow the study by signing informed consent and information for consent. Criteria for the exclusion of the subjects were in acute exacerbations, had a history of diabetes mellitus, had a malignancy or history of malignancy, and had a history of stroke with limited motor function.

Measurement of Serum Adiponectin

Adiponectin is a 30 kDa glycoprotein that is secreted primarily by adipocytes and induces wide ranging paracrine and endocrine eff ects on metabolism and inflammation. Adiponectin circulates in the blood with a high concentration as total adiponectin[18].

Adiponectin measurement in this study used a quantitative ELISA method from venous blood samples in μg / ml units. Blood samples were taken as much as 5 ml and put into Vacuette Z Serum Sep Clot Activator tubes and store inside the cooler box with a temperature of 2–4° C, to be processed and separated the serum part in less than 24 hours by centrifugation. The total adiponectin was measured using a commercial tool kit Sekisui Medical Co., Ltd. The normal value of adiponectin serum was a range between 2.54–6.06 μg / mL. Type of data is a ratio data.

Samples were taken by consecutive sampling. A total of 38 samples were obtained[19]. All data were entered into the computer through the statistical program R version 3.1.2. Data on general

characteristics of the samples according to age, sex, level of education, degree of COPD, smoking history, and comorbid history were presented descriptively in tabular form. Subjects' specific characteristics data including body mass index, MMSE score, handgrip strength, 15 feet walking test, and PASE scores are presented in tables and graphs. Types of data were ordinal (categorical) data for frailty degrees and ratio (numeric) data for serum adiponectin levels, so we used One-Way Anova test if the parametric statistical test requirements were met, or the Kruskal-Wallis test if the parametric statistical test requirements were not met. Subanalysis was conducted to determine the relationship of serum adiponectin levels with Fried's five frailty components.

RESULTS AND DISCUSSION General

Characteristics of the Subjects

The number of subjects in this study were 38 COPD patients in the Pulmonary and Geriatric Outpatient Unit, Dr. Soetomo Hospital, Surabaya, Indonesia, who had fulfilled the inclusion and exclusion criteria. Table 1 shows the general characteristics of the study subjects.

Most subjects were found in the 70-74 years age range. The mean age of the subjects in robust group was 69.69 ± 7.85 years, in prefrail group 70.50 ± 6.85 years, and in frailty group 70.85 ± 8.19 years. All of the subjects (100%) were male.

The degree of COPD obstruction used in this study was based on the 2014 GOLD criteria which divided into 4 groups, mild (GOLD 1), moderate (GOLD 2), severe (GOLD 3) and very severe (GOLD 4) obstruction[20]. We obtained mostly COPD patients with mild obstruction degrees as many as 16 (42.1%) patients, and no subjects with COPD had very severe obstruction degrees.

Increased serum adiponectin level was found in COPD patients with severe obstruction. However, the comparative test did not show differences in adiponectin levels in various degrees of COPD obstruction. Table 3 shows that under frail conditions serum adiponectin levels increase. The comparative test showed differences in serum adiponectin levels between degrees of frailty with p=0.015 (p <0.05). Further post-hoc analysis showed significant differences in serum adiponectin levels between frail and prefrail patients, and between robust and frail patients. Furthermore, analysis with Spearman's correlation between serum adiponectin levels and

Table 1. General characteristics of the subjects

Characteristics	Total
Age. year (Mean ± SD)	(70.26 ± 7.52)
(Min-Max)	(60 - 84)
Education. n (%)	
No formal education	3 (7.9%)
Elementary	12 (31.6%)
Junior Secondary	7 (18.4%)
Senior Seconday	15 (39.5%)
High Education	1 (2.6%)
Nutritional Status (BMI)	
Low (BMI<18.5)	10 (26.3%)
Normal (BMI 18.5-25.0)	21 (55.3%)
High (BMI>25.0)	7 (18.4%)
Smoking History	
Yes	38 (100%)
No	0
Comorbidities	
Hypertension	4 (10.5%)
Heart disease	1 (2.6%)
Renal disease	0
Liver disease	0
Degree of COPD obstruction	
Mild	16 (42.1%)
Moderate	14 (36.8%)
Severe	8 (21.1%)
Very Severe	0

Table 2. Particular characteristics of the subjects

Frailty components	Frequency	Percent
Fatigue (CESD)		
Yes	23	60.5
No	15	39.5
Weight loss		
Yes	11	28.9
No	27	71.1
PASE		
Yes	10	26.3
No	28	73.7
Slowness (*Walking*)		
Yes	11	28.9
No	27	71.1
Muscular weakness (*Handgrip*)		
Yes	0	0.0
No	38	100.0

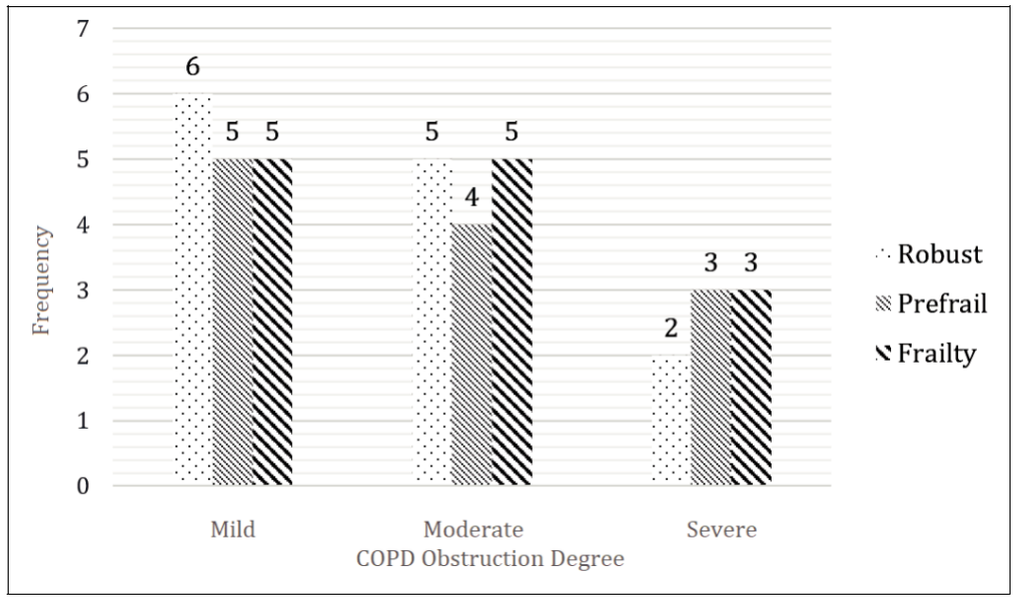

Figure 1. Relationship between the degree of COPD obstruction and frailty frequency.

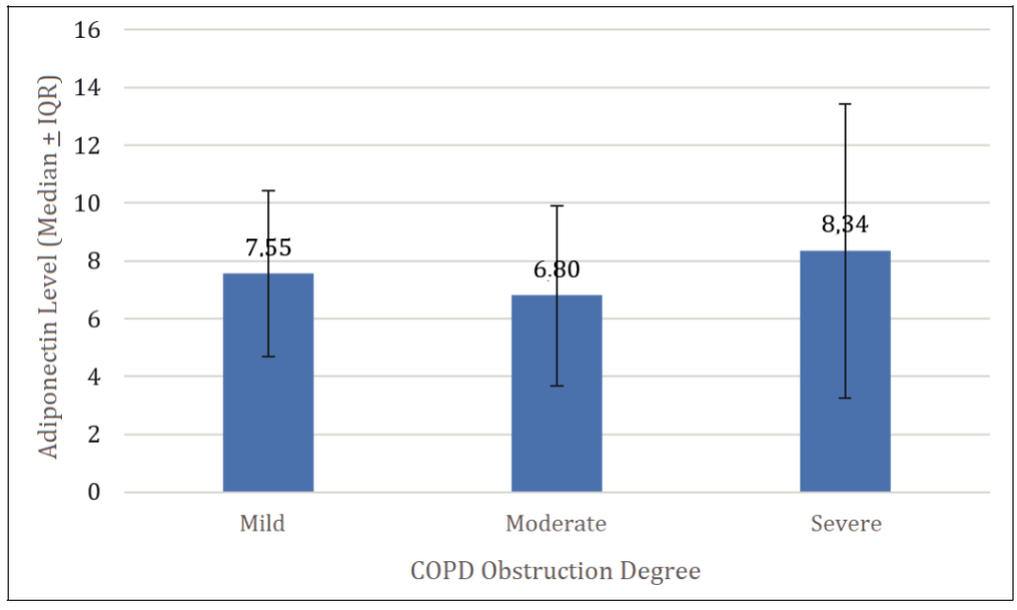

Figure 2. Relationship between the degree of COPD obstruction and adiponectin levels

Table 3. Adiponectin levels at various frailty levels

Degree of Frailty	n	Adiponectin Level					p
		Mean	SD	Median	Minimum	Maximum	
Robust	13	6.84	2.66	5.94[a]	3.68	11.59	
Prefrail	12	6.58	4.27	5.30[a]	2.70	18.31	0.015*
Frail	13	11.62	4.90	11.36[b]	2.97	17.56	

frailty degrees showed Spearman's correlation coefficient rs=0.368 with p=0.023 (p <0.05), showing the relationship between serum adiponectin levels and frailty degrees. The analysis showed that the higher the degree of frailty, the higher the adiponectin level.

Characteristics of the Subjects

This study was conducted to determine the relationship between serum adiponectin levels and degrees of frailty in COPD patients from mild (GOLD 1) to very severe (GOLD 4) obstruction non-exacerbations with age limited to ≥ 60 years. In this study, the mean age was 70.26 + 7.52 years with an age range between 60-84 years. According to Fried frailty phenotype/CHS system as many as 13 (34.2%) of the total 38 samples included in the robust group, 12 (31.6%) in the prefrail group, and 13 (34.2%) in the frail group. Based on Fried's phenotype criteria and their various modified versions, the prevalence of frailty in adult populations aged 65 years or older in the United States ranges from 7% to 12% and increases according to the age group of 3.9% in 65 to 74 the age group, and increased to reach 25% in age group above 85 years.[21] This is similar to the findings in this study, that the robust group was found in the age range of 60-69 years while most of the frail group were over the age of 70 years.

Subjects in this study were all male, although the authors did not limit only one sex. In this study, 21 patients (55.3%) had normal BMI, 10 patients (26.3%) with low BMI, and 7 patients (18.4%) with high BMI. A study conducted by Vestbo et al in 2008 also reported that 96.9% of the COPD population had a normal or high BMI. We also find similar findings. A population-based epidemiological study conducted by de Oca who examined BMI in COPD patients conducted in 5 cities in Latin America showed that most Asian ethnicities had normal BMI, compared with less and more BMI.

In this study the most comorbidity was hypertension, which was as much as 10.5%, followed by heart disease of 2.6%. Other comorbidities such as diabetes mellitus were excluded in this study because diabetes mellitus can affect the results of adiponectin levels. In diabetes mellitus the level of adiponectin is low. In this study, various degrees of frailty were found in various degrees of COPD, it was apparent that that prefrail and frail conditions were more common in COPD subjects (Table 2)

Determining the Degree of Frailty in Elderly COPD Patients

In this study, COPD subjects were obtained with various degrees of frailty, both in COPD with mild, moderate and severe obstruction. This shows that the higher the degree of obstruction, the higher the increase of prefrail and frail conditions. In a study conducted by Lahouse in 2014 on the risk of frailty in elderly, as many as 28.8% of COPD patients were found to be frail, 16.4% prefrail and 14.1% robust.[22] This was different from this study's finding, where frail and robust had the same prevalence. This could be caused by age. The robust patients were mostly in the age range of 60 years while the frail ones were mostly in the age range of 70 years. In a study conducted by Lahouse, the average age was 70 years. If the degree of obstruction was categorized based on GOLD classification, out of 402 COPD subjects, patients with mild obstruction were 200 subjects (49.8%), moderate obstruction 174 subjects (43.3%) and severe obstruction 28 subjects (7,0%). In this study, the prevalence of frailty was strongly related to the severity of COPD, according to the degree of obstruction based on GOLD classification. The higher the degree of COPD obstruction, the frailer condition obtained, as compared to robust and prefrail conditions.[22]

Measuring Adiponectin Level in Elderly COPD Patients

This study found elevated levels of adiponectin in COPD patients, with a median of 7.55 μg/ ml in mild obstruction, 6.80 μg/ml in moderate obstruction, and 8.34 μg/ml in severe obstruction. The highest increase was found in COPD with severe obstruction. Chan, who examined serum levels of adiponectin in COPD patients in 2010, found that COPD subjects who smoked had significantly higher levels of adiponectin, IL-6 and CRP than healthy smokers and nonsmokers. This study found that the higher the degree of COPD, the higher the serum adiponectin level. Serum adiponectin, IL-6 and CRP levels were negatively correlated with FEV1 (% predicted) in COPD patients and healthy smokers.[23] Similar

to this study and Chan's study, Tomoda et al. examined the levels of adiponectin in COPD with low and normal body weight, also found increased levels of adiponectin in COPD subjects.[6]

Relationship between Serum Adiponectin Levels and Degree of Frailty in Elderly COPD Patients

In this study, the median serum adiponectin levels in the robust, prefrail, and frailty groups were 5.9 μg/ml (3.68-11.59), 2.70 μg/ml (2.70-18,31), and 11.36 μg/ml (2.97-17.56) respectively. These results indicated that higher serum adiponectin levels are found at a higher degree of frailty. This study also found differences in adiponectin level between degrees of frailty and, in addition, also found a relationship between levels of adiponectin with degrees of frailty with Spearman's correlation coefficient of 0.368 and p=0.023 (p <0.05), showing a relationship between adiponectin levels and the degree of frailty. The analysis showed that the higher the level of adiponectin, the higher the degree of frailty.

In a study conducted by Tsai, who examined the relationship between adiponectin levels and frailty components, 168 subjects were found to be 65-90 years old, and 83 (49.4%) were male. Serum adiponectin levels differed significantly between the three subgroups (p=0.012). The results of the study showed that plasma adiponectin levels were positively related to an increase in frailty components in older men.[10] In contrast to our study, the subjects in Tsai's study were elderly (>60 years), and Tsai's study as well as this study showed an increase in adiponectin levels. This indicates that in the elderly the adiponectin level is increasing.

This study did not find female respondents because female COPD sufferers were rarely found. However, the data in this study, as those of Tsai's and Huang's findings showed that sex was an important factor that could have affected not only blood adiponectin levels, but also the severity of frailty.[24,25]

CONCLUSIONS

Serum adiponectin level in all subjects was found to increase with median in robust, prefrail, and frailty groups. The highest increase was found in severe degree COPD. A weak positive relationship was found between adiponectin level and the degree of frailty.

REFERENCES

1. Kirkwood TB, 2005. Understanding the odd science of aging. *Cell;* 120: 437–447.
2. Halbert RJ, Natoli JL, Gano A, Badamgarav E, Buist AS & Mannino DM, 2006. Global burden of COPD: systematic review and meta-analysis. *European Respiratory Journal*; 28: 523–532.
3. Incalzi R, Scarlata S , Pennazza G, Santonico M & Pedon C, 2014. Chronic obstructive pulmonary disease in the elderly. European Journal of Internal Medicine; 25: 320–328.
4. Kobayashi S, Yanai M, Hanagama M & Yamanda S, 2014. The burden of chronic obstructive pulmonary disease in the elderly population. Respiratory Investigation; 52: 296–301.
5. Fantuzzi G,2005. Adipose tissue, adipokines, and inflammation. *Journal Allergy Clinical Immunology*; 115: 911–919.
6. Tomoda K, Yoshikawa M,Takefumi Itoh T, Tamaki S, Fukuoka A Komeda K & Kimura H, 2007. Elevated circulating plasma adiponectin in underweight patients with COPD. CHEST; 132: 135–140.
7. Oraby SS, Ahmed ES, Farag TS, Zayed AE & Ali NK, 2014. Adiponectin as inflammatory biomarker of chronic obstructive pulmonary disease. *Egyptian Journal of Chest Disease and Tuberculosis*: 1–5.
8. Iwabu M, Okada-Iwabu M, Yamauchi T & Kadowaki T, 2015. Adiponectin/adiponectin receptor in disease and aging. *npj Aging and Mechanism of Disease; 1–4.*
9. Park SK, Richardson CR, Holleman RG & Larson JL, 2013. Frailty in people with copd, using the national health and nutrition evaluation survey dataset (2003– 2006). Heart & Lung: The Journal of Acute and Critical Care; 42: 163–170.
10. Maddocks M, Kon SSC, Caravan JL, Jones SE, Nolan CM, Labey A, Polkey IM & Man WD, 2016. Physical frailty and pulmonary rehabilitation in COPD: a prospective cohort study. *Thorax*; 1–8
11. Mittal N, Raj R, Ebtesam Ataya Islam EA & Nugent K, 2015. The Frequency of frailty in ambulatory patients with chronic lung diseases. Journal of Primary Care & Community Health; 7(1): 10–15.

12. de Oca MM, T'alamo C, Perez-Padilla R, B. Jardim JR, Muino A, Lopez MV, Valdivia G, Pertuze J, Moreno D, J. Halbert R & B. Menezes AM, For the PLATINO Team, 2008. Chronic obstruc tive pulmonary disease and body mass index in five Latin America cities: The PLATINO study. *Respiratory Medicine*: 642–650.
13. Brusik M, Ukropec J, Joppa P, Ukropcova B, Skyba P, Balaz M, Pobeha P, Kurdiova T, Klimes I, Tkac I, Gasperikova D & Tkacova R, 2012. Circulatory and adipose tissue leptin and adiponectin in relationship to resting energy expenditure in patients with chronic obstructive pulmonary disease. Physiological research: 469–480.
14. Breyer MK, Rutten EPA, Locantore NW, Watkins ML, Miller BE & Wouters EFM, 2012. Dysregulated adipokine metabolism in chronic obstructive pulmonary disease. *European Journal Clinical Investigation; 42(9): 983–91*.
15. Mohamed NA, Fawzy MA, Reda Elgamry R , Gad DM & Ibraheem HA, 2013. Role of adiponectin and other inflammatory biomarkers in COPD patients. *Egyptian Journal of Chest Diseases and Tuberculosis*; 62: 45–50.
16. Omar MM, Isa HA, Abdelsadek A & Abd-Elhamid MA, 2014. Serum adiponectin level in obese and non-obese COPD patients during acute exacerbation and stable conditions. *Egyptian Journal of Chest, Diseases and Tuberculosis; 63*: 313–319.
17. Rockwood K, Song X, McKnight C, Bergman H, Hogan DB & Mc Dowell I, 2005. A global clinical measure of fitness and frailty in elderly people. *Canadian Medical Association Journal*; 173: 489–495.
18. Wang ZV, Scherer PE, 2016. Adiponectin, the past two decades. J Mol Cell Biol. Apr; 8(2): 93–100. doi: 10.1093/jmcb/mjw011. Epub 2016 Mar 18.
19. Global Initiative for Chronic Obstructive Lung Disease (GOLD), 2014. Chapter 2. Diagnosis and assessment. in: *global strategy for the diagnosis, management, and prevention of chronic obstructive ling disease updated 2014*. 2014 Global Initiative for Chronic Obstructive Lung Disease Inc.
20. Fried LP, Ferrucci L, Darer J, Williamson JD & Anderson G, 2004. Untangling the concepts of disability, frailty, and comorbidity: implications for improved targeting and care. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences; 59A: 255–263.
21. Lahousse L, Maes B, Ziere G, Loth DW, Verlinden VJA, Zillikens MC, Uitterlinden AG, Rivadeneira F, Tiemeier H, Franco OH, Ikram MA, Hofman A, Brusselle GG, Stricker BH, 2014. Adverse outcomes of frailty in the elderly: the Rotterdam Study. European Journal of Epidemiology; 29(6): 419–427.
22. Chan KH, Yeung SC, Yao TJ, Ip MS, Cheung AH, Chan-Yeung AH, Mak JC, and the COPD Study Group of the Hongkong Thoracic Society, 2010. Elevated plasma adiponectin levels in patients with chronic obstructive pulmonary disease. International Journal Of Tuberculosis And Lung Disease; 14: 1193–1200.
23. Tsai JS, Wu CH, Chen SC, Huang KC, Chen CY, Chang CI, Chuang LM & Chen CY. 2013. Plasma adiponectin levels correlate positively with an increasing number of components of frailty in male elders. Plos one; 1–8.
24. Huang C, Niu K & Momma H, 2014. Inverse association between circulating adiponectin levels and skeletal muscle strength in Japanese men and women. Nutrition, Metabolism & Cardiovascular Diseases; 24: 42–49.
25. Huang C, Momma H & Niu K,2016. High serum adiponectin levels product incident falls among middle-aged and older adults: a prospective cohort study. Age and ageing; 45: 366–371.

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Original Article

Association Between Sepsis Risk Calculator and Infection Parameters for Neonates with Risk of Early-Onset Sepsis

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ABSTRACT

C-reactive protein (CRP) is an acute-phase reactant protein that is primarily induced by the IL-6 action during the acute phase of an inflammatory or infectious process. The bacterial infection is a potent stimulus, leading to a rapid elevation of CRP levels within hours while the CBC and symptom are often misleading and/or absent. American Academy of pediatrics (AAP) is recommended routine blood examination test Complete Blood Count (CBC), C-reactive protein (CRP), and blood culture along with empirical antibiotic in neonates with early onset sepsis risk (EOS) risk even asymptomatic. The previous study is showed there were no correlation of CRP and EOS risk. This study aims to evaluate the CRP and CBC profile in neonate with risk of EOS. Methods of this study are using the sepsis risk calculator (SRC) to calculate the probability of neonatal early ons5et sepsis (EOS) based on maternal risk and infant's clinical presentation. Neonates with ≥34 weeks of gestation who were started on antibiotic treatment after laboratory examination and blood culture were taken. EOS risk estimation were compared including CRP, leukocyte, and thrombocyte count. ANOVA applied to distinguished laboratory examination between stratified risk groups. The result is showed using 82 subjects who met the inclusion and exclusion criterias, The EOS risk level was stratified into green, yellow, and red group. The p-value of CRP level, platelets, white blood cells were 0.35,0.54 and 0.48 where p-value was considered as significant if < 0.05. The conclusion of this study is there were no correlation of CRP level and EOS risk

Keywords: *Sepsis risk calculator, infection parameter, risk of early onset sepsis, C-reactive protein, Complete Blood Count*

ABSTRAK

C-reactive protein (CRP) adalah suatu reaksi fase akut protein yang diinduksi oleh aktivasi dari IL-6 selama fase akut dari inflamasi atau proses infeksi. CRP adalah sebuah indikator yang penting pada pasien dengan risiko sepsis. Infeksi bakterial adalah suatu stimulus yang berpotensi meningkatkan kadar CRP dalam beberapa jam dimana darah lengkap dan klinis pasien seringkali tidak berubah secara signifikan. American Academy of paediatrics (AAP) merekomendasikan pemeriksaan darah rutin antara lain darah lengkap, CRP dan kultur darah bersamaan dengan pemberian antibiotik namun penelitian sebelumnya menemukan bahwa tidak didapatkan hubungan antara kadar CRP dengan risiko sepsis. Tujuan dari penelitian ini adalah untuk mengevaluasi kadar CRP dan darah lengkap pada bayi baru lahir dengan risiko sepsis awitan dini. Metode yang digunakan pada penelitian ini dengan menggunakan sepsis risk calculator (SRC) untuk menghitung probabilitas risiko sepsis awitan dini berdasarkan risiko ibu dan klinis pasien. Bayi baru lahir dengan risiko sepsis awitan dini dengan usia gestasi ≥34 minggu dilakukan pengambilan darah lengkap, kultur darah dan CRP sebelum pemberian antibiotic. Laboratorium yang dibandingkan diantara ketiga kelompok risiko sepsis termasuk CRP, leukosit, dan jumlah trombosit. ANOVA diterapkan untuk menilai perbedaan antara kelompok risiko. Hasil dari penelitian ini yang melibatkan 82 subjek yang memenuhi kriteria inklusi dan eksklusi, Kelompok berdasarkan rekomendasi SRC dikelompokkan menjadi kelompok hijau, kuning, dan merah. Nilai p dari CRP, trombosit, sel darah putih adalah

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0,35,0,54 dan 0,48 di mana nilai p dianggap signifikan jika <0,05. Kesimpulan dari penelitian ini adalah tidak didapatkan hubungan antara risiko sepsis awitan dini dan CRP.

Kata kunci: *sepsis risk calculator, parameter infeksi, risiko sepsis awitan dini, C-reactive protein, darah lengkap*

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INTRODUCTION

Early onset sepsis (EOS) can be related to microorganisms obtained from the mother where pathogenic colonization occurs in the perinatal period. With rupture of the amniotic membrane, microorganisms in the vaginal flora or other pathogenic bacteria can reach the amniotic fluid and fetus.[1] Increasing risk of early onset of sepsis is in line with increasing of maternal temperature (≥ 37.5°C), rupture duration of the membranes (≥ 18 hours) along with gestational age (less than 34 weeks and more than 40 weeks of gestation) and also low birth weight.[2] American Academy of Pediatrics (AAP) recommends neonates from chorioamnionitis mother, to take laboratory examination and received antibiotic treatment even if the baby is asymptomatic.[3] This CBC counts and C-reactive proteins (CRPs) recommendation can be used as guidance of antibiotic treatment decisions in well-appearing infants, and the potential utility of clinical examination to identify EOS in at-risk infants.[4]

The use of antibiotics may cause several complications, longer length of stay on NICU, several pain procedures, lower rate of breastfeeding, changes of intestinal microbes, necrotizing enterocolitis and antibiotic resistance.[5]

Sepsis Risk Calculator (SRC) is the interactive calculator produces the probability of Early Onset Sepsis per 1000 babies by entering values for the specified maternal risk factors along with the infant's clinical presentation.[6] SRC can be calculated in an infant born ≥ 34 weeks gestation. After entering the clinical presentation (well-appearing, equivocal, and clinical illness), SRC recommendation were assessed and considered in each group (green, yellow and red). The red group is the most vulnerable to suff er or have higher probability of EOS.

Sepsis Risk Calculator (SRC) is originally introduced by Kaiser Permanente, and a validated tool which has been used and studied in many countries in predicting EOS.[7-8] Kerste *et al* on 2016 study the implementation of SRC, there were reduced of antibiotics used 50%.[9] Even the SRC was promising tools, the comparison between each group has not been evaluated yet.

The aim of this study is to evaluate the result of SRC on Complete Blood count and CRP level in neonates with Early Onset of Sepsis.

MATERIALS AND METHODS

The study was approved by the Ethical Committee in Health Research of Dr. Soetomo Academic-Teaching Hospital Surabaya (625/ Panke.KKE/x/2017). This observational study with the cross-sectional design was conducted in NICU Dr. Soetomo Academic-Teaching Hospital from November 2017 until April 2018, on newborns with gestational age ≥ 34 weeks who had EOS risks and were born in this hospital within the study period. The subject was selected using a consecutive total sampling method and sample size was determined using a prospective-cohort calculation. Routine laboratory examination comprising of CBC and CRP was performed in all subjects. Blood culture was only obtained in 42 subjects. The inclusion criteria of this study were newborns who had gestational age≥ 34 weeks, EOS risks, appropriate gestational age (AGA). Subject are excluded if any of major congenital abnormality.

Neonatal Sepsis Risk Calculator: SRC can be accessed through *https://neonatalsepsiscalculator.kaiserpermanente.org/* website or smartphone. The required information in SRC application are the incidence of EOS was set as at 0.5/1000 live births according to CDC national incidence. Group B streptococcus (GBS) status was set as unknown because GBS status was not routinely assessed in Dr. Soetomo Academic-Teaching Hospital Surabaya. The score will be shown as personal risk stratification of EOS for each newborn according to the clinical presentation (well-appearing, equivocal, and clinical illness) and EOS risk level (green, yellow, dan red). With the SRC method, the baby will be grouped based on three groups, namely the green, yellow and red groups. Where the green group is the group that does not need blood tests or antibiotics. In the yellow group, patients are recommended to do a blood culture examination without empirical antibiotics and it is recommended to monitor vital signs in the NICU. In patients who enter the red group, empirical antibiotics are recommended to be given immediately

Blood Culture: As blood culture is a gold standard of bacteremia we also observed the characteristic of the patient and the result of CBC and CRP between SRC group. The blood will be obtained through a peripheral vein (equal to 1 cc) as the gold standard diagnosis of EOS. BacT system was used as the microbial culture method and transferred into the Mullerhinton agar to check antimicrobial susceptibility (AST) in Vitex 2 Compact.

Abnormal leukocytes: Leukocyte abnormality values are less or more than normal values. Less if <5,000 / mm3 and more if> 34,000 / mm3 in infants aged 0 days - 1 week. Blood counts measurement is using CELLPACK DCL from Sysmex. Blood count were taken before antibiotic admission, in the first 12 hours of life.

C-Reactive protein (CRP) is expressed in units of mg / L. Normal CRP value is <10 mg / L and abnormal if more than 10 mg/L. Measurement of CRP using Flex® cartridge from Sysmex. CRP were taken before antibiotic admission, in the first 12 hours of life.

Statistics

Data were analyzed using SPSS (Statistical Package for the Social Sciences). The value was presented as the mean ± standard deviation (SD). Normality test was tested using Kolmogorov-Smirnov test. If the data distribution was normal, T-paired test would be used and Wilcoxon test would be performed if the distribution was not normal. Chi-Square test was utilized to assess the homogenity of the subjects according to the demographic characteristic and laboratory examination.

RESULTS AND DISCUSSION

The population of this study is infants who had the risk of early onset sepsis (born to mothers who had a history of premature rupture of membranes for more than 18 hours, mothers with chorioamnionitis and had indications for intrapartum prophylactic antibiotics but inadequate). There were 82 patients were included in this study but only 42 patients that have blood culture results. Characteristics of the subject that have blood culture were described in Table 1. An inadequate intrapartum antibiotic is the most cause of risk of Early Onset of Sepsis.

Inadequate intrapartum antibiotics are the higher percentage of EOS risk in this study population. Gestational age, maternal highest temperature, and PROM have a nonlinear correlation with EOS risk. [10]Previous study is

Table 1. Characteristics of the Subject

Characteristics (n=42)	
Maternal	
Chorioamnionitis n (%)	4 (9.5%)
Rupture of the membrane ≥ 18 Hours n (%)	22 (53%)
Inadequate intrapartum antibiotic n (%)	26 (62%)
Infant	
Mean gestational age, (week)	36.7 ± 2.2
Mean Body weight, gram	2523 ± 566.3
Mean heart rate (time/minute)	150 ± 155
Mean Respiratory Rate (time/minute)	46 ± 47.6
Oxygen Support (Mechanical ventilation)	4

* Data are in number and percentage. This is the characteristic of 42 patients, the most EOS risk in this study was an inadequate intrapartum antibiotic. Four patients with needed oxygen support more than room oxygen.

showed that an adequate antibiotic which used by mothers with premature rupture of membranes will reduce the risk of infection in neonates with *Relative Risk* [RR] = 0.67, 95% CI 0.52–0.85) [11] An inadequate antibiotic in patient with PROM will increase the risk of EOS with OR 37.0 (95% CI 9.7–140.9). The mean of gestational age on the population are below than 37 weeks, this event increases the incident of Early Onset Of Sepsis with incidence 3.0 cases per 1000 birth life.[12]

Sepsis risk calculator recommends the management of the patient with EOS risk according to clinical presentation such as vital sign (tachycardia, tachypnea, and abnormality of body temperature), usage of mechanical ventilation used and vasoactive drugs. In this study, vital signs on the red group had abnormal mean Heart Rate (166.4(6.2)) and Respiratory Rate 64.3(4.38).

CBC and CRP Analysis between SRC Groups were described in Table 2. The laboratories were Complete Blood Count (CBC) values and CRP in 82 patients, where all blood samples were taken 8 hours after birth 1 time and repeated if the clinical deterioration has occurred. In this study, there were no significant differences as a statistic between the three groups of both CBC and CRP values with mean values still in the normal range. Similar to the previous study, Acthen et al 2017 found EOS risk was not correlated with changes in infection parameters. They found negative correlations between both EOS risk, CRP level and leukocyte count within 6 h of the start of antibiotics, as well as CRP level between 6 and 24 h after start of treatment.[13] CRP production is a non-specific response to disease and cannot be used alone as a diagnostic test for septicaemia. The sensitivity and specificity of CRP (at 72 hours of admission) in diagnosis of acute neonatal sepsis were 76.92% and 53.49% respectively while it had a positive predictive value of 80% and negative predictive value of 48.94%. Over all the diagnostic accuracy of CRP in diagnosis of neonatal sepsis was 70.07%.[14]

Patient with positive blood culture's characteristics, and laboratory results were described in Table 3. This study is found that two patients with positive blood culture have a normal level of CRP and one patient on the green group have abnormal CRP level. Contradiction with this result, A study in India (2016) have found the abnormality of CRP in 92.95% of positive culture cases. There is also a statistically significant relationship between positive blood cultures and CRP. The CRP test is positive at 64.34% of early onset sepsis and 35.66% of late onset sepsis.[15]

In the study by Carola et al 2017, the management recommendations based on the EOS calculator after clinical evaluation are presented including the 5 neonates with culture-proven sepsis and 142 neonates with culture-negative sepsis who were treated with antibiotics for ≥7 days. Empiric antibiotics would have been recommended in 23.5% of the neonates in the Cohort. Blood culture only was recommended for 8.9% of the neonates. No empiric antibiotics or laboratory evaluations were recommended for the remaining 66.7%. In that cohort, 142 neonates were treated with prolonged antibiotics (7 days or more) for suspected culture-negative sepsis.

Table 2. CBC and CRP Analysis between SRC Groups

Laboratory results	Groups			
	Green	Yellow	Red	p
Complete Blood Count (mean ± SD)				
Haemoglobin	16.7 (2.28)	15.8 (2.37)	15.49 (1.85)	0.19
White Blood Cell	18747 (6472)	15646 (4712)	14817 (7331)	0.54
Platelet	242043 (59622.7)	252875 (70656)	250909 (87464)	0.48
C –Reactive protein (mg/L)	2.73(8.6)	0.45(0.56)	8.36 (31.75)	0.35

* Data are in number and P values are the results of ANOVA. Patients on the red group had higher CRP level than green and yellow group.

Table 3. Patient with Positive Blood Culture Characteristics and Laboratory Results

Initial/Culture result	SRC Groups	BW/GA	Hb	WBC	PLT	CRP
N.S/*Micrococcus Luteus*	Green	2600/37	21.5	24370	360000	0.66
M/*Acinetobacterlwofii*	Green	2600/38	16	11680	190000	13.68
N.F/*Aerococcusviridans*	Red	3600/41	16.2	23030	296000	2.56

* BW = Body weight, GA = Gestational Age, Hb = haemoglobin, WBC = white blood cell, PLT = platelet, CRP = C-reactive protein.

All 5 neonates with positive blood cultures had abnormal CBC and CRP values.[8]

The sensitivity and the specificity of each CRP was 92.96% and 50.39%.[16] C-reactive protein has the best predictive value when measured within 24 to 48 hours after infection. In healthy individuals, the CRP level is generally below 2 mg/L but can be up to 10 mg/L. There may be slight variations with age, sex, and race. It has a half-life of approximately 19 hours, begins to rise after 12–24 hours, and peaks within 2–3 days. Normal CRP values at two examinations (8 to 24 hours after birth and 24 hours later) were shown to have 99.7% negative predictive values and negative likelihood ratios of 0.15 which were proven to be sepsis.[17]

In the diagnosis of early-onset sepsis, previous studies are reported on widely diff ering sensitivities and specificities of CRP ranging from 29 to 100% and from 6 to 100%, respectively. The delayed induction of the hepatic synthesis of CRP during the inflammatory response to infection lowers its sensitivity during the early phases of sepsis.[18]

From the results of Complete Blood Count results, there were no significant differences between the three groups and in patients with positive blood cultures only one in three patients had a positive CRP score. Total white blood cells have a low Positive Predictive Value (PPV) for sepsis while platelet counts are insensitive or specific for the diagnosis of sepsis and are not very helpful for monitoring response to therapy.[19]

The blood culture results of patients belong to green group, positive culture was found in 2 patients (*Micrococcus Luteus and Acinetobacter lwofii*), while in red group, 1 patient had positive blood culture (Multistrain resistant *Aerococcus viridans)*. All patients with positive blood culture, had risk factor of meconial amniotic fluid with inadequate antibiotics treatment. Meconial amniotic fluid could be sign of chorioamnionitis, which may enhanced the growth of bacteria in amniotic fluid and caused both maternal and neonatal infections.[20]

Among 42 patients there were 3 patients with positive blood cultures (7.5%). The results of blood cultures obtained were *Micrococcus luteus* (1), *Acinetobacter lwofii* (1), *Aerococcus viridans* (1) which had more than one class of antibiotic resistance. Blood culture is the gold standard for the diagnosis of sepsis, and when the adequate volume is obtained, culture has excellent sensitivity even when the baby has a very low level of bacteremia. However, many culture results were found to be negative especially when the baby appeared ill or antibiotics were received before culture was obtained. Based on the recommendation at least 1 mL of blood, either in 1 or divided into 2 0.5 mL cultures, obtained from infants with suspected sepsis before initiation of antibiotic therapy. However, sampling is limited by blood volume, especially in very low birth weight babies, who are at the highest risk for sepsis but have the lowest total blood volume. However, the sensitivity of blood culture decreased by 10% to 40% when 0.5 mL was inoculated compared to 1 mL. Therefore, adequate volume for culture must be ensured.[21] The sensitivity of blood culture is almost 100% when 1 mL is inoculated and the baby has bacteremia concentration of at least 4 colony-forming units (CFU) per milliliter. The optimal time for culture taking in bacteremic conditions is as soon as possible in fever episodes based on heat followed by bacteremia or endotoxaemia in one or two hours. In newborns often have a shorter threshold for the commencement of antibiotics,

which results in low opportunities for isolated organisms in blood culture. This coincides with the low specificity of signs of sepsis in newborns compared to children and adults that contribute to a low positive rate in blood culture.[22-23]

Two patients had gram-positive blood cultures and one patient with gram-negative on this study has normal blood count and have an inadequate antibiotic as a risk factor. Newborns with mothers who received Intrapartum Antibiotic Prophylaxis (IAP) due to colonization of group B streptococci or chorioamnionitis, had a lower risk for Early Onset Sepsis than infants with mothers who did not receive an adequate IAP.[24] The classic study focusing on *Escerchia coli* infection, newborns were found to have bacteriemia with high colonies. However, more recent studies include pathogens other than *Eserchia coli* in infants. A newborn with a risk of sepsis found that 68% of septic infants had bacteremia with a low colonization rate (≤ 10 Colony-forming units (CFU) / ml) and 42% had a 1 CFU / ml colony count. Calculation of low bacterial colonies will cause as much as 60% of culture to be false negative with a sample volume of 0.5 ml. Many blood cultures can help improve these test results, but studies in the newborn period have shown conflicting results.[25]

On this study patient with red group, there were only 1 patient who had positive culture, These results diff er from those of other researchers where a clinical evaluation of sepsis compared with blood cultures in patients diagnosed with sepsis which is showed sensitivity (62.5% [95% confidence interval (CI): 35.43- 84.80%]}, specificity [63% (95% CI: 47.55-76.79%)], positive predictive value [37% (95% CI: 19.40-57.63%)] and value negative predictive [82.8% (95% CI: 66.35 -93.44%)]. There were statistically significant diff erences between blood culture results and clinical sepsis (p 0.014).[26]

One patient with clinically ill appearance had the results of an *Aerococcus viridans* culture that had multi-resistance to antibiotics also have a normal range of blood count and CRP. Patients with *Aerococcus viridans* culture results in this study had risk factors for meconeal amniotic fluid and inadequate antibiotic administration. The organisms most commonly involved in early-onset sepsis in term infants and fewer term infants are GBS and *Escherichia coli*, which account for around 70%. Additional pathogens are other streptococci (viridans group *streptococci, Streptococcus pneumoniae*).[27] *Aerococcus, abiotrophia* which is a gram-positive-*coccus bacteria* - catalase negative is a group of rarely isolated bacteria as opportunistic agents of infection, although this organism can become a pathogen in immunocompromised patients. *Aerococcus* is an environmental isolate that can also be found in human skin. These bacteria have low virulence and only become opportunistic pathogens in immunocompromised hosts. Infection that occurs is often in the form of tissue damage (for example a heart valve) or may be nosocomial and is associated with prolonged hospitalization, antibiotic therapy, invasive procedures and the presence of foreign objects. The association of infection with *Aerococcus Viridans* in humans found an almost significant value in those with rupture of membranes during childbirth (P 0.073), prolonged rupture of membranes (P 0.058), those receiving Intrapartum Antibiotic Prophylaxis (IAP) (P 0.059) and women who smoked during pregnancy (P 0.062)[28]There were several limitations of this study. First, the number of samples was relatively small. Second, the lack of GBS status data of the subject's mother, since this test is not a routine in Indonesia. hird, due to financial restraints, blood culture test were only performed in half of the subjects.

In this study, two neonates with green recommendation had positive blood culture. The SRC tools incidence input in this study follows CDC recommendation (0.5 ‰). The result is similar to retrospective cohort study of Carola et al, in which, from 1159 infants born to mothers with clinical chorioamnionitis, the calculator would have missed 2 of 5 infants with culture-proven, early-onset sepsis. The SRC tools has been updated to enable the possibility of EOS incidence, as high as 4‰. This update would enable to capture the two missed sepsis infants into the right risk and management category.

CONCLUSIONS

The results of Complete Blood Count and CRP levels between each group of SRC recommendation shown no significant diff erences. The analysis indicate that CRP level is uncorrelated with EOS risk, thus clinical judgement is necessary to accompany laboratory examination.

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CONFLICT OF INTEREST

There is no conflict of interest of this paper.

REFERENCES

1. Tita, a. T. and Andrew W. Diagnosis and management of clinical chorioamnionitis. Clin perinatol. 2010; 37**:** 339–54.
2. Puopolo KM, Draper D, Wi S, Newman TB, Zupancic J, Lieberman E, et al. Estimating the probability of neonatal early-onset infection on the basis of maternal risk factors. J Pediatr. 2011; 128: 1155–63.
3. Polin RA, Papile L-A, Baley JE, et al. Management of neonates with suspected or proven early-onset bacterial sepsis. J Pediatr. 2012; 129
4. Joshi N, Gupta A, Allan J, et al. Clinical monitoring of well-appearing infants born to mothers with chorioamnionitis; J Pediatr. 2018; 141: 1–10
5. Brecht, m., Clerihew, l. and Mcguire, W. Prevention and treatment of invasive fungal infection in very low birthweight infants. Arch dis child fetal neonatal. 2009; 94: 65–9.
6. Escobar, g. J., Puopolo, k. M. and Wi, s. Stratification of risk of early-onset sepsis in newborns ≥ 34 weeks' gestation. J Pediatr. 2014; 133: 30–6.
7. Warren S, Garcia M, Hankins C. Impact of neonatal early-onset sepsis calculator on antibiotic use within two tertiary healthcare centers. Nat Publ Gr [Internet]. 2016; (October): 1–4. Available from: http://dx.doi. org/10.1038/jp.2016.236
8. Carola D, Vasconcellos M, Sloane A, Mcelwee D, Edwards C, Greenspan J, et al. Utility of Early-Onset Sepsis Risk Calculator for Neonates Born to Mothers with Chorioamnionitis. J Pediatr. 2017; 11: 1–6
9. Kerste, M., Corver, J., Sonnevelt, M. C., et al. Application of sepsis calculator in Refer to Author guideline newborns with suspected infection. J matern fetal neonatal med. 2016; 29: 3860–5.
10. Puopolo K, Draper D, Wi, S, dan Newman, et al. Estimating the probability of neonatal early-onset infection on the basis of maternal risk factors. J Pediatr. 2011; 128: 1155–63.
11. Kenyon S, Boulvain M, Neilson J. Antibiotics for preterm rupture of membranes. Cochrane Database Syst Rev. (Abstrak). 2010
12. Weston J, Pondo T dan Lewis M, et al. The burden of invasive early-onset neonatal sepsis in the united states, 2005-2008. Pediatr infect dis j. 2011; 30: 937–41.
13. Achten N, Zonneveld R, Tromp E, Plötz F. Association between sepsis calculator and infection parameters for newborns with suspected early onset sepsis, J Clin Neonatol. 2017; 6: 159–162
14. Hisamuddin E, Hisam A, Wahid S, Raza G. Validity of C-reactive protein (CRP) for diagnosis of neonatal sepsis. Pak J Med Sci. 2015; 31(3): 527–531.
15. Bhatia S, Verma C, Tomar B, et al. Correlation of CRP and Blood Culture in evaluation of Neonatal Sepsis. IJBAMR. 2016; 6: 663–70
16. Kamble R and Rajesh Ovhal R. Bacteriological profile of neonatal septicemia. Int.J.Curr.Microbiol App.Sci. 2015; 2: 172–182
17. Meem, M., Modak, J. K., Mortuza, R., Morshed, M., Islam, M. S. dan Saha, S. K. Biomarkers for diagnosis of neonatal infections: a systematic analysis of their potential as a point-of-care diagnostics. J Glob Health. 2011; 1: 201–9.
18. Hofer N, Zacharias E, Müller W, Resch B. An Update on the Use of C-Reactive Protein in Early-Onset Neonatal Sepsis: Current Insights and New Tasks. J Clin Neonatol. 2012; 102: 25–36
19. Manzoni P, M. M., Galletto P, Gastaldo L, Gallo E, Agriesti G, dan Farina D. Is thrombocytopenia suggestive of organism-specific response in neonatal sepsis? J Pediatr. 2011; 51: 206–10.
20. Siriwachirachai T, Sangkomkamhang US, Lumbiganon P, Laopaiboon M. Antibiotics for meconium-stained amniotic fluid in labour for preventing maternal and neonatal infections. Cochrane Database Syst Rev. (Abstrak). 2014
21. Nora H, Eva Z, Wilhelm M, and Bernhard R. An Update on the Use of C-Reactive Protein in Early-Onset Neonatal Sepsis: Current Insights and New Tasks. J Clin Neonatol. 2012; vol 102: 25–36.
22. Derek S. Wheeler, M.D., Hector R. Wong, M.D., and Basilia Zingarelli. Pediatric Sepsis – Part I: "Children are not small adults. Open Inflamm J. 2011; 4: 4–15

23. James L. Wyn. Defining Neonatal Sepsis. Curr Opin Pediatr. 2016; 28(2): 135–140.
24. Jonathan M. Wortham, Nellie I, Stephanie J, Schrag, et al. Chorioamnionitis and Culture-Confirmed, Early-Onset Neonatal Infections. J Pediatr. 2016; 137(1): 1–11
25. Alonso T dan Theresa O. Challenges in the diagnosis and management of neonatal sepsis. J Trop Pediatr. 2015; 61: 1–13
26. Somaia E, Mervat E, Reem H, Doaa A, Qassem, dan Gameel. The Role of 16S rRNA Gene Sequencing in Confirmation of Suspected Neonatal Sepsis. J Trop Pediatr. 2016; 62: 75–80
27. Simonsen, K. A., Anderson-Berry, A. L., Delair, S. F. dan Davies, H. D, 2014. Early-onset neonatal sepsis. Clin microbiol rev, 27: 21–47.
28. Rasmussen. Aerococcus: an increasingly acknowledged human pathogen. Clin Microbiol Infect. 2015; 22: 22–7

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Research article

Differences of Interleukin-18 and Interleukin-10 Levels in Pulmonary Rifampicin Resistant dan Rifampicin Sensitive Tuberculosis Patients in Dr. Soetomo Hospital Surabaya

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ABSTRACT

Rifampicin is an anti-tuberculosis drug used in short-term treatment regimen for tuberculosis (TB) patients. Resistance to rifampicin causes the prolonged duration of tuberculosis treatment. Interleukin-18 (IL-18) is a pro-inflammatory cytokine which acts in controlling the growth of M. tuberculosis, while Interleukin-10 (IL-10) is an anti-inflammatory cytokine which acts in limiting tissue damage and maintain tissue homeostasis. IL-18 and IL-10 is important in explaining the different degrees of inflammation (mild, moderate and severe) in rifampicin-resistant (RR) and rifampicin-sensitive (RS) pulmonary TB patients. The purpose of this study is to determine different levels of IL-18 and IL-10 in new TB patients with RR and RS. A retrospective cohort study with a cross-sectional design. 50 subjects were examined and grouped into two groups, namely pulmonary TB with RR (n = 25) and pulmonary TB with RS (n = 25). IL-18 and IL-10 were measured using the ELISA Method. Differences in IL-18 and IL-10 levels between groups were analyzed using the Mann-Whitney test. The mean level of IL-18 (pg/ml) in RR and RS pulmonary TB patients were 1273.53±749.86 and 787.96 ±589.28 respectively. The mean level of IL-10 (pg/ml) in RR and RS pulmonary TB patients were 125.25±118.32 and 128.81±135.77 respectively. The mean level of IL-18 in RR and RS pulmonary TB patients were found to have a significant difference, while the mean level of IL-10 did not have a significant difference. This circulating level of IL-18 and IL-10 can be used as a marker of inflammation degrees in pulmonary RR-TB and RS-TB patient.

Keywords: *Interleukin-18, Interleukin-10, Tuberculosis, Rifampicin Resistant, Rifampicin Sensitive*

ABSTRAK

Rifampisin adalah rejimen dasar pengobatan jangka pendek untuk penderita tuberculosis (TB). Resistensi terhadap rifampisin menyebabkan durasi pengobatan tuberculosis menjadi lebih lama. Interleukin-18 (IL-18) adalah sitokin Proinflamsi yang berperan dalam mengontrol pertumbuhan M. tuberculosis, sedangkan Interleukin 10 (IL-10) adalah sitokin anti-infl amasi yang berperan membatasi kerusakan jaringan dan mempertahankan homeostatis jaringan. IL-18 dan IL-10 berperan penting untuk menjelaskan derajat inflamasi (ringan, sedang dan berat) yang berbeda pada penderita TB paru dengan rifampicin resistant (RR-TB) dan rifampcin sensitive (RS-TB). Tujuan penelitian ini adalah mengetahui perbedaan kadar IL-18 dan IL-10 pada penderita RR-TB dan RS-TB. Penelitian ini merupakan penelitian cohort retrospektif dengan rancangan cross-sectional. Sebanyak 50 subjek penelitian diperiksa dan dikelompokkan menjadi dua kelompok yaitu kelompok RR-TB (n=25) dan kelompok RS-TB (n=25). Pemeriksaan IL-18 dan IL-10 dilakukan dengan Metode ELISA. Perbedaan kadar IL-18 dan IL-10 antara kelompok dianalisis menggunakan Mann-whitney. Rerata kadar IL-18 (pg/ml) pada penderita RR-TB dan RS-TB adalah 1273.53±749.86 dan 787.96±589.28. Rerata kadar IL-10 (pg/ml) pada penderita RR-TB dan RS-TB adalah 125.25±118.32 dan 128.81±135.77. Rerata kadar IL-18 pada penderita RR-TB dan RS-TB ditemukan memiliki perbedaan signifikan, sedangkan

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memiliki perbedaan yang signifi kan. Nilai kadar IL-18 dan IL-10 ini dapat digunakan sebagai penanda derajat infl amasi pada penderita RR-TB dan RS-TB.

Kata Kunci: *Interleukin-18, Interleukin-10, Tuberculosis, Rifampicin Resistant, Rifampicin Sensitive*

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INTRODUCTION

In 2018, The World Health Organization (WHO) was stated that Tuberculosis (TB) is one of the top ten causes of death worldwide. About 10.4 million people suffer from TB and 1.7 million people die from this disease. More than 95% of deaths from TB occur in low and middle-income countries. India, Indonesia, China, the Philippines, Pakistan, Nigeria, and South Africa are countries that accounted the most cases of TB.[1] According to the Basic Health Research of Indonesia the prevalence of patients diagnosed with TB in 2013 was 0.4% with the five highest provinces which are West Java, Papua, DKI Jakarta, Gorontalo, Banten and West Papua. Of the entire population diagnosed with TB, only 44.4% were treated with a program medicines.[2]

Rifampicin Resistant is defined as a TB case that is declared resistant to rifampicin. TB strains resistant to rifampicin may be either sensitive or also resistant to isoniazid, which for the latter is considered as Multidrug Resistant-Tuberculois (MDR-TB) based on the GeneXpert test results. This is due to the lower mutation rate of isoniazid (2.56×10^8 CFU / ml *M. tuberculosis* colonies) compared to the mutation rate of rifampicin (6×10^{10} CFU / ml *M. tuberculosis* colonies), so that it can be said that TB patients that are resistant to the rifampicin drug are also resistant to isoniazid, but this comparison varies greatly between countries and patient groups.[3,4] Rifampicin is an antibiotic that has efficient antimicrobial action which combined with isoniazid which considered to be the basis of a short-term treatment regimen for TB. Rifampicin in *M. tuberculosis* targets the RNA polymerase β-subunits by binding and inhibiting the extension of RNA messenger. The role of rifampicin is to inhibit active growth and slow metabolism (slow-growing) of bacilli.[3]

Interleukin-18 (IL-18) was first described and used in rat serum which was intraperitoneally inoculated with endotoxin and was referred to as "Interferon-gamma (IFN-J) inducing factor".[5] Inside the human body, IL-18 is constitutively expressed by several cells, namely macrophages, kupff er cells, keratinocytes, osteoblasts, adrenal cortex cells, intestinal epithelial cells, microglial cells, and synovial fibroblasts.[6] IL-18 is a pro-inflammatory cytokine that works synergistically with Interleukin-12 (IL-12) to induce IFN-J production. The expression of IL-18 is regulated in chronic inflammatory diseases mediated by Th1. IL-18 can also contribute to the protection against mycobacteria. It is found that rats with IL-18 deficiency also have a decrease in IFN-J levels.[7]

Interleukin-10 (IL-10) is an anti-inflammatory cytokine which has a crucial role in preventing inflammatory, pathological autoimmune[8] and allergies.[9] Deficiency or decreased expression of IL-10 can increase the inflammatory response to microbes but on the other hands, it can also cause the development of infectious diseases such as TB and several of autoimmune diseases.[8] IL-10 can also increase the continuity of *M. tuberculosis* and its growth in macrophages by suppressing the partial maturation of phagosomes which depend on the activity of the signal transducer and activator of transcription 3 (STAT3)[10]. Currently, IL-10 increases survival and intracellular growth Mycobacteria by suppressing innate and adaptive immune responses.[11]

This study will describe how diff erent levels of IL-18 and IL-10 in pulmonary TB patients with rifampicin resistant and rifampicin sensitive,

where IL-18 and IL-10 can play an important role in explaining the different degrees of inflammation between these two groups.

MATERIALS AND METHODS

Study Population

This study was conducted in the Department of Clinical Pathology, Dr. Soetomo Hospital from August to November 2018. This study included 50 patients who were selected from the TB-DOTS/MDR Clinic of Dr. Soetomo Hospital. The study protocol has been approved by the Ethical Review Committee of Dr. Soetomo Hospital (0488/KEPK/VIII/2018). The data of all patients were collected after taking informed consent from patients. The age of patients ranged from 17 to 75 years old. The patients were assigned into two groups. The first group consisted of 25 patients with rifampicin-resistant pulmonary TB and the second group also consisted of 25 patients with rifampicin-sensitive pulmonary TB. Patients with HIV-AIDS, hepatitis, autoimmune diseases, diabetes mellitus, liver and kidney disease were excluded from this study. Also, patients treated with corticosteroid or immunosuppressive drugs were excluded, along with patients who had received anti-tuberculosis for more than one month because it can cause bias in the results of the examination

Sample Preparation

Four milliliters of blood were drawn aseptically from the basilic vein of each patient. Blood specimens were collected by using vacutainer venipuncture then stored in the serum separator tube. The tube contains a separation gel in the base of the tube which separates the serum from the whole blood. The blood sample was collected then was centrifuged at 3000 rpm for 10 minutes, the serums were then stored and freeze at -80°C for further use.

Enzyme-linked Immunosorbent Assay (ELISA) Analysis

The frozen serums were thawed at room temperature and cytokine IL-18 and IL-10 levels were then measured using the Human Sandwich-ELISA kit from *Elabscience*® done as the manufactures instructions. The cytokine concentrations in samples were calculated using the standard curve generated from recombinant cytokines, and the results are expressed in picograms per milliliter (pg/ml).

Statistical Methods

The result is presented as the mean ± s.d. Statistical significance was calculated by the *Mann-Whitney test* to see differences between IL-18 and IL-10 in patients with pulmonary RR-TB and pulmonary RS-TB. The *p values*< 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Clinical Characteristics of Subjects

The clinical characteristics of the 25 patients with pulmonary RR-TB and 25 patients with pulmonary RS-TB are summarized in Table 1. The clinical type of all TB patients were all pulmonary TB.

IL-18 Level

The highest level of IL-18 found in pulmonary RR-TB patients was 2486 pg/ml, and the lowest 58.39 pg/ml, while the highest level of IL-18 in pulmonary RS-TB patients was 1990 pg/ml and the lowest was 106.06 pg/ml. The mean, standard deviation, and *p-values* of IL-18 levels in these two groups are shown in Table 2. The mean level of IL-18 between pulmonary RR-TB and RS-TB patients were showed significant differences ($p < 0.05$). The differences of IL-18 in pulmonary RR-TB and pulmonary RS-TB patients are shown in the boxplot in Figure 1.

Table 1. Clinical Characteristics of the Population Studied.

	Pulmonary RR-TB	Pulmonary RS-TB
Gender, male/female	18/7	11/14
Median age (range)	37.00 (23-67)	43.00 (18-63)

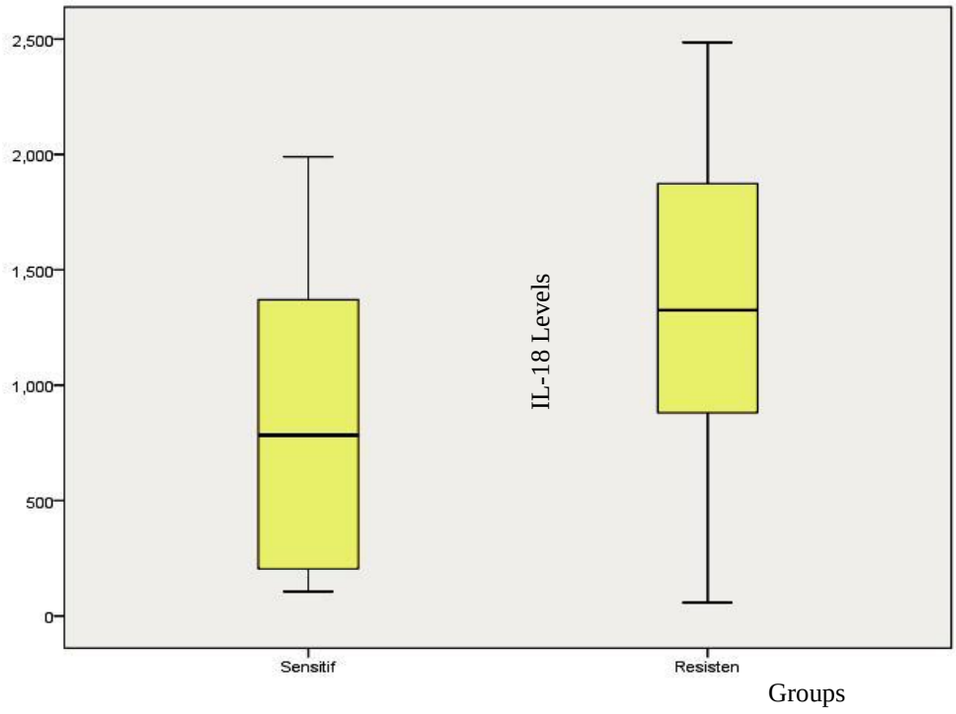

Figure 1. IL-18 Levels in Pulmonary RR-TB and Pulmonary RS-TB. This result shows that an increase in IL-18 levels in the blood was found to be significantly higher in pulmonary RR-TB patients compared to pulmonary RS-TB, meaning a higher increase in the inflammatory process for pulmonary RR-TB patients compared to pulmonary RS-TB patients. This results is also accordance with the result of Wang et al[12] study.

Table 2. The Mean and Standard Deviation of IL-18 in Pulmonary RR-TB and Pulmonary RS-TB

Group	N	Mean	Standard deviation	p-value
Pulmonary RR-TB	25	1273.53	749.86	0.017
Pulmonary RS-TB	25	787.96	589.28	

n = number of samples
p < 0,05 = significant

The IL-18 level between pulmonary RR-TB and RS-TB patients found in this study has a mean of 1273.53 ± 749.86 pg/ml and 787.96 ± 589.28 pg/ml respectively. This shows that the increasing level of IL-18 in the blood was found to be significantly higher in pulmonary RR-TB than in pulmonary RS-TB. This results in this study also in accordance with the result of Wang et al[12] study. Wang et al[12] also stated that the IL-18 serum was found to be higher in patients with pulmonary RR-TB (131.03 ± 94.92) compared to drug sensitive TB (94.28 ± 57.10) and healthy controls (61.66 ± 24.78). The resistance to rifampicin in TB is caused by mutations in the bacterial chromosome (rpoE gene). Mutations in this gene will cause changes in the structure and activity of drug targets that results in generating bacterium *M. tuberculosis* that cannot be eliminated using rifampicin which has an impact on increasing the number of said bacteria in the host body.[13] This increase in the number of bacteria causes macrophages as a first-line defense against the invasion of these bacteria and mediates the innate immune response through the introduction of pathogens and an increase in inflammatory reactions. Increased macrophage activation in RR pulmonary TB infection will increase the production of proinflammatory cytokines that play a role for the mechanism of killing *M. tuberculosis.*[14]

Rifampicin plays an important role in TB treatment because of its bactericidal effect that can eliminate *M. tuberculosis*.[15] When pulmonary TB patients are resistant to rifampicin, the

growth of *M. tuberculosis* will increase and cannot be controlled. Macrophages as the first-line defense will fight the bacterial invasion and mediate innate immune responses through the introduction of pathogens and the activation of inflammatory reactions. Macrophages will polarize to various functional conditions such as M1 which is classically activated and M2 which is alternatively activated. Macrophage polarization into M1 is important for the elimination of intracellular *M. tuberculosis*. Activation of M1 macrophages through the TLR2 signal pathway can be beneficial for the host to inhibit growth and the survival of *M. tuberculosis.*[16,17] Increased activation of M1 macrophages in newly infected

RR pulmonary TB will produce pro-inflammatory cytokines which play a role in the mechanism of eliminating *M. tuberculosis*. This causes the level of pro-inflammatory cytokines to be higher in RR pulmonary TB serum compared to RS pulmonary TB. The level of pro-inflammatory cytokines in both RR and RS pulmonary TB is found to be higher compared to the level of anti-inflammatory cytokines to suppress growth and the survival of *M.tuberculosis.*[12]

Increased level of IL-18 in the patients' serum is also suspected to indicate that there has been a leak of cytokines from the tissues to the circulation. This is supported by various studies which stated that a high concentration of IL-18 are found in TB patients with advanced disease, high fever, and extensive radiographic infiltrates.[7, 18] Increased levels of IL-18 as a pro-inflammatory cytokine in RR pulmonary TB patients are associated with various pathological conditions in the patients themselves. Patients with pulmonary RR-TB with high levels of IL-18 were also found to have higher ESR and CRP levels compared to pulmonary RS-TB patients and healthy people. ESR and CRP have been used as markers for the diagnosis of pulmonary TB that reflect pathological processes in the patient's body. Increased CRP and ESR indicate that an acute inflammatory process has occurred in pulmonary TB patients.[12] Higher IL-18 levels found in pulmonary RR-TB patients compared to pulmonary RS-TB patients in this study confirmed various previous studies which stated that IL-18 levels were significantly increased in patients with severe pulmonary TB.

IL-10 Level

The highest level of IL-10 in pulmonary RR-TB patients was 465.77 pg/ml, and the lowest was 1.57 pg/ml, while the highest level of IL-10 in pulmonary RS-TB patients was 552.11pg/ml and the lowest level was 1.36 pg/ml. The mean, standard deviation, and *p-values* of IL-10 level in these two groups are shown in Table 3. The mean of IL-10 level between patients showed no significant differences ($p>0.05$). The differences of IL-10 in pulmonary RR-TB and pulmonary RS-TB patients are shown in the boxplot in Figure 2.

IL-10 is an anti-inflammatory cytokine that works by inhibiting the ability of myeloid cells such as macrophages and dendritic cells to activate Th1 cells. Initially, IL-10 is known to be secreted by antigen-stimulated Th2, but it is now known that IL-10 is not only secreted by Th2, but also secreted by a subset of CD4 + T cells, including Th1 and Th17, B cells, neutrophil cells, and macrophages.[17] IL-10 is generally thought to modulate the ability of the immune response and allow bacterial elimination without damaging the host tissue, but in some cases the absence of IL-10 makes the immune response more effective in eliminating pathogens, but resulting in more damage to the tissue and affects the survival of the host.[20, 21]

The mean level of IL-10 in pulmonary TB patients with RS and RR in this study were 128.81 ± 135.77 pg/ml and 125.15 ± 118.32 pg/ ml respectively. This shows that IL-10 levels in RS were found to be higher than in RR pulmonary

Table 3. The Mean and Standard Deviation of IL-10 in Pulmonary RR-TB and Pulmonary RS-TB

Group	N	Mean	Standard deviation	p-value
Pulmonary RR-TB	25	125.15	118.32	0.961
Pulmonary RS-TB	25	128.81	135.77	

n = number of samples
p > 0,05 = not significant

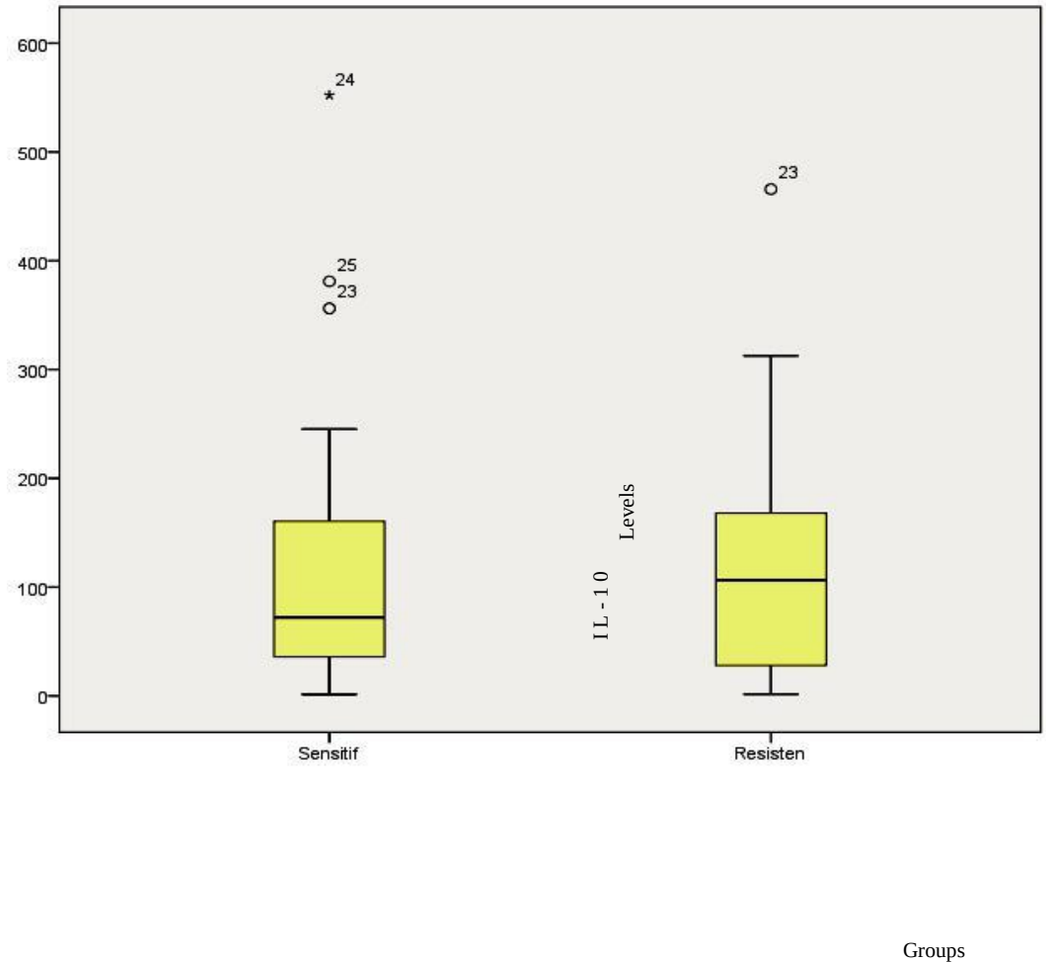

Figure 2. IL-10 Levels in Pulmonary RR-TB and Pulmonary RS-TB Patients. The results shows showed no significant differences between these two groups.

TB, although statistically did not have a significant difference ($p > 0.05$). The results of this study are following a study conducted by Butov et al[22] which stated that the mean level of IL-10 in MDR-TB patients'serum before and after 2 months of treatment were found to be lower when compared to non-MDR TB patients and healthy people. This result is in accordance with the result of Lihawa[23] and Peñaloza[24] study. Lihawa and Yudhawati[23] in Dr. Soetomo Hospital showed that descriptively IL-10 levels in MDR-TB patients were found to be lower than non-MDR TB, but statistically no significant differences were found. Peñaloza[24] was stated that during non-MDR *M. tuberculosis* infection, IL-10 production is important for host survival, but the role of IL-10 in the immune response of patients with MDR pulmonary TB molecularly has not been found with certainty. This insignificant difference in IL-10 may indicate a tendency of static state occuring during the acute phase of TB levels IL-10 due to the role of macrophages which secrete more proinflammatory cytokines to protect the host from M. tuberculosis. It is evidenced in this study by the discovery of IL-18 levels that were higher than the IL-10 levels in each group. High levels of IL-10 can only be found in chronic TB infections.[25]

CONCLUSIONS

The level of IL-18 is higher in patients with pulmonary RR-TB compared to pulmonary RS-TB. This circulating level of IL-18 and IL-10 can be used as a marker of inflammation degrees in pulmonary RR-TB and RS-TB patients.

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CONFLICT OF INTEREST

There is no conflict of interest that has to be declared in this study.

REFERENCES

1. WHO. Global Tuberculosis Report 2018. Geneva, Switzerland: World Health Organization; 2018.
2. Kemenkes RI. Pedoman Nasional Pengendalian Tuberculosis. Jakarta: Kementrian Kesehatan Republik Indonesia; 2014.
3. Dasilva P, Palomino J. Molecular basis and mechanisms of drug resistance in Mycobacterium tuberculosis: classical and new drugs. J Antimicrob Chemother. 2011; 66(7): 1417–30. doi: 10.1093/jac/dkr173
4. Kurbativa EV, Cavanaugh JS, Shah SN, Wrisht A, Kim HJ, Metchock B. Rifampicin-resistant Mycobacterium tuberculosis susceptibility to isoniazid and other anti-tuberculosis drugs. Int J Tuberc Lung Dis. 2012; 16(3): 355–7. doi: 10.5588/ijtld.11.0542.
5. Wawrocki S, Druszczynska M, Kowalewics M.K, Rudnicka W. Interleukin 18 (IL-18) as a target for immune intervention. Acta Biochim Pol. 2016; 63(1): 59–63. doi: 10.18388/abp.2015_1153.
6. Dinarello C, Novick D, Kim S, Kalplanski G. Interleukin-18 and IL-18 binding protein Front Immunol. 2013; 4: 289. doi: 10.3389/fimmu.2013.00289.
7. Han M, Yue J, Lian Y, Zhao Y, Wang H, Liu L. Relationship between single nucleotide polymorphism of interleukin-18 and susceptibility to pulmonary tuberculosis in the Chines Han population. Microbiology and Immunology. 2011: 55: 388–93. doi:10.1111/ j.1348-0421.2011.00332.x
8. Iyer SS, Cheng G. Role of Interleukin 10 Transcriptional Regulation in Inflammation and Autoimmune Disease. Crit Rev Immunol. 2012; 32(1): 23–63.
9. Ng TH, Britton GJ, Hili EV, Verhagen J, Burton BR, Wrauth DC. Regulation of adaptive immunity; the role of interleukin-10. Front Immunol. 2013; 4; 129. doi:10.3389/fimmu.2013.00129
10. O'Leary S, O'Sullivan MP, Keane J. IL-10 blocks phagosome maturation in mycobacterium tuberculosis-infected human macrophages. Am J Respir Cell Mol Biol. 2011; 45: 172–80.
11. Abdalla AE, Lambert N, Duan X, Xie J. Interleukin-10 Family and Tuberculosis: An Old Story Renewed. Int J Biol Sci 2016; 12(6): 710–717. doi:10.7150/ ijbs.13881
12. Wang Y, Chunmei H, Zailang W, Hui K, Weiping X, Hong W. Serum IL-1Ε and IL-18 Correlate with ESR and CRP in Multi-drug Resistant Tuberculosis Patients. J Biomed Res. 2015; 29(5): 426–28. doi: 10.7555/ JBR.29.20150077
13. Amalia E, Nindatama M.R, Hayati L, Handayani D. (2015). Identifikasi Mutasi Gen rpob Ser531Leu Mycobacterium tuberculosis yang Berhubungan Dengan Resistensi Rifampsin. Biomed J of Indo, Vol. 1 No.1.
14. Domingo-Gomzales R, Prince O, Cooper A, Khader S. Cytokines and chemokines in Mycobacterium tuberculosis infection. Microbiol Spectr. 2016; 4(5). doi: 10.1128/microbiolspec.TBTB2-0018-2016.
15. Zhang, X., & Guo, J. Advances in the treatment of pulmonary tuberculosis. J Thoracic Dis. 2012; 4(6): 617–623.
16. Lim YJ, Yi MH, Choi JA, Lee J, Han JY, Jo SH, et al. Roles of endoplasmic reticulum stress-mediated apoptosis in M1-polarized macrophages during mycobacterial infections. Sci Rep. 2016; 6:37211DOI: 10.1038/srep37211
17. Wang S, Zhang J, Sui L, Xu H, Piao Q, Qu X, et al. Antibiotics induce polarization of pleural macrophages to M2-like phenotype in patients with tuberculous pleuritis. Sci Rep. 2017; 7(1): 14982. doi: 10.1038/ s41598-017-14808-9.
18. Elarab AE, Garrad H. Serum level of interferon gamma (INF-J), IL-12, and IL-18 in active pulmonary. AAMJ. 2012; 10(3).
19. Redford P, Murray J, O'Garra A. The Role of IL-10 in Immune Regulation during *M. tuberculosis* Infection. Mucosal Immunol. 2011; 4(3): 261–70. doi: 10.1038/ mi.2011.7.
20. Peñaloza HF, Schultz BM, Nieto PA, Salazar GA, Suazo I, Gonzalez PA, et al. Opposing roles of IL-10 in acute bacterial infection. Cytokine Growth Factor Rev. 2016; 32: 17–30. doi: 10.1016/j.cytogfr.2016.07.003.
21. Ng TH, Britton GJ, Hill EV, Verhagen J, Burton BR, Wraith DC. Regulation of adaptive immunity; the role of interleukin-10. Front Immunol. 2013; 4: 129. doi: 10.3389/fimmu.2013.00129
22. Butov DO, Mykhalio K, Kuzhko BT. Interleukin-10 Gene Polymorphisms is Associated with Multi-drug resistance Tuberculosis in Ukranian Population. Intl J of Mycobac. 2016; 5: 152–3.

23. Lihawa N, Yudhawati R. Hubungan Kadar IL-10 dan Tuberculosis Multi-drug Resistant. Jurnal Respirasi. 2015; 1(2): 41–47
24. Penaloza H, Noguera L, Riedel C, Bueno S. Expanding the Current Knowledge About the Role of Interleukin-10 to Major Concerning Bacteria. Front Microbiol. 2018; 9: 2047. doi: 10.3389/fmicb.2018.02047
25. O'Garra, Redford P.S, McNab F.W, Bloom C.I, Wilkinson R.J, Berry M. The Immune Response in Tuberculosis. Annurev Immunol. 2013; 31: 475–527.

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Research article

Anti-Hepatitis C Activity and Toxicity of *Scoparia Dulcis* Linn. Herb

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ABSTRACT

Hepatitis C Virus (HCV) infection is a serious public health problem since HCV is the ribonucleic acid (RNA) virus that easy to mutate. The HCV standard treatment has rapidly developed but the possibility of resistance and effectiveness of treatment needs to be considered. The medicinal plants are a source of various compounds that may potentially cure diseases including infectious diseases. Since a long years ago, medicinal plants were famous as an inherited treatment that believed to cure the disease. One of the medicinal plants is Scoparia dulcis (S. dulcis) that belongs to Scrophulariaceae family and traditionally used as remedies for digestive problems, hypertension, diabetes mellitus, bronchitis, and as an analgesic & antipyretic agent. The previous report showed that S. dulcis was known active as an antiviral against Herpes Simplex Virus (HSV) type 1 in vitro and in vivo. The aim of the study is to determine the biactivity potential of S. dulcis against HCV. Scoparia dulcis was extracted using 80% ethanol (EE) then further separated by liquid-liquid fractionation using dichloromethane (DCMF), ethyl acetate (EAF), butanol solvent (BF) and water (WF). The in vitro anti-HCV analysis was performed with Huh7it cells and HCV JFH1 (genotype 2a) by determining inhibition concentration 50 (IC_{50}). The toxicity (Cytotoxicity Concentration 50, CC_{50}) test was performed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and mechanism of action were analyzed using time addition experiment. Phytochemical groups as the suspected active compounds of S. dulcis were identified by Thin Layer Chromatography (TLC) and observed under UV 254 nm, UV 365 nm, before and after sprayed using H_2SO_4 10% and heated at 105^{o}C for 5 minutes. The IC_{50} test result of 80% EE and DCMF showed anti-HCV activity with a value of 12.7±4.8 μg/ml and 5.8±0.69 μg/ml, while EAF, BF, and AF respectively resulted in IC_{50} value of >100 μg/ml that suggested there was no inhibition effect on HCV JFH1. The DCMF was the most active fraction but toxic to the cell with CC_{50} value >23 μg/ml and selectivity index (SI) >3.9. According to the time addition experiment data, DCMF of S. dulcis inhibited post entry step HCV JFH1 infection that it means the possibility was to inhibit virus replication and or virion release. Scoparia dulcis contain chlorophyll, flavonoids and terpenoids as the suspected active compounds for inhibition of HCV JFH1 infecton. Futher study of post-entry inhibitions of HCV infection was needed.

Keywords: *Scoparia dulcis, anti-HCV, toxicity, Huh7it, HCV JFH1*

ABSTRAK

Infeksi Virus Hepatitis C (VHC) merupakan masalah kesehatan yang serius di dunia dikarenakan VHC adalah virus RNA yang mudah untuk bermutasi. Pengobatan VHC telah berkembang pesat namun kemungkinan adanya resitansi dan efektivitas pengobatan perlu untuk dipertimbangkan. Tanaman obat adalah sumber dari berbagai macam senyawa yang potensial untuk mengobati penyakit termasuk penyakit infeksi. Sejak bertahun-tahun sebelumnya tanaman obat dikenal untuk pengobatan turun temurun yang dipercaya dapat menyembuhkan penyakit. Salah satu dari tanaman obat adalah Scoparia dulcis (S. dulcis) yang berasal dari famili Scrophulariaceae dan secara tradisional digunakan untuk pengobatan masalah pencernaan, hipertensi, diabetes mellitus, bronkitis, dan sebagai agent analgesik dan

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diketahui aktif sebagai antiviral terhadap Herpes Simplex Virus (HSV) secara in vitro dan in vivo. Tujuan dari penelitian ini adalah mengetahui potensi aktivitas dari S.dulcis terhadap HCV. Scoparia dulcis diekstraksi menggunakan etanol 80% (EE) dan dilanjutkan pemisahan menggunakan metode fraksinasi cair-cair dengan pelarut diklorometana (DCMF), etil asetat (EAF), butanol (BF), dan air (AF). Analisis antiHCV secara in vitro dilakukan dengan menggunakan sel Huh7it dan VHC JFH1 (genotip 2a) dengan menentukan inhibition concentration 50 (IC_{50}). Uji toksisitas (Cytotoxicity Concentration 50, CC_{50}) dilakukan dengan metode 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dan analisis mekanisme aksi menggunakan uji time addition. Berbagai kelompok fitokimia yang diduga sebagai komponen aktif S. dulcis diidentifikasi dengan Thin Layer Chromatography (TLC) dan dilihat dibawah sinar UV 254 nm, UV 365 nm, sebelum dan sesudah disemprot dengan H_2SO_4 10% serta dipanaskan pada 105^oC selama 5 menit. Hasil uji IC_{50} menunjukkan 80% EE dan DCMF memiliki aktivitas anti-VHC dengan nilai IC_{50} 12,7±4,8 μg/ml dan 5,8±0,69 μg/ml, sedangkan EAF, BF, and AF berturut-turut menghasilkan nilai IC_{50} lebih dari 100 μg/ml yang menunjukkan tidak adanya hambatan terhadap VHC JFH1. Fraksi paling aktif adalah DCMF namun toksik terhadap sel dengan nilai CC_{50} >23 μg/ml dan selectivity index (SI) >3,9. Berdasarkan data pengujian time addition, DCMF S. dulcis menghambat infeksi VHC JFH1 pada post entry step yang berarti kemungkinan menghambat replikasi virus dan atau pelepasan virion. Scoparia dulcis terbukti mengandung klorofil, berbagai flavonoid dan terpenoid yang diduga sebagai komponen aktif penghambat infeksi HCV JFH1. Diperlukan penelitian lebih lanjut terhadap berbagai hambatan post entry pada infeksi VHC.

Kata kunci: *Scoparia dulcis, anti-VHC, toksisitas, Huh7it, VHC JFH1*

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INTRODUCTION

Hepatitis C Virus (HCV) is one of the causative agents of liver disease that potentially develop to liver cirrhosis and hepatocellular carcinoma (HCC).[1] More than 185 million people worldwide were infected by HCV, and 350.000 of them die every year.[2] In Indonesia, there had been estimated that 24 million people were infected with Hepatitis B (HBV) and HCV then 14 million of them had potentially become a chronic liver disease. Patients who had developed into chronic liver disease, around 1.4 million of them potentially develop into liver cancer.[2]

Until now there was no vaccine available for HCV infection. Various genotype and subtype of HCV probably caused difficult vaccine development. The current therapy of HCV infection is direct-acting antiviral agents (DAAs) combined with Interferon (IFN). The HCV infection therapy has improved Sustained Virological Response (SVR) >90%. Many therapies of HCV infection have been developed, but therapeutic efficacy still needs to be improved especially for high-risk populations with relatively low income. The important issues such as drug resistance and safety for long usage also need to be considered. Therefore, it is essential to develop effective, safe, inexpensive, and well-tolerated drugs for HCV infection.[3,4] Medicinal plants are a source of promising drug candidates for HCV infection.[5] Some plants were reported to have an antiviral activity of such as *Phyllantus amarus*, *Acacia nilotica*, *Boswellia carterii*, *Embelia schimperi*, *Piper cubeba*, *Quercus infectoria*, *Trachyspermum ammi*, and *Syzygium aromaticum.*[6]

Scoparia dulcis is a medicinal plant that belongs to Scrophulariaceae family. *Scoparia dulcis* traditionally used to treat some diseases such as digestive problems, hypertension, and diabetes. Another study reported that *S. dulcis* active as an antiviral against herpes simplex virus type 1 (HSV).[7] The phytochemical screening was showed that *S. dulcis* contained coumarin,[8] phenol,[9] saponins,[10] tannins,[11] flavonoids,[12] terpenoids,[13] and catecholamines.[14] In the previous publications, phytochemical groups terpenoids i.e Scopadulcic acid B was reported had antiviral activity against herpes simplex virus (HSV)[15] ; and the extract was reported to reduce virus titer of Coxsackie B1-B6 virus.[16] Some compounds of *S. dulcis*, Scopadulcic acid A was reported had antimalarial activity against *Plasmodium falciparum* in vitro and Scopadulcic

acid B exhibited inhibition of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA).[7, 17] Furthermore, some compounds of *S.dulcis* were reported as an antitumor or anticancer agents such as Scopadulcic Acid B[18]; Scopadulcic acid C[19]; Benzoxazinoids[20]; and Betulinic acid.[21] Based on the above background, further study was conducted to determine the activity of anti-HCV and toxicity for extract and fractions from *S. dulcis* then analyzed their mechanism of action by time addition experiment, and to identify the presence of active compounds as antiviral of *S. dulcis*.

MATERIALS AND METHODS

Plant Material

Scoparia dulcis herb was obtained from Wain River Protection Forest Region of Balikpapan, East Kalimantan in September 2015 and determined at Lembaga Ilmu Pengetahuan Indonesia (LIPI) Purwodadi, Pasuruan, East Java.

Extraction and fractionation.

Simplicia of *S. dulcis* was extracted by the ultrasonic-assisted extraction method using 80% ethanol as a solvent. The extract was homogenized using ultrasonic then it was separated by filtration by three-time repetition. The Filtrate was collected then the solvent was evaporated by a rotary evaporator. The extract was dried in an oven at a temperature of 40°C and fractionated using dichloromethane 100%, ethyl acetate 100%, butanol 100%, and water successively.

Virus and cells.

Huh7it cells, a clone of human hepatocellular carcinoma-derived from Huh7 cell,[22] were cultured in Dulbecco's Modified Eagle Medium (Wako, Osaka, Japan) completed with 10% Fetal Bovine Serum (FBS, GIBCO), Non-Essential Amino Acids (NEAA, GIBCO), and 0.15 mg/ml kanamycin solution (SIGMA). A cell culture-adapted HCV variant (JFH1 strain of genotype 2a)[22] was propagated with Huh7it cells, suspended in 4ml medium containing JFH1 (1.8x107 ffu, Multiplication of Infection (MOI) 0.1), and incubated at 37 °C in 5% CO_2 for 4 hours with agitation every 30 minutes. Culture supernatant was harvested and removed cell debris by centrifugation on the third day. The supernatant was concentrated using Amicon-Ultra-15 centrifuge filter.[22]

Anti Hepatitis C Virus (Anti-HCV) activity.

Huh7it cells ($5.2x10^4$) were seeded for 24 hours before HCV infection. Hepatitis C virus with MOI of 0.1 was mixed with different concentrations of the plant extract/ fractions (100; 50; 25; 12.5; 6.2; 3.1μg/ml) and then inoculated into the Huh7it cells. After 2 hours of absorption, the cells were washed with medium and further incubated in the medium containing the same extracts for 46 hours.[23] Cultures supernatant were collected to assess the mode-of-action of the samples tested. The 50% inhibitory concentration (IC_{50}) effect was calculated and analyzed by SPSS probit. All experiments were conducted for three times replication to collect Standard Deviation (SD).

Cytotoxicity assay.

The cytotoxicity of the samples was assessed by 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. Huh7it cells ($2.3x10^4$) in 96 well plates were treated with various concentrations of extract/ fractions for 48 hours. The medium was replaced with MTT 10% 150 μl/well containing medium and incubated for 4 hours. Insoluble precipitates were dissolved in Dimethyl Sulfoxide (DMSO) and measured the reaction color at 560 nm absorbance. Percentages of cell viability were compared to the control and calculated for 50% cytotoxic concentration (CC_{50}) values.[24] The ratio of CC_{50} and IC_{50} was calculated to obtain the Selectivity Index (SI) to determine the best candidate among the sample. The best one of *S. dulcis* extract or fraction according to the highest selectivity index was chosen for a time addition experiment.

Time addition experiment.

Time in addition experiments using much concentration of chosen extract/fraction were

performed for HCV JFH1 and Huh7it host cell culture by three set experiments: 1. The virus was inoculated to the cell after pretreatment cell with *S. dulcis* has chosen extract/fraction for 2 hours; 2. Virus was inoculated first (2hr incubation) then continued by adding *S. dulcis* chosen extract/fraction sample after virus fusion;
3. The chosen extract/fraction of *S. dulcis* was added before and after HCV JFH1 infection. All three set experiments were stained using 3,3'-Diaminobenzidine (DAB) staining (Thermo, UK) to visualize the cell infection.[5]

Identification of phytochemical groups in *S. dulcis*.

The identification of phytochemical groups contains in the *S. dulcis* extract and fraction was conducted by Thin Layer Chromatography (TLC). The profile was obtained using silica gel F254 as a stationary phase and chloroform: methanol (9:1 v/v) as a mobile phase. The plate was observed under UV 254 nm, UV 365 nm, and UV 365 after sprayed using H_2SO_4 10% and heated at 105^{o}C for 5 min.

RESULTS AND DISCUSSION

There were five samples resulted from *S. dulcis* separation i.e 80% Ethanol Extract (EE), Dichloromethane fraction (DCMF), Ethyl acetate fraction (EAF), Butanol fraction (BF), and Aqueous fraction (AF). The result of anti-HCV (IC_{50}), toxicity (CC_{50}), and Selectivity Index (SI) as a ratio of CC_{50} and IC_{50} of *S. dulcis* extract/fraction was presented in Table 1.

The result in Table 1 showed that 80% EE was active inhibited JFH1 with IC_{50} value of 12.7±4.8 µg/ml and less toxic with CC_{50} >100 µg/ ml. Further analysis of fraction showed the most active fraction of 80% EE was DCMF with IC_{50} value of 5.8±0.69 µg/ml meanwhile the EAF, BF, and AF didn't show inhibition with IC_{50} value of >100 µg/ml.

Based on dose-dependent inhibition and cytotoxicity activity, it was showed that the anti HCV activity of DCMF from *S. dulcis* increased after concentration > 6.25 ug/ml but it was also followed by increased toxicity in cells (Figure 1). According to toxicity data, DCMF has the strongest toxicity among four fractions. The toxicity on DCMF may disturb HCV infection to the Huh7it or/and affected directly to virus inhibition.

To determine the anti-HCV mechanisms, a time of addition the experiment was performed in this study. *Scoparia dulcis* DCMF was analyzed for a mechanism of action at various dose extract during inoculation and post-inoculation. The results revealed that the mechanism of HCV JFH1 inhibition was dominantly in post-entry inhibition (post-inoculation) with IC_{50} value of 9.25 µg/ml (Table. 2) than entry inhibition (during inoculation).

The result in Table 2 was demonstrated the possible inhibition process in the assembly or/ and release progeny virions. The inhibition of the virion replication and release can be affected by all virus life cycles and disturbed virus infection in the cells. Further analyzed on the specific inhibition on post-entry-step in host cells were needed.

The result of identification of phytochemical groups contained in the *S. dulcis* extract/fraction showed in Figure 2. Chlorophyll was identified as one of the phytochemical compounds contained in the EE and DCMF. It can be indicated by red bands at TLC profile when observed under UV 365 nm in figure 2B and 2D; and indicated by dark bands when observed under UV 254 nm in figure 2A (white arrows).[25,26]

Table 1. Anti-HCV activity (IC_{50}), CC_{50}, and SI of *S. dulcis* extract/fraction

***S. dulcis* Extract/ Fraction**	**IC_{50} (µg/ ml) ± SD**	**CC_{50} (µg/ml)**	**SI (CC_{50}/ IC_{50})**
80% EE	12.7±4.8	>100	>7.87
DCMF	5.8±0.69	>23	>3.97
EAF	>100	>800	>8
BF	>100	>800	>8
AF	>100	>800	>8

IC_{50} : 50% Inhibition concentration of HCV JFH1 infection in Huh7it culture
CC_{50} : 50% Cytotoxicity concentration in Huh7it culture
SI : Selectivity index

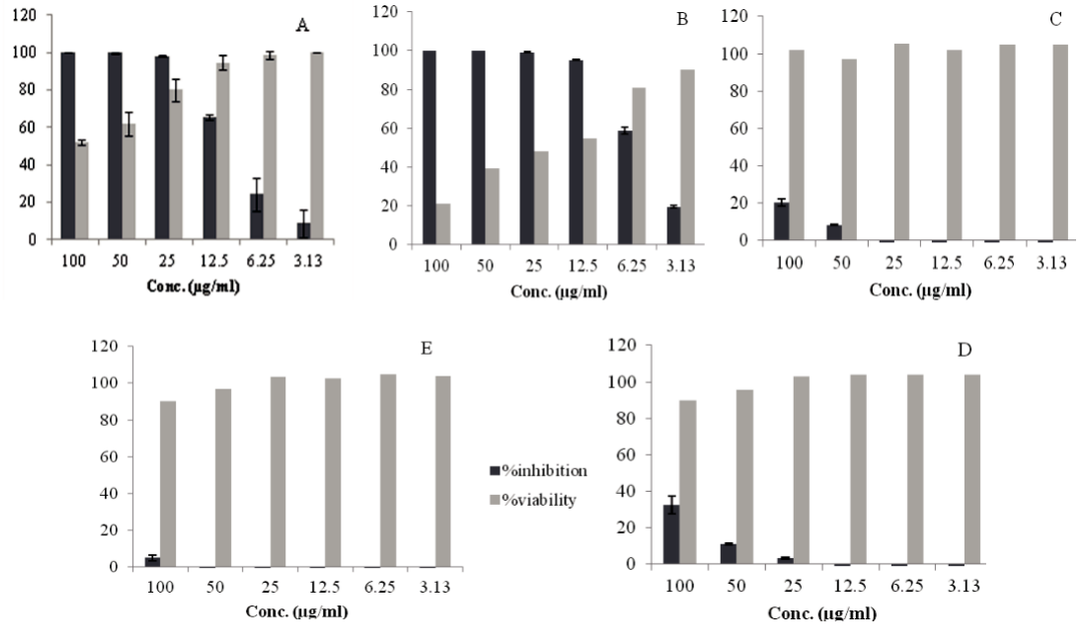

Figure 1. Dose-dependent Inhibition and Toxicity of *S. Dulcis;* A. 80% EE, B. DCMF, C. EAF, D. BF, E. AF.

Table 2. Mode of action of DCMF from *S. dulcis*

DCMF Concentration (ug/mL)	During and Post inoculation %Inhibition	During inoculation %Inhibition	Post inoculation %Inhibition
100	100	98.68	100
50	100	71.58	100
25	99.67	57.37	100
12.5	92.07	30.27	71.58
6.25	64.31	8.79	24.32
3.125	29.28	-3.11	20.03
IC_{50}	5.43 ug/mL	21.64 ug/mL	9.25 ug/mL

The identification of flavonoids and terpenoids compounds, after running TLC was taken using H_2SO_4 10% spray reagent which followed by heating at 105°C for 5 minutes. In Figure 2C and 2D, EE and DCMF were found to have a similar profile. Purple bands and yellow-brownish bands were identified in both samples. In figure 2C, the bands indicated flavonoids (yellow brownish band, white arrow) and terpenoids (purple bands, yellow arrow) compounds contained in EE and DCMF as well.[22, 25] Both samples were active and contain similar phytochemical compounds. Secondary metabolites such as flavonoids, alkaloids, coumarins, and terpenoids/polyphenol compounds have been reported to possess antiviral effects including anti-HCV activities.[3] The similarity of phytochemical compounds contained in both samples matched with the anti-HCV activities. Chlorophyll, terpenoids and flavonoids compounds in EE and DCMF were possible to have a role as anti-HCV active agents.

CONCLUSIONS

Scoparia dulcis EE and DCMF showed antiviral inhibition against HCV with the IC_{50} value of 12.7±4.8 and 5.8±0.69 µg/ml, respectively. Meanwhile, EAF, BF, and AF were not active as anti-HCV with IC_{50} value of >100 µg/ml. The DCMF was the most active fraction as anti-HCV but toxic to the host cells with CC_{50} value of >23

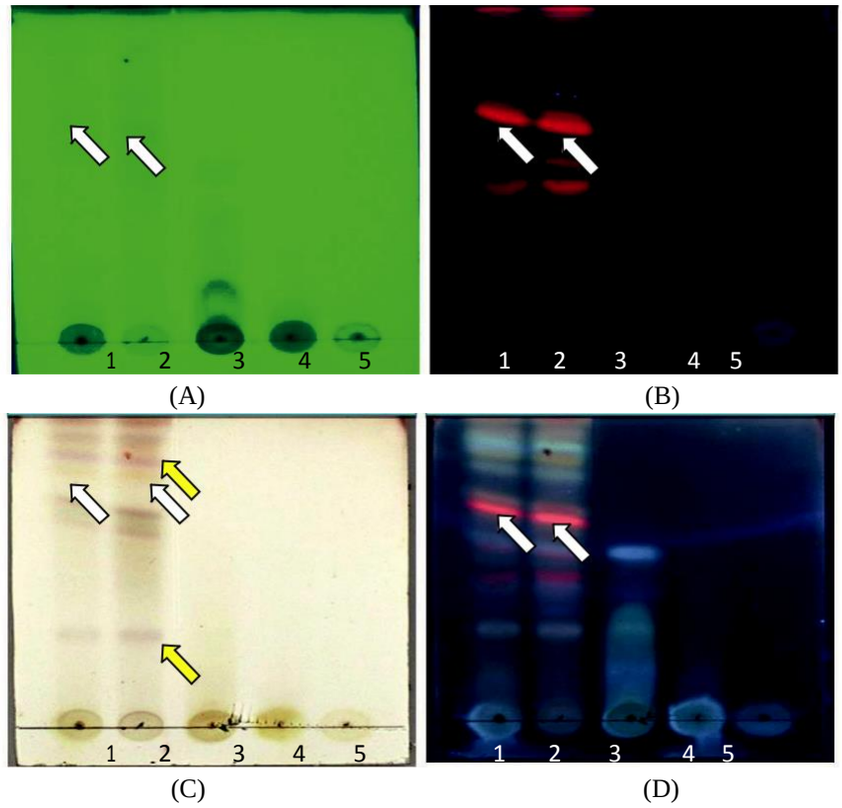

Figure 2. Thin Layer Chromatography profile of S. dulcis. Thin Layer Chromatography (TLC) profile of 1. 80% EE; 2. DCMF; 3. EAF; 4.BF; and 5. AF. The figures were observed in: A. Under UV 254 nm; B. Under UV 365 nm; C. Under visible lamp after sprayed using H2SO4 10% and heated at 105°C for 5 min; D. Under UV 365 nm after sprayed using H2SO4 10% and heated at 105°C for 5 min.

µg/ml and SI >3.97. The time addition experiment showed DCMF was inhibited on post-entry-step of HCV infection, it means the inhibition probably was on virus construction or/and virus release. Chlorophyll, terpenoids and flavonoids compounds in EE and DCMF were suspected to have a role as anti-HCV active agents.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

No conflict of interest of this paper.

REFERENCES

1. WHO. Hepatitis C 2002 [Available from: http://www.who.int/csr/disease/hepatitis/whocdscsrlyo2003/en/index.html.
2. Guidelines for the Screenin, Care, and Treatment of Person with Hepatitis C Infection [Internet]. WHO Geneva. 2014. Available from: http://www.who.int/hepatitis/publications/hepatitis-c-guidelines-2016/en/.
3. Wahyuni TS, Aoki C, Hotta H. Promising Anti-Virus hepatitis C Compounds from Natural Resources. Natural product communications. 2016; 11(8): 1193–200.
4. Manns MP, Foster GR, Rockstroh JK, Zeuzem S, Zoulim F, Houghton M. The way forward in VHC treatment — finding the right path. Nat Rev Drug Discov. 2007; 6: 991–1000.
5. Wahyuni TS, Tumewu L, Permanasari AA, Apriani E, Adianti M, Rahman A, et al. Antiviral activities of Indonesian medicinal plants in the East Java region against hepatitis C virus. Virology journal. 2013; 10: 259.

6. Lee J, Lim S, Kang SM, Min S, Son K, Lee HS, et al. Saponin inhibits hepatitis C virus propagation by up-regulating suppressor of cytokine signaling 2. PloS one. 2012; 7(6): e39366.
7. Murti K, Panchal M, Taya P, Singh R. Pharmacological Properties of Scoparia dulcis: A Review. Pharmacologia. 2012; 3(8): 3.
8. Ratnasooriya WD, Jayakody JR, Premakumara GA, Ediriweera ER. Antioxidant activity of water extract of Scoparia dulcis. Fitoterapia. 2005; 76(2): 220-2.
9. Le QU, Lay HL, Wu MC. Phenolic Composition, in Vitro Antioxidant and Anticancer Activities of Hypericum Japonicum Thunb and Scoparia Dulcis L. Herbal Medicine Journal. 2019; 4(1).
10. Jedage HD. Pharmacognostic, phytochemical investigation & pharmacological evaluation of scoparia dulcis linn. Plant extracts for nephro-protective activity. International Journal of Pharmaceutical Sciences and Research. 2014; 5(8): 3342–6.
11. Wankhar W, Srinivasan S, Rajan R, Rathinasamy S. Phytochemicals screening and antimicrobial efficacy of Scoparia dulcis Linn (Scrophulariaceae) against clinical isolates. Journal of Pharmacognosy and Phytochemistry. 2015; 3(6): 17–21.
12. Mishra MR, Behera RK, Jha S, Panda AK, Mishra A, Pradhan DK. A Brief Review on Phytoconstituents and Ethnopharmacology of Scoparia dulcis Linn. (Scrophulariaceae). International Journal of Phytomedicine 2011; 3: 422–38.
13. Krishnamurthy PT, Bajaj J, Sharma A, Manimaran S, Ravanappa PK, Pottekad V. Hepatoprotective activity of terpenoids and terpenoid fraction of Scoparia dulcis. L. Oriental Pharmacy and Experimental Medicine. 2010; 10(4): 163–270.
14. Freire SM, Torres LM, Souccar C, Lapa AJ. Sympathomimetic effects of Scoparia dulcis L. and catecholamines isolated from plant extracts. The Journal of pharmacy and pharmacology. 1996; 48(6): 624–8.
15. Hayashi K, Niwayama S, Hayashi T, Nago R, Ochiai H, Morita N. In vitro and in vivo antiviral activity of scopadulcic acid B from Scoparia dulcis, Scrophulariaceae, against herpes simplex virus type 1. Antiviral research. 1988; 9(6): 345–54.
16. Kuriakose J, Ramamurthy N. Antiviral Effect of Scoparia dulcis against coxsackie B1-B6 virus. 23nd Swadeshi Science Congress 2013.
17. Riel MA, Kyle DE, Milhous WK. Efficacy of scopadulcic acid A against Plasmodium falciparum in vitro. Journal of natural products. 2002; 65(4): 614–5.
18. Nishino H, Hayashi T, Arisawa M, Satomi Y, Iwashima A. Antitumor-promoting activity of scopadulcic acid B, isolated from the medicinal plant Scoparia dulcis L. Oncology. 1993; 50(2): 100–3.
19. Nkembo KM, Lee JB, Hayashi T. Selective enhancement of scopadulcic acid B production in the cultured tissues of Scoparia dulcis by methyl jasmonate. Chem Pharm Bull (Tokyo). 2005; 53(7): 780–2.
20. Wu WH, Chen TY, Lu RW, Chen ST, Chang CC. Benzoxazinoids from Scoparia dulcis (sweet broomweed) with antiproliferative activity against the DU-145 human prostate cancer cell line. Phytochemistry. 2012; 83: 110–5.
21. Ahsan M, Islam SK, Gray AI, Stimson WH. Cytotoxic diterpenes from Scoparia dulcis. Journal of natural products. 2003; 66(7): 958–61.
22. Hafid AF, Permanasari AA, Tumewu L, Adianti M, Aoki C, Widyawaruyanti, et al. Activities of Ficus fistulosa Leave Extract and Fractions Against Hepatitis C Virus. Procedia Chemistry. 2016; 18: 179–84.
23. Apriyanto DR, Aoki C, Hartati S, Hanafi M, Kardono LB, Arsianti A, et al. Anti-Hepatitis C Virus Activity of a Crude Extract from Longan (Dimocarpus longan Lour.) Leaves. Japanese journal of infectious diseases. 2016; 69(3): 213–20.
24. Aoki C, Hartati, S., Santi, M.R., Lydwina, T., Firdaus, R., Hanafi, M., Kardono, L.B.S., Shimizu, Y., Sudarmono, P., Hotta, H. Isolation and identification of substances with anti-hepatitis C virus activities from Kalanchoe pinnata. Int J Pharm Pharm Sci. 2014; 6(2).
25. Daniel M, Bhattacharya SD, Arya A, Raole VM. Analytical methods for natural dyes, Natural Dyes: Scope and challenges: Scientific Publishers; 2019. 3 p.

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Case Report

Recurrent Giant *Condylomata Acuminata* Caused by *Human Papilloma Virus* in HIV with Homosexual Male

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ABSTRACT

Perianal giant condylomata acuminate (GCA) is a rare clinical condition associated with low-risk Human papillomavirus (HPV) type 6 and 11 infections. Human Immunodeficiency Virus (HIV) infection is one of the risk factors for GCA, that can increase the condylomata acuminate incidence and spread caused by HPV. A 28-year-old man came with a cauliflower-like mass complaint in his perianal and anal since 2 months ago. The patient did not complain of pain or itching on the mass but often bled when defecating. The patient is a male who has sex with men (MSM) and often changes partners. He has been diagnosed with HIV since 11 months ago and regularly taking anti-retroviral drugs, Efavirenz 600 mg daily. He was also diagnosed having lung tuberculosis at the same time, got 6 months treatment and was declared cured. The venereological examination of the perianal and anal region revealed erythematous and grayish stem-shaped vegetation and papules, verrucous surface, multiple, well defined, with 3 x 1.5 x 2 cm in size. A positive act of white examination was obtained. Blood tests revealed CD^+ 4 230 cells /μL. Polymerase chain reaction (PCR) examination for HPV obtained HPV types 6 and 11 infections. Histopathologic examination revealed acanthosis, papillomatosis, and hyperkeratotic epidermis and koilocytotic cells. The patient was treated with electrodesiccation three times but obtained mass in anal getting bigger with a size of 6 x 3 x 3 cm. Therefore, he agreed to be referred to the surgical department with an extensive surgical excision plan. Screening of GCA using PCR is not a routine examination but PCR has high sensitivity and specificity for determining the type of HPV, is useful for determining GCA prognosis and therapy, and is recommended for malignant and possible GCA recurrence detection.

Keywords: *Giant condylomata acuminate, HPV, recurrent, HIV, MSM*

ABSTRAK

Perianal Giant condylomata acuminata merupakan kondisi klinis yang jarang dan dihubungkan dengan infeksi rekuren Human Papillomavirus (HPV) low-risk tipe 6 dan 11. Infeksi Human Immunodeficiency Virus (HIV) merupakan salah satu faktor risiko GCA, yang dapat meningkatkan risiko kejadian kondilomata akuminata dan penyebaran yang disebabkan oleh HPV. Laki-laki 28 tahun datang dengan keluhan benjolan seperti bunga kol di anus dan sekitar anus sejak 2 bulan yang lalu. Pasien tidak mengeluhkan nyeri maupun gatal pada benjolan tersebut, namun sering berdarah saat buang air besar. Pasien berhubungan seksual dengan sesama jenis dan sering berganti pasangan. Pasien telah didiagnosis HIV sejak 11 bulan yang lalu dan rutin minum anti-retroviral, Efavirenz 600 mg setiap hari.Pasien juga didiagnosis menderita tuberculosis paru pada saat yang bersamaan, mendapatkan 6 bulan terapi dan dinyatakan sembuh. Pemeriksaan venereologis pada regio perianal dan anal didapatkan vegetasi bertangkai serta papul-nodul eritematous dan keabu-abuan, permukaan verukosa, multipel, batas tegas, ukuran 3 x 1,5 x 2 cm. Pemeriksaan acetowhite positif. Pemeriksaan darah CD^+ 4 230 sel/μL. Pemeriksaan Polymerase chain reaction (PCR) untuk HPV, didapatkan hasil HPV tipe 6 dan

11. Pemeriksaan histopatologis didapatkan epidermis akantosis, papilomatosis, hyperkeratosis dan sel-sel koilositosis. Pasien diterapi dengan elektrodesikasi sebanyak 3x namun didapatkan benjolan semakin membesar dengan ukuran 6 x 3 x 3 cm sehingga pasien setuju dirujuk ke bagian bedah dengan rencana wide surgical excision. Skrining GCA dengan menggunakan PCR bukanlah pemeriksaan yang

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rutin dilakukan, namun pemeriksaan PCR ini mempunyai sensitivitas dan spesifitas tinggi untuk menentukan tipe HPV yang berguna untuk menentukan prognosis serta terapi GCA dan disarankan untuk deteksi keganasan serta deteksi kemungkinan rekurensi GCA.

Kata kunci: *giant condylomata acuminata, HPV, rekurensi, HIV, LSL*

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INTRODUCTION

The incidence of anogenital condylomata acuminata (CA) has increased in the past decades and is, to date, the most common sexually transmitted disease in Western countries. Condylomata acuminata is correlated with low-risk human papillomavirus (HPV) type 6 and 11 infections, whereas high-risk HPV type 16 is frequently present in anogenital malignant lesions.[1]

Perianal giant condyloma acuminatum (GCA) is a rare clinical condition related to HPV infection and characterized by a circumferential, exophytic, cauliflower-like mass with an irregular warty surface localized in the anal region.[1]

The giant form of this disease has a rare event rate, no more than 0.1% genital warts. Most of the incidence attacks middle-aged men, with a male-to-female ratio at 3:1.[2] Risk factors for GCA include anoreceptive intercourse, Human Immunodeficiency Virus (HIV), and immunosuppression.[3] Human Immunodeficiency Virus infection is a predisposition that increases the CA incidence and spread caused by HPV.[4]

Many examination techniques are used to detect HPV infection. To mention one is polymerase chain reaction (PCR) technique. By PCR, it is now possible to amplify enzymatically specific target Deoxyribonucleic acid (DNA) sequences to higher levels so that they are now readily detectable by additional methods to detect the type of HPV.[5] Detection and subsequent HPV types have a profound role in assessing the prognosis and therapy of genital lesions and evaluation of efficacy therapy.[5] Classification of HPV infection types is important for the identification of patients at risk of developing malignant transformation and for the detection recurrence rates of GCA.[5,17]

A case of giant condylomata acuminata caused by HPV types 6 and 11, identified by PCR techniques in a 28-year-old male patient with HIV-infected who had sex with men (MSM).

CASE REPORT

A man, 28 years old, came to dermato-venereology's outpatient clinic of Saiful Anwar Regional General Hospital (RSSA) Malang with a complaint of cauliflower-like mass on his anal and perianal since 2 months ago. It initially appeared as a small bump that got bigger in both anal and perianal, and some reddish and some brownish-gray in color. There was no itching or pain in the bumps. The cauliflower-like mass was rapidly enlarged. Three days before his visit, the patient felt difficult to defecate due to the mass getting bigger and bled after defecation, accompanied by an unpleasant odor.

The patient had a history of similar complaints 2.5 years ago, initially obtained small bumps around the anal, enlarged within a year. The bump in the anal was also getting bigger, and the patient complained often of bleeding after defecation. He checked to the private hospital and was diagnosed as "giant condylomata acuminate." He was referred to the surgical department in RSSA and performed surgery in August 2017 (6 months ago). The complaint reappeared 4 months later.

The patient had been diagnosed with HIV since March 2017, and routinely taking antiretroviral (ARV), Efavirenz 600 mg daily, from Internal Department's outpatient clinic of a private hospital in Malang. He was also diagnosed

with pulmonary tuberculosis (TB) at the same time, received complete TB treatment for up to 6 months and was declared cured in September 2017.

The patient has had sex with men (MSM) since his age of 17 years. The patient acts as a "bottom". He claimed to have had a pair of 7 men known through social media applications. The Patient and his couple rarely use condoms during intercourse. The last time he had sex was around 2.5 years ago. Currently, the patient works as an entrepreneur.

A general examination of the patient showed mild illness. Vital signs were within normal limits. Venereological examination of the corpus penis, glans penis, ostium urethra external, and scrotum was within normal limits. Preputium has been circumcised. The perianal and anal region revealed stemmed vegetation and erythematous to grayish papules, verrucous surfaces, multiple, well defined, varying in size with the largest size at 3 x 1.5 x 2 cm (Figure 1).

Acetowhite test using 5% acetic acid revealed the mass changed becoming paler. Blood and urinalysis examination revealed normal limits, while $CD^{+}4$ was 230 sel/µL. HPV DNA genotyping was performed using the PCR method, as tissue was taken from warts in the anal region. It found that the mass was due to types 6 and 11HPV infection.

Histopathologic examination taken from mucocutaneous lesions in the perianal region, found: acanthosis, papillomatosis, and hyperkerathosisepidermis. There were also koilocytosis cells, whereas in the dermis layer there was no abnormality. No malignancy was found in the tissues. The conclusion was a condylomata acuminata.

Having diagnosed as Giant Condylomata Acuminata, the patient was treated with electrodesiccation on genital warts in the perianal. Meanwhile, in anal warts due to extensive bleeding, electrodesiccation was done gradually. He was educated to routinely treat wounds and maintain hygiene.

The evaluation was done every two weeks. In the second week, evaluation for the rest of the electrodesiccation had dried up. After three times electrodesiccation, the mass in the anal region was getting bigger and bled easily with a size of 4 x 2.5 x 2.5 cm. Since the patient went abroad, the electrodesiccation was postponed.

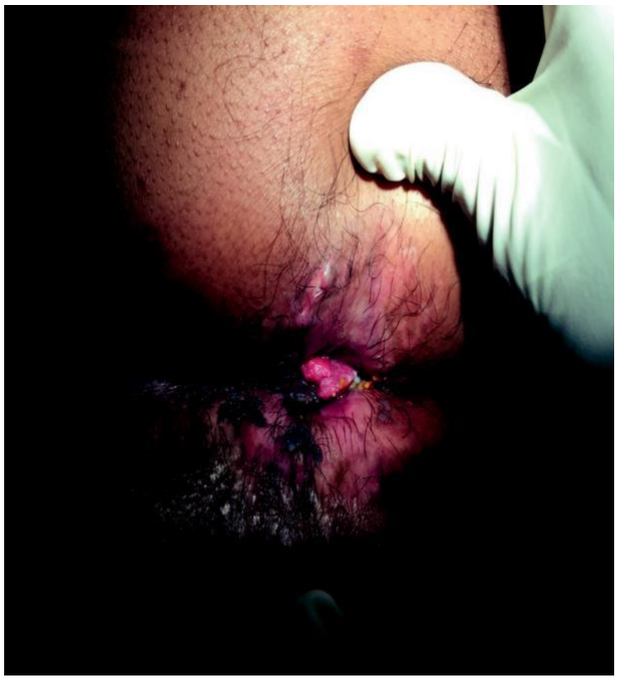

Figure 1. Anal and perianal region revealed cauliflower-like mass.

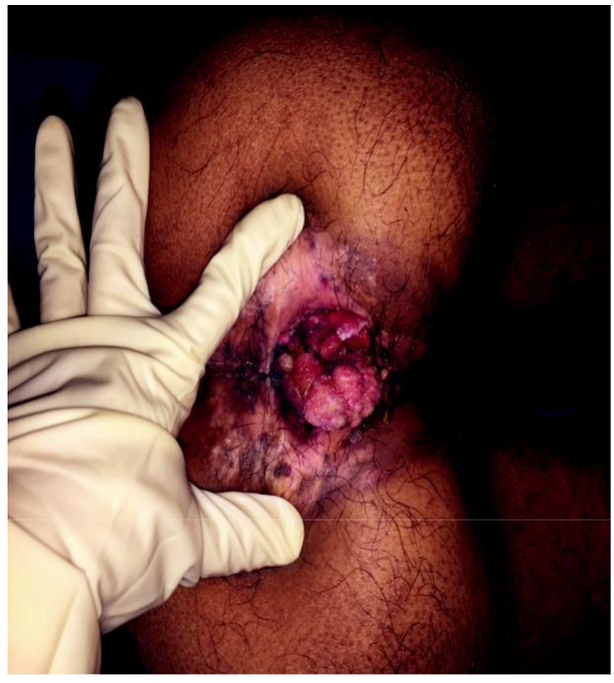

Figure 2. Follow up of the 5^{th} week, mass in the anal area grew larger by 6 x 3 x 3 cm.

At the follow-up, five weeks after the patient back from abroad, mass in the anal region grew larger by 6 x 3 x 3 cm (Figure 2). Therefore, the patient agreed to be referred to the surgical department with an extensive surgical excision plan.

DISCUSSION

Giant Condylomata Acuminata (GCA) is a slow-growing, large, cauliflower-like tumor with locally destructive behavior that typically appears in the anogenital region.[3,6,8] Originally described as a penile lesion by Buschke in 1896 and Löwenstein in 1925, it is a genital infection caused by Human Papillomavirus (HPV) types 6 and 11.[7] The first description of anorectal GCA was by Dawson et al. in 1965. Giant condylomata acuminata is a rare lesion tending to present in the fifth decade with a 2.7:1 male: female ratio. For patients under 50 years old, this ratio increases to 3.5:1.[3,8] In some cases, series of these lesions have a high recurrence rate of between 18% and 67%, with an overall mortality rate of 21%.[9] According to some literature, GCA is a low-grade and well-differentiated squamous cell carcinoma. Giant condylomata acuminata or verrucose carcinoma should be considered as a differential diagnosis in lesions larger than 1 cm.[7]

Risk factors of GCA include anoreceptive intercourse, HIV and immunosuppression. The prevalence of HPV infection in the anal is very high, around 57% in men with Human immunodeficiency virus (HIV)-negative who have sex with men (MSM); and among people, with HIV-positive infections, the incidence rate is about 60 times higher than in the general male population.[9]

In this case report, the patient is experiencing an MSM for approximately 8.5 years, acted as a "bottom" and rarely used condoms. There were lesions in the form of stemmed vegetation with the largest size of 3 x 1.5 x 2 cm in the perianal and anal region. The patient was also diagnosed with HIV-positive and took ARV daily.

The anal disease is a common disease in patients with HIV infection, especially in MSM patients.[10] Anal HPV infection and anal intraepithelial neoplasia (AIN) are more common in HIV-positive compared to HIV-negative MSM.[11] Recurrent anal condylomata are stronger with HIV and CD^+4 lymphocytopenia compared to HPV persistence indicating that people with HIV-negative can clear the virus more easily.[12]

Presumably, there is a complex interaction between HIV, HPV and local mucosal immune mechanisms. HIV increases HPV transcription and resets HPV E7 which affects cellular differentiation, leading to higher amounts of HPV DNA in the tissue.[9] Furthermore, HPV causes a decrease in the number of local macrophages, Langerhans and CD^+4 cells and decreases local cytokine production, which results in impaired local immunity control against HPV infection.[9]

Since HIV appears to increase HPV replication, one would expect that antiretroviral therapy initiation with future suppression of HIV viral load should lead to a decrease in the amount of HPV in the infected mucosa, followed by clinical improvement. It has been reported that a paradoxical case illustrates the impairment of GCA as a consequence of immune reconstitution syndrome after ARV, in patients with low CD^+4 counts at the beginning of treatment (50 / mm3).[13] A study of HIV positive women showed that antiretroviral drugs could reduce the incidence of genital warts and vulvar intraepithelial neoplasia and this effect was mediated through an increase in CD^+4 count and HIV viral load reduction.[14]

Histologically, GCA appears to be similar to condyloma, but grows both upward and downward and indicates a local invasion.[15] In a limited biopsy, the pathologist may only see hyperkeratotic benign epithelium, but the fully developed lesions exhibit an exophytic and endophytic growth pattern.[15] Knowledge of HPV is obtained through several examinations such as cytological examination, histopathology, immunohistochemistry, molecular hybridization, and PCR.[16]

Polymerase chain reaction techniques have high sensitivity and specificity. They can be used to amplify and sequence DNA viral processes and to determine the type of HPV that is defined as DNA sequence homology. PCR

examination requires only 10 copies of HPV.[16] Because information on the type of HPV is clinically useful for prognosis and treatment of condyloma, molecular epidemiology of HPV using the PCR method has been widely used. Clinical classification of HPV types is important for identifying patients at risk of developing malignant transformation and detection risk of GCA recurrence.[7]

The result of the PCR examination of the patient showed that his GCA was caused by multiple infections, namely types 6 and 11 HPV. Cong X et al. (2015)[17] conducted a study of HPV type correlation and clinical features in patients with CA in China and found out that multiple HPV infection results in the formation of larger-size ofCA (GCA) and associated with higher recurrence rates, and extended disease course.[17] This corresponds to a patient's history that 6 months ago the patient had undergone surgery at RSSA for his GCA in the anal region and then started growing again 4 months later.

The patient, in this case, was then referred to the surgical department for wide surgical excision. The treatment choice for the management of GCA is considered a wide surgical excision.[18] Surgical excision alone has been shown to result in a disease-free state in up to 46% of cases.[18,19] Oral and topical chemotherapeutic modalities can be used as an adjuvant, to surgery. Some factors that need to be taken into account during treatment choice include the size and thickness of the lesion, anatomic site, associated HPV subtype, and immune status.[18,19,20,21]

The direct-applied modalities that are targeted to remove warts locally do not destroy all the very small or subclinical lesions in the surrounding healthy-looking skin and this may be the cause of recurrence.[20,21,22]

The polymerase chain reaction was not a routine examination for GCA. Nevertheless, HIV-infected men with anal condylomatous lesions were at high risk of having high-grade squamous intraepithelial lesions and harboring multiple HPV infections involving high-risk HPV types in the canal anal in comparison to HIV-infected men without condylomata. These data emphasize the importance of screening and follow-up of condylomata in the anal canal in HIV-infected men. One of the screenings is using PCR to determine the type of HPV.[23,24,25]

CONCLUSIONS

The 28-year-old male patient, MSM, has been reported with recurrent giant condylomata acuminata and HIV positive. The patient was then referred to the digestive surgical department for wide surgical excision. Recurrent GCA in this patient may root in his immunosuppression condition due to HIV infection, multiple infections of some HPV types, or previous operations that were not completely clean. Polymerase chain reaction genotyping of HPV DNA obtained types 6 and 11HPV. Screening of GCA using PCR is not a routine examination but it is very important to determine prognosis, therapy and possible of GCA recurrence.

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CONFLICT OF INTEREST

There is no conflict of interest of this study.

REFERENCES

1. Guttadauro A, Chiarelli M, Macchini D, Frassani S, Maternini M, Bertolini A, Gabrielli F. Circumferential anal giant condyloma acuminatum: a new surgical approach. *Diseases of the Colon & Rectum*. 2015; 58(4): e49–52.
2. Akhavizadegan H. Electrocautery resection, shaving with a scalpel, and podophyllin: a combination therapy for giant condyloma acuminatum. *The world journal of men's health*. 2015; 33(1): 39–41.
3. De Toma G, Cavallaro G, Bitonti A, Polistena A, Onesti MG, Scuderi N. Surgical management of perianal giant condyloma acuminatum (Buschke-Löwenstein tumor). *European Surgical Research*. 2006; 38(4): 418–22.
4. Murtiastutik D. Kelainan Infeksi Menular Seksual pada Infeksi HIV. Dalam: Barakbah J, Lumintang H,

Martodihardjo S, editors. *Buku Ajar Infeksi Menular Seksual*. Surabaya: Airlangga University Press; 2008. h. 260–268.
5. Mills A, Balasubramaniam R, Longacre T, Kong C, Pinsky B. Laboratory-Developed L1 Sequencing and Type-Specific, Real-Time Polymerase Chain Reaction for the Detection and Typing of Human Papillomaviruses in Formalin-Fixed, Paraffin-Embedded Tissues. Archives of Pathology & Laboratory Medicine. 2013; 137(1): 50–54.
6. Chao MW, Gibbs P. Squamous cell carcinoma arising in a giant condyloma acuminatum (Buschke-Lowenstein tumor). *Asian Journal of Surgery*. 2005; 28(3): 238– 40.
7. Komohara Y, Jinushi M, Takeya M. Clinical significance of macrophage heterogeneity in human malignant tumors. Cancer Science. 2013; 105(1): 1–8.
8. Papiu HS, Dumnici A, Olariu T, Onita M, Hornung E, Goldis D, Aiordachioae G, Vasca V. Perianal giant condyloma acuminatum (Buschke-Löwenstein tumor). Case report and review of the literature. Chirurgia (Bucur). 2011 Jul 1; 106(4): 535–9.
9. Mudrikova T, Jaspers C, Ellerbroek P, Hoepelman A. HPV-related anogenital disease and HIV infection: not always 'ordinary' condylomata acuminata. *Neth J Med*. 2008; 66(3): 98–102.
10. Bazouti S, Zizi N, Dikhaye S. Perianal giant condyloma Acuminatum-Buschke-Löwenstein tumor. La Presse Médicale. 2019; 48(5): 584–585.
11. Marks D, Goldstone S. Electrocautery Ablation of High-Grade Anal Squamous Intraepithelial Lesions in HIV-Negative and HIV-Positive Men Who Have Sex With Men. JAIDS Journal of Acquired Immune Deficiency Syndromes. 2012; 59(3): 259–265.
12. Arany I, Evans T, Tyring SK. Tissue-specific HPV expression and downregulation of local immune responses in condylomas from HIV seropositive individuals. *Sexually transmitted infections*. 1998; 74(5): 349–53.
13. Moussa R, Stephenson I, Fisk P, Dhar J, Nicholson KG, Wiselka MJ. Buschke–Loewenstein lesion: another possible manifestation of immune restoration inflammatory syndrome?. *Aids*. 2004; 18(8): 1221–3.
14. Massad LS, Silverberg MJ, Springer G, Minkoff H, Hessol N, Palefsky JM, Strickler HD, Levine AM, Sacks HS, Moxley M, Watts DH. Eff ect of antiretroviral therapy on the incidence of genital warts and vulvar neoplasia among women with the human immunodeficiency virus. *American Journal of Obstetrics & Gynecology*. 2004; 190(5): 1241–8.
15. Martin JM, Molina I, Monteagudo C, Marti N, Lopez V, Jorda E. Buschke-Lowenstein tumor. *Journal of dermatological case reports*. 2008; 2(4): 60.
16. Koutsky LA, Kiviat NB. Genital human papillomavirus. In: Holmes KK, Sparling PF, Lemon SM, Stamm WE, Piot P, Wasserheit JN, editors. *Sexually Transmitted Disease*. 3rd ed. New York: Mc Graw-Hills; 1999. p. 347–59
17. Cong X, Sun R, Zhang X, Wang Y, Wang L, Yu Y. Correlation of human papillomavirus types with clinical features of patients with condyloma acuminatum in China. *International journal of dermatology*. 2016; 55(7): 775–80.
18. Lilungulu A, Mpondo BC, Mlwati A, Matovelo D, Kihunrwa A, Gumodoka B. Giant Condyloma Acuminatum of Vulva in an HIV-Infected Woman. *Case reports in infectious diseases*. 2017; 2017.
19. Mistrangelo M, Cornaglia S, Pizzio M, Rimonda R, Gavello G, Dal Conte I, Mussa A. Immunostimulation to reduce recurrence after surgery for anal condyloma acuminata: a prospective randomized controlled trial. *Colorectal Disease*. 2010; 12(8): 799–803.
20. Silvera RJ, Smith CK, Swedish KA, Goldstone SE. Anal condyloma treatment and recurrence in HIV-negative men who have sex with men. *Diseases of the Colon & Rectum*. 2014; **57(6)**: 752–61.
21. Leszczyszyn J, Lebski I, Lysenko L, Hirnle L, Gerber H. Anal warts (condylomata acuminata)-current issues and treatment modalities. *Adv Clin Exp Med*. 2014; 23(2): 307–11.
22. Ockenfels HM. Therapeutic management of cutaneous and genital warts. JDDG: Journal der Deutschen Dermatologischen Gesellschaft. 2016 Sep; 14(9): 892–9.
23. Thomas R, Steben M, Greenwald Z, Stutz M, Rodier C, DeAngelis F, Rampakakis E. Recurrence of human papillomavirus external genital wart infection among high-risk adults in Montréal, Canada. Sexually transmitted diseases. 2017 Nov 1; 44(11): 700–6.
24. Darwich L, Cañadas MP, Videla S, Coll J, Piñol M, Cobarsi P, Molina-López RA, Vela S, García-Cuyás F, Llatjos M, Sirera G. Condylomata, cytological abnormalities and human papillomavirus infection in the anal canal in HIV-infected men. HIV medicine. 2012 Oct 1; 13(9): 549–57
25. Zou H, Tabrizi SN, Grulich AE, Hocking JS, Bradshaw CS, Cornall AM, Morrow A, Prestage G, Law MG, Garland SM, Chen MY. Site-specific human papillomavirus infection in adolescent men who have sex with men (HYPER): an observational cohort study. The Lancet Infectious Diseases. 2015 Jan 1; 15(1): 65–73.

Indonesian Journal of
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