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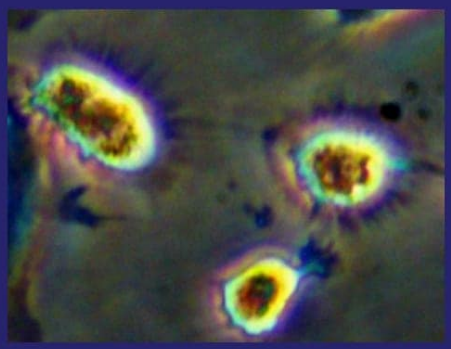

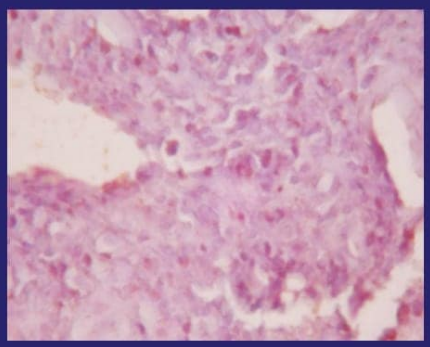

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CONTENTS

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Research Report

ANALYSIS ON SECONDARY INFECTION-TRIGGERING MICROORGANISMS IN HIV/AIDS PATIENTS AS A MODEL FOR POLICY CONTROL

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ABSTRACT

HIV infection is associated with immune-compromised and rising in opportunistic infection (secondary infection). Therefore, the number of mortality caused by HIV/AIDS is increasing. The use of ARV and development of HIV/AIDS management are expected to suppress the progress of HIV infection into AIDS and, therefore, the mortality can be diminished, while in fact most of the patients eventually suffer from AIDS due to secondary infection that commonly causes death. There should be a management by analysing microorganisms that trigger secondary infection. The method of this study was observational descriptive with cross sectional design. HIV infected blood samples were using ELISA Antibody (IgG and IgM) and Polymerase Chain Reaction (PCR) on laboratory test. The result showed correlation between HIV/AIDS severity and the amount and types of secondary infection. The most common secondary infections were toxoplasm (96.77%), hepatitis C (22.58%), tuberculosis (19.35%), and hepatitis B (3.22%). Other less frequent secondary infections, which were quite difficult to diagnose and not commonly found in Indonesia, were West Nile Virus (25.81%), Japanese Encephalitis Virus (3.22%), and Enterovirus (3.22%). Due to MDGs (Millenium Development Goals) target and the results above, researchers are highly demanded to contribute in decreasing mortality related to AIDS through early detection of secondary infection, including type of infection which have not been commonly found in Indonesia, such as West Nile Virus and Nipah Virus. The discovery of secondary infection in this study was not enough to suppress the occurrence of infection in HIV/AIDS patients. Antimicrobes and good nutrition are required. Moreover, there should be either a primary or secondary prophylaxis to prevent secondary infection that raises the number of mortality and morbidity of HIV/AIDS patients. The result of this study was to meet the target of MDGs by establishing new policies in handling HIV/AIDS infections and have potential as model for policy control in HIV/AIDS.

Key words: Microorganisms, secondary infection, HIV/AIDS, model, policy control

ABSTRAK

Infeksi HIV berkaitan dengan immune-compromised dan peningkatan infeksi oportunis (infeksi sekunder). Oleh karena itu angka kematian yang disebabkan HIV/AIDS semakin meningkat. Penggunaan ARV dan pengembangan penatalaksanaan HIV/AIDS diharapkan dapat menekan perkembangan HIV menjadi AIDS. Oleh karena itu tingkat kematian pun dapat berkurang, meskipun pada kenyataannya mayoritas pasien pada akhirnya mengidap AIDS karena infeksi sekunder yang umumnya mengakibatkan kematian. Diperlukan adanya sebuah penatalaksanaan dengan menganalisa mikroorganisme yang memicu terjadinya infeksi sekunder. Metode yang digunakan pada kajian ini merupakan pengamatan deskriptif dengan desain bagi silang. Sampel darah yang terinfeksi HIV dilakukan uji laboratorium menggunakan antibodi ELISA (IgG dan IgM) serta Polymerase Chain Reaction (PCR). *Hasil penelitian menunjukkan adanya korelasi antara tingkat keparahan HIV/AIDS dengan jumlah dan jenis infeksi sekunder. Infeksi sekunder yang paling umum terjadi ialah toksoplasma (96.77%), hepatitis C (22.58%), tuberkulosis (19.35%) dan hepatitis B (3.22%). Infeksi sekunder lainnya dengan frekuensi lebih rendah yang jarang ditemui di Indonesia saat ini adalah Virus West Nile (25.81%), Virus Japanese Encephalitis (3.22%) and Enterovirus (3.22%). Berdasarkan target Millenium Development Goals (MDG) dan hasil penelitian tersebut di atas, peneliti sangat*

dituntut untuk berkontribusi dalam menurunkan tingkat kematian yang berkaitan dengan AIDS melalui deteksi dini infeksi sekunder, termasuk jenis infeksi yang belum lazim ditemui di Indonesia seperti Virus West Nile dan Virus Nipah . Penelitian infeksi sekunder dalam kajian ini belum cukup untuk menekan terjadinya infeksi pada pasien HIV/AIDS. Antimikroba dan gizi yang baik sangat diperlukan. Selain itu diperlukan adanya profilaksi baik primer maupun sekunder untuk mencegah infeki sekunder yang dapat meningkatkan angka kematian dan morbiditas pasien HIV/AIDS. Hasil dari kajian ini adalah untuk memenuhi target MDGs dengan mengadakan kebijakan baru dalam penanganan infeksi HIV/AIDS dan berpotensi sebagai model untuk kebijakan kontrol pada HIV/AIDS.

Kata kunci: *Mikroorganisme, infeksi sekunder, HIV/AIDS, model, kebijakan kontrol*

INTRODUCTION

HIV infection is associated with decreased endurance and increased incidence of opportunistic infections that in a given period of time raises a set of disease called Acquired Immunodeficiency Syndrome (AIDS).[1] Human Immunodeficiency Virus (HIV) remains a global health problem, including in Indonesia. World Health Organization (WHO) reported that 2001 up to 58 million people worldwide have been infected with HIV, while in Indonesia until 2009 there were an estimated 186,000 HIV-positive people. The death rate from HIV/AIDS infection is reported quite high. Until 2000 it was reported that there were 22 million deaths related to HIV/AIDS.[2]

Indonesia was ranked first in the transmission of new cases of HIV and AIDS in Asia. Data from the Ministry of Health said there were 15,372 new HIV cases and 3541 new AIDS cases in January to September 2012. Majority of the patients were male in productive age. The highest transmission is through sexual contact, followed by needles and drug users, and it is reported that the number of patients is increasing sharply compared to ten years ago. Along with increased capacity for early detection, screening programs and increased public awareness of HIV disease, we will find more new cases. The area with the highest number of new cases is DKI Jakarta, followed by Papua and East Java.[3]

Currently, with the developing management of HIV/AIDS infection and increasingly widespread use of antiretroviral drugs, the progression of HIV infection to AIDS and death from AIDS should be reduced. In fact, most of the patients fell into AIDS as a result of the emergence of secondary infections (opportunistic infections) that often leads the patients to death.[1] In the decline of immune status, especially when the CD4 cells less than 200 cells/mL, a variety of microorganisms such as bacteria, viruses, protozoa and fungal infections also appear tend to be easy to grow and reproduce, causing secondary infections in the body of the people with HIV/AIDS.[4] The lower the CD4 cell count, the more types of microorganisms involved in secondary infection of HIV/AIDS. Fungal infections can occur simultaneously with bacterial infections, viruses and protozoa.[2,5] The main problem faced by people with HIV/AIDS is an opportunistic infection caused by a secondary infection.[6] The more advanced the severity of HIV/AIDS, the more increasing the potential incidence of secondary infections and death. Analysis of microorganisms triggering the secondary infection, as is often seen in people with HIV/AIDS and other viruses, is associated with CD4 count and viral load. Some secondary infections include CMV (Cytomegalovirus), Mycobacterium tuberculosis, West Nile virus, hepatitis B and C virus, and Candida sp.[7,8] It was not clear whether any people with HIV/AIDS will be infected by all these microorganisms.

Patients with infections are often followed by clinical conditions, such as malnutrition and wasting syndrome, which also will result in a decrease in CD4 T lymphocytes count. The condition results in a decrease of T lymphocytes count in patients susceptible to the incidence of secondary infections (opportunistic infections), such as hepatitis C, hepatitis B, hepatitis C, CMV, toxoplasmosis, Japanese encephalitis, West Nile virus, Nipah virus, all of which can be detected with CD4 and HIV RNA viral load. In HIV patients with secondary infections the increase of HIV progression is taking place. Therefore, HIV management policies is including promotion, prevention of secondary and tertiary infections, and complete therapy in accordance with the MDGs 2014, which comprises the absence of HIV-related deaths, the absence of new infections and the absence of discrimination.

This study aims to analyze how far the correlation between HIV/AIDS severity with the involvement of the type and number of secondary infections. The results are expected to be a new policy on HIV/AIDS, thereby supporting the achievement of the MDG targets in the field of infectious diseases of HIV/AIDS is zero new infections, zero discrimination, and zero AIDS-related deaths. From the laboratory results can be seen how far the relationship between the severity of HIV/AIDS with the involvement of the type and number of secondary infections. Based on the analysis of microorganisms triggers the secondary infection, according MDGs (Millennium Development Goals) in the field of infectious diseases HIV/AIDS, the results of this study are expected to contribute in lowering AIDS deaths through early detection of secondary infection, including an infection that has not been commonly detected in Indonesia, West Nile Virus Infection and Nipah virus. The results of this study are also expected to be a new policy on HIV/AIDS, thus supporting the achievement of the MDG targets, and can generate a new reference in the information and health sciences in the form of a journal.

METHODS

This study was a descriptive observational using cross-sectional design. Blood samples were taken from HIV-infected patients in Hospital Universitas Airlangga (RSUA), and Infectious Disease Intermediate Care Unit (*Unit Perawatan Intermediet Penyakit Infeksi*, UPIPI) Dr Soetomo Hospital, then we conducted laboratory tests to determine secondary infections experienced by the patients. From the laboratory results, we could see how far the relationship between the severity of HIV/AIDS with the involvement of the type and number of secondary infections.

The laboratory tests were carried out at the Institute of Tropical Diseases (ITD), Universitas Airlangga. The study was conducted for three months. The population in this study was HIV-positive patients who have received antiretroviral therapy, whereas the samples in this study were part of the whole object under study who met the inclusion criteria. Criteria for inclusion in this study were as follows: willingness to involve, HIV-positive, and has received antiretroviral therapy. To obtain accurate results, this research was conducted using Antibody (IgG and IgM) ELISA and PCR.

RESULTS & DISCUSION

The number of patients included in this study was 31 patients. The mean age of patients in this study was 35.06 ± 11.20 years, with the youngest two years old and the oldest 54 years of age. The highest number of the patients in age group of 31–45 years was 22 patients (70.96%), the least in the age group of < 16 years was 2 patients (6.46%), whereas the age group of 16-30 years was 3 patients (9.67%), and 46–60 years was 4 patients (12.91%) (Figure 1).

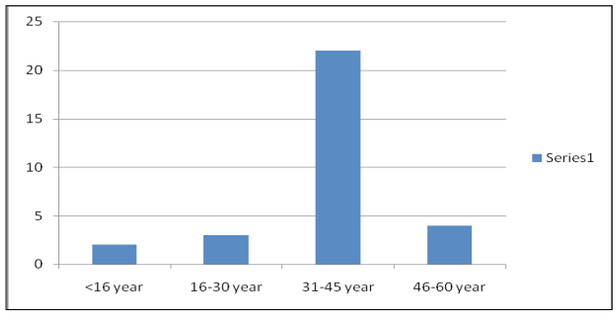

Figure 1. HIV patients distribution by age group.

Characteristics of the subjects by sex showed that the males were 15 patients (48.38%) and females 16 patients (51.62%). A total of 26 (83.87%) patients had married and 5 (16.13%) unmarried (Figure 2). Characteristics of study subjects based on tribes revealed Javanese of 27 (87.12%), Arabic 1 (3.22%), Chinese 1 (3.22%), Banjarese 1 (3.22%), and Tetungs 1 (3.22%).

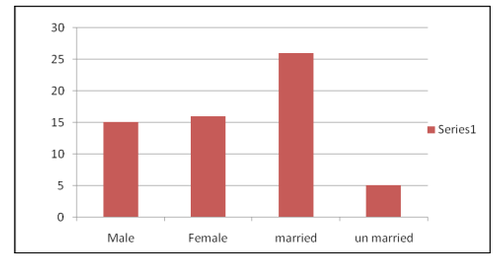

Figure 2. Distribution of HIV patients by sex and marital status.

In this study, HIV/AIDS transmission through sex was 21 patients (67.74%), through IDU (Intravenous Drug Users) was 8 (25.81%), and through vertical mother to child transmission (MTCT) was 2 patients (6.45%) (Figure 3).

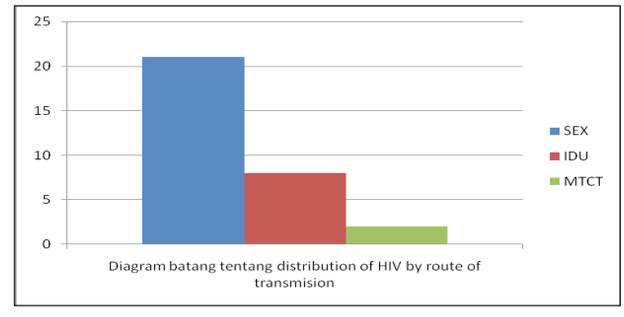

Figure 3. Distribution of HIV by Route of Transmission.

A total of 29 (93.54%) patients had received antiretroviral drug therapy and 2 (6.46%), while the rest had not received (Figure 4).

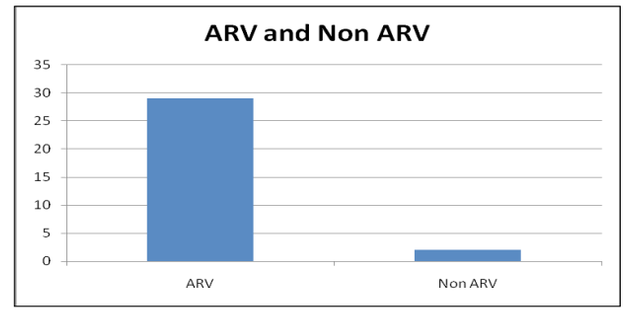

Figure 4. Distribution of HIV patients by antiretroviral therapy.

According to the length of antiretroviral therapy, 7 (24.14%) of the patients had received antiretroviral therapy for < 1 year, 11 (37.94%) patients for 1–3 years, 6 (20.68%) patients for 3–5 years and 5 (17.24%) patients for > 5 years (Figure 5).

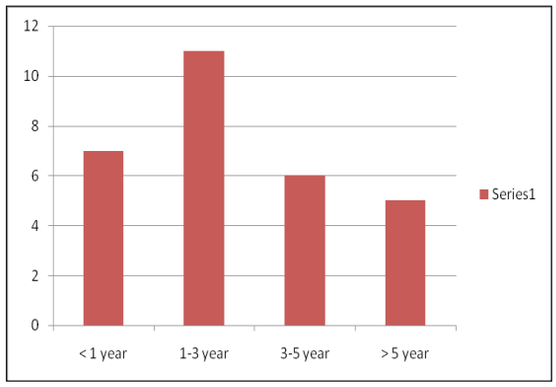

Figure 5. Distribution of HIV patients by the length of antiretroviral therapy.

Based on HIV/AIDS clinical stage according to WHO in 2010, this study found that 13 (41.94%) of the patients were at stage I, 11 (35.48%) patients at stage II, 7 (22.58%) patients at stage III and there were no patients at stage IV (Figure 6).

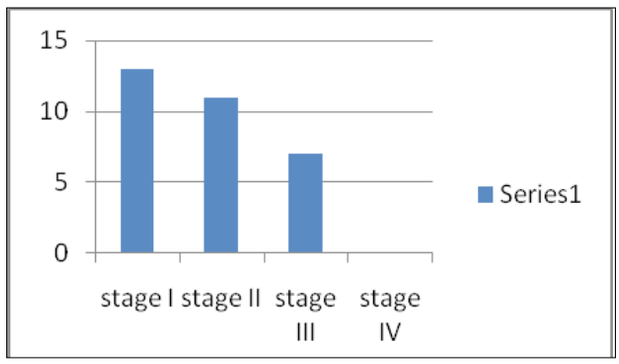

Figure 6. Distribution of patients based on WHO's clinical stage of HIV/AIDS.

In this study, most patients with undetected HIV viral load test results were 23 (74.19%) patients. Results of viral load < 4 × 102 copies/mL were in 2 (6.45%) patients and viral load > 4 × 102 copies/mL were in 5 (16.12%) patients (Figure 7).

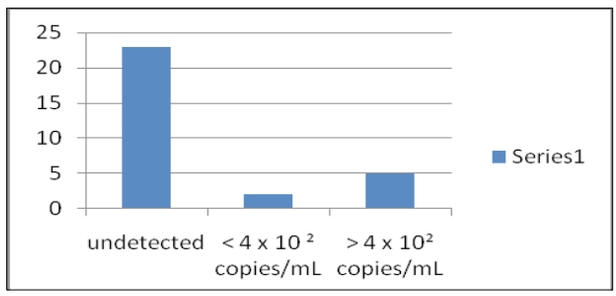

Figure 7. Patients distribution by HIV viral load.

Secondary Infection in Subjects Research

In this study we performed examination of secondary infections in people with HIV/AIDS. The results showed that 6 patients (19.35%) of the patients were with secondary infection of tuberculosis of 7 (22,58%) patients of the patients had secondary infection of hepatitis C, 1 (3.22%) of the patients had secondary infection of hepatitis B, 30 (96.77%) with secondary infections Toxoplasma, 8 (25.81%) with West Nile Virus, 1 (3.22%) patients with Japanese encephalitis virus, 2 (6.45%) patients with Enteroviruses, and 1 (3.22%) patients with secondary infections of dengue virus. There were no patients with secondary infection of cytomegalovirus (Figure 8).

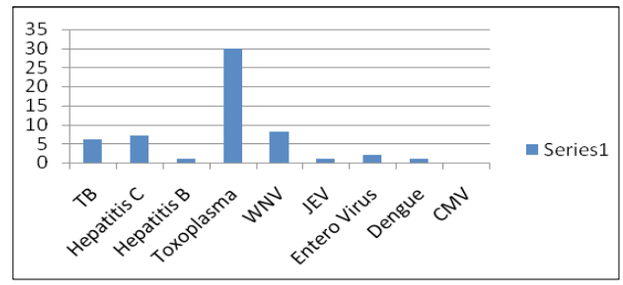

Figure 8. Distribution of secondary infection in the subjects.

Secondary Infection in the Subjects by HIV/AIDS Clinical Stage

In this study, we performed examination on secondary infections in people with HIV/AIDS. Based on the clinical stage according to the WHO in 2010 the secondary infections appeared on stage I was tuberculosis of 3 patients (23.07%), Hepatitis C of 4 patients (30.76%), West Nile Virus of 2 patients (15.38%), and Toxoplasma of 12 patients (92.31%). In stage II the secondary infections were Tuberculosis of 3 patients (27.27%), Hepatitis C of 1 patients (9.09%), Hepatitis B of 1 patients (9.09%), West Nile Virus of 6 patients (54.55%), Japanese encephalitis virus of 1 patients (9.09%), enterovirus of 1 patients (9.09%), dengue virus of 1 patients (9.09%) and Toxoplasma of 11 patients (100%). Whereas, stage II the secondary infections were Hepatitis C of 2 patients (28.57%), enterovirus of 1 patients (14.28%) and Toxoplasma of 7 patients (100%).[7]

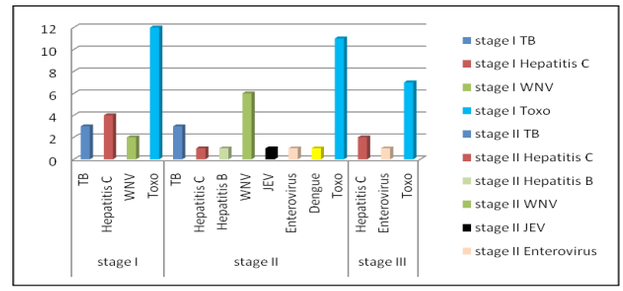

Figure 9. Distribution of secondary infection by HIV/AIDS Clinical Stage.

Secondary Infection in the Subjects by HIV Viral Load

We carried out examination of secondary infections in people with HIV/AIDS. Based on HIV viral load, the secondary infections in patients with HIV viral load < 4 × 102 copies/mL was toxoplasma of 2 (100%). Patients with HIV viral load > 4 × 102 copies/mL the secondary infections were tuberculosis in 2 (40.0%), hepatitis C 2 (40.0 patients, West Nile Virus of 1 patients (20.0%), Japanese encephalitis virus of 1 patients (20.0%), and enterovirus by 1 patients (20.0%), and Toxoplasma of 4 patients (80.0%). Whereas, in patients with undetectable HIV viral load, the secondary infections were tuberculosis in 4 (17.39%) patients, hepatitis C 5 (21.73%), hepatitis B 1 (4.34%), West Nile Virus 7 (30.43%), enterovirus 1 (4.34%), Dengue virus 1 (4.34%) and toxoplasma in 3 (100%) (Figure 10).

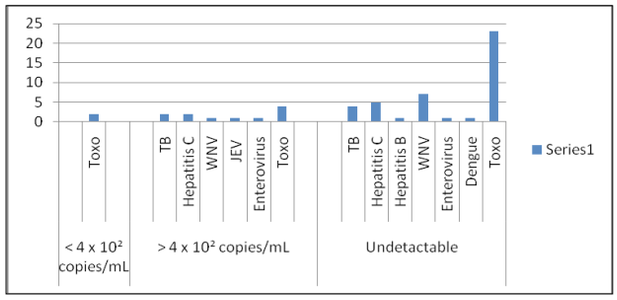

Figure 10. Distribution of secondary infection by HIV viral load.

Characteristics of the study population

In this study, the subjects were patients with HIV/AIDS of 31 patients. The mean age of the patients was 35.06 ± 11.20 years, with the youngest 2 years old and the oldest 54 years of age. The age distribution of the patients showed those of < 16 years were 6.46%, 16–30 years were 9.67%, 31–45 years were 70.96%, and aged 46–60 years were 12.91%. The proportion of males was 48.38% and females 51.52%, mostly (83.87%) were married and 16.13% unmarried. This figure showed that the incidence of HIV/AIDS infections is more common in reproductive age, which is along with the data from the Ministry of Health of Indonesia in 2011 that the highest percentage of HIV/AIDS was at the age of 20–29 years with a ratio of men and women 3:1.

Indonesia ranked first in the transmission of new HIV and AIDS cases in Asia. Data report of the Directorate General of PP & PL, the Ministry of Health, mentioned that there were 15,372 new cases and 3541 new cases of AIDS in January to September 2012. The majority of sufferers were of childbearing age and men. Highest transmission was through sexual contact followed through needles of drug users. The number increased significantly when compared to ten years ago, along with an increase in the ability of the government to detect, to carry out screening programs and increase public awareness of HIV disease, then there will be more new cases to be found. Areas with highest number of new cases are Jakarta, followed by Papua and East Java. Since 1999 a new phenomenon in HIV/AIDS dispersion occurred, that was the predisposition of transmission through blood contact, especially among Intravenous Drug Users (IDUs). Transmission in IDUs occurs rapidly due to the use of shared needles. In 2000 there was a significant increase in the spread of HIV pandemic among sex workers in Indonesia (Indonesian Ministry of Health, 2011). In this study, sexual transmission of HIV/AIDS was 67.74%, through IDU (Intravenous Drug Users) was 25.81%, and through mother-to-child vertical transmission or MTCT (Mother to Child Transmission) was 6.45%. This indicates that the highest incidence rates of HIV/AIDS transmission was through unhealthy sexual relationships.

The discovery of antiretroviral drugs (ARVs) in 1996 led to a revolution in the treatment of PLWHA (People Living with HIV and AIDS). Although antiretroviral therapy has not been able to cure the disease and the presence of major challenges in terms of side effects of drugs and the incidence of chronic resistance to antiretroviral drugs, such therapy can dramatically reduce mortality and morbidity, and improve the quality of life of people living with HIV. Currently HIV/AIDS has been accepted as a disease that can be controlled and no longer considered a dread disease.[2] In this study shown that antiretroviral therapy has been widely used in patients with HIV/AIDS in Indonesia, and with quite a number of study subjects who had received antiretroviral therapy for more than 5 years showed the role of ARVs in increasing the life expectancy of people with HIV/AIDS.

The findings in this research indicated that the majority of the study subjects were at an early stage (stage I and II) of HIV/AIDS infection. Undetectable viral load results indicated that the use of antiretroviral therapy in the majority of study subjects could control and suppress HIV/AIDS progress and improve the quality of life of the patients. This is in accordance with the policy on HIV/AIDS in Indonesia, which includes 4 pillars, all of which are aimed at bringing about a paradigm of zero new infection, zero AIDS-related death and zero discrimination:[9,10] (1) prevention: includes prevention of HIV transmission through sexual behavior and syringe, prevention in prisons and detention centers, prevention of mother-to-child transmission (PMTCT), prevention of transmission among sex workers and others, (2) Maintenance and support treatment (PDP): includes the strengthening and development of health services, prevention and treatment of opportunistic infections, ARV treatment, as well as support and education, training people living with HIV. PDP program is primarily intended to reduce morbidity and hospitalization, mortality related to HIV-AIDS and improve the quality of life of people living with HIV, (3) Mitigation of the impact of psychosocial and economic support, (4) creation of a conducive environment (creating the enabling environment) which includes institutional strengthening and management, program management and policy alignment.

With growing HIV/AIDS management infection and increasingly widespread use of antiretroviral drugs,

progression of HIV infection to AIDS and death from AIDS should have been suppressed. In fact, most of the patients fell into the emergence of AIDS as a result of secondary infections that often lead to death. Declining CD4 cell count to some extent (< 200 cells/mm^3) will open up opportunities for a secondary infection. The more advanced severity of HIV/AIDS, the more increase the potential incidence of secondary infection and death.[1] Based on Figure 8, there were no patients with secondary infection of cytomegalovirus. In this study, the encountered secondary infections were mostly Toxoplasma, as many as 96.77%. High toxoplasma infection in HIV/AIDS is related to the deterioration of the immune system.[11,12,13]

The parasite *Toxoplasma gondii* can reactivate again when CD4 lymphocyte count decreases to below 100 cells/mcl. The incidence of toxoplasma seroprevalence in a group of non-HIV individuals and groups of individuals with HIV/AIDS is almost the same, which is about 10–40%. In the United States, 67% of people with HIV/AIDS have positive Toxoplasma antibodies. However, the possibility of reactivation is 30% higher in people with HIV/AIDS.[11] Secondary infection of tuberculosis in this study was found to be 19.35%. This finding is in line with secondary tuberculosis infection data on HIV/AIDS. Tuberculosis is a secondary infection most often found in people with HIV and is the largest cause of morbidity and mortality in HIV infection in the world. More than 11 million HIV infections is accompanied with TB.[14,15,16] Thirty percent of is the cause of death in people with HIV is TB.[17] Data in UPIPI Dr. Soetomo Hospital showed that manifestations of AIDS due to secondary infection of pulmonary TB reaches 25–83%.[17]

Hepatitis B and hepatitis C are blood-borne diseases, together with HIV transmission. Both are secondary infections commonly found in people living with HIV who are injecting drug users (IDUs). Coinfection of hepatitis C and HIV among injecting drug users were 40–90%, whereas coinfection of hepatitis B and HIV in sexual transmission was 77%.[18] In this study, secondary infection of 3.22% with hepatitis B and hepatitis C was 22.58%. This is because the transmission of HIV infection in the study was largely through sexual transmission (67.74%) and through injecting drug use (IDU) (25.81%).

Another finding in this study was the secondary infection that is rare and often undiagnosed in Indonesia, such as Japanese encephalitis virus, West Nile virus (WNV), enterovirus, and dengue viruses.[19] In this study, secondary infection of West Nile Virus was found to be 25.81%, which is quite a high figure for a rare viral infection and rarely diagnosed in Indonesia. WNV infection is a viral infection that is transmitted through mosquito bites, self-limited with mild symptoms such as flu-like syndrome that can occur more severe in HIV co-infection with neurological manifestations such as meningoencephalitis. There has been no report on the epidemiological data of WNV and HIV coinfection rate. Only in the United States some cases of WNV and HIV co-infection was reported with manifestations of severe encephalitis.[20]

As WNV, Japanese encephalitis virus (JEV) is also a coinfection virus that can be found in HIV. Often found in Asian countries including Indonesia, JEV is a flavivirus transmitted by mosquito bite with severe neurological manifestations of encephalitis and high mortality rate up to 60%.[21] In this study, a secondary infection of JEV was found to be 3.22%. Although the data on JEV findings is low, these findings need attention because of the limitations of the study that was only in Surabaya (which is a reference to Eastern Indonesia). Thus the molecular epidemiological studies are necessary to get the database on JEV infections that accompany HIV/AIDS so that the mortality rate of patients with HIV/AIDS can be prevented early. Enterovirus is a virus that is identified as one of the causes meningoencephalitis in patients with HIV. Neurological deficits often appear along with a decrease in CD4 cell counts. More common in children, enteroviruses are often associated with complaints of diarrhea in people with HIV.[22]

Dengue virus has been reported to coinfect with HIV. With the decline in immune status in HIV and high infection rates in dengue endemic areas, the incidence of co-infection becomes possible.[23] There have been no reports of dengue and HIV coinfection rate, but in this study, the rate was found to be 3.22%. The findings of secondary infection in this study showed ARVs alone is not sufficient to reduce the incidence of secondary infection in HIV/AIDS, so that it requires antimicrobial therapy and adequate nutritional support. There should also be a primary or secondary prophylactic measures to combat secondary infections that can increase mortality and morbidity of patients with HIV/AIDS. Primary prophylaxis is given to prevent an infection that has never been suffered, while secondary prophylaxis is a treatment given to prevent the repetition of an infection which never been suffered before. For primary prophylaxis we can give cotrimoxazole tablets of 960 mg/day single dose for 2 years, while for secondary prophylaxis the treatment was given in accordance with arising secondary infections.

CONCLUSION

Toxoplasma (96.77%) which is the most common secondary infection is higher than other infection. The benefits of this research to the patients is that they know the type and number of secondary infections associated with the severity of HIV/AIDS suffered, so they may immediately seek treatment in order to have better prognosis. By proving relationships between HIV/AIDS severity and the involvement of microorganisms in HIV/AIDS secondary infection, we can take strategic policy to reduce the transmission rate of secondary infections and related deaths.

ACKNOWLEDGEMENT

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Research Report

OPTIMATION OF 48 kHz ULTRASONIC WAVE DOSE FOR THE INACTIVATION OF *Salmonella typhi*

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ABSTRACT

This study was aimed to determine the effect of ultrasonic dose exposure which could decrease the viability of Salmonella typhi by using the variation of exposure time (15, 20, 25, and 30 minutes) and volume of bacterial suspension (2, 4, 6, and 8 ml) at constant power. The sample used was Salmonella typhi. Ultrasonic wave transmitter was a piezoelectric tweeter with 0,191 watts of power and 48 kHz frequency generated by the signal generator. Piezoelectric tweeter was a kind of transducer which converted electrical energy into ultrasonic energy. This research was an experimental laboratory with a completely randomized design. The decrease of bacterial percentage was calculated by using TPC (Total Plate Count). Data were analyzed by using One Way Anova. The results showed that the variation of exposure time and volume of bacterial suspension gave significant effect on the percentage of Salmonella typhi kill. The most optimal of ultrasonic dose exposure to kill Salmonella typhi was 281.87 J/ml with 100% bacterial kill.

Key words: Ultrasonic dose exposure, ultrasonic wave, piezoelectric tweeter, Salmonella typhi, total plate count

ABSTRAK

Penelitian ini bertujuan untuk menentukan efek dosis paparan ultrasonik yang dapat mengurangi viabilitas Salmonella typhi dengan menggunakan variasi paparan waktu (15, 20, 25, and 30 menit) dan volume suspense bakteri (2, 4, 6, and 8 ml) pada kekuatan konstan. Sampel yang digunakan ialah Salmonella typhi. Transmiter gelombang ultrasonik ialah tweeter piezoelectric dengan daya 0,191 watt dan frekuensi 48 kHz yang dihasilkan oleh signal generator. Tweeter piezoelectric ialah sejenis tranduser yang mengubah energi listrik menjadi energi ultrasonik. Penelitian ini ialah percobaan laboratorium dengan desain random lengkap. Pengurangan persentase bakteri dihitung dengan menggunakan teknik pengujian total bakteri. Data dianalisis menggunakan Anova satu arah. Hasil menunjukkan bahwa variasi paparan waktu dan volume suspensi bakteri memberikan efek yang signifikan pada persentase Salmonella typhi yang mati. Dosis paparan ultrasonik untuk membunuh Salmonella typhi yang optimal ialah 281.87 J/ml dengan 100% bakteri yang mati.

Kata kunci: *Dosis paparan ultrasonik, gelombang ultrasonik, tweeter piezoelectrik, Salmonella typhi, pengujian total bakteri*

INTRODUCTION

Food is an important requirement for organisms because food serves as a source of carbohydrates, proteins, fats, vitamins, minerals, and other essential substances needed by organisms for growing process, developing process, and repairing damaged cells. Food and beverages consumed by humans must have good quality and free from pathogenic bacterial.

Pathogenic bacterial which often contaminate water, food, eggs and meat, fish and meat, milk and its processed products is Salmonella typhi.[1] Salmonella typhi is very dangerous because it is pathogenic to humans and causes fever.[2]

Most effort to obtain sterile food and beverage is using sterilization process. The method is used on sterilization process is heating. However, this method has the disadvantage because it reduces some nutrients contained

in the food during the sterilization process. Besides heating, another sterilization method which is often used is ultraviolet radiation that can cause mutagenic damage to DNA. Ultraviolet radiation is very harmful for humans when exposed directly.[3] Therefore, other alternatives are needed in the sterilization process that is ultrasonic wave's exposure.

Ultrasonic waves are very effective on materials sterilization process from bacterial,[4,5,6,7,8,9] This method is very safe because it is free from chemical substances and selectively to reduce bacterial viability without giving bad effects to humans and environment.

Ultrasonic wave exposure on bacterial suspensions showed that exposure time is proportional to the decrease of the number of Salmonella typhi colonies. The bacterial kill after ultrasonic wave's exposure occurs due to cavitation effects. Cavitation is the formation of bubble collapse which is a continuous stretch and eventually will be destroyed when it reaches the limit of its elasticity.[9] Ultrasonic wave's exposure on bacterial with causes mechanical stress on the bacterial cell wall so the cell wall stretches beyond the limits of its elasticity. Stretching of cell wall can lead to rupture of the cell wall, lyse, and ended in the death of the bacterial.[7] Cavitation occurs due to local pressure in the sound wave drops to a low enough pressure. It causes rupture of the cell as indicated by the following relationship:

$$\rho = P - P_0 \quad (1)$$

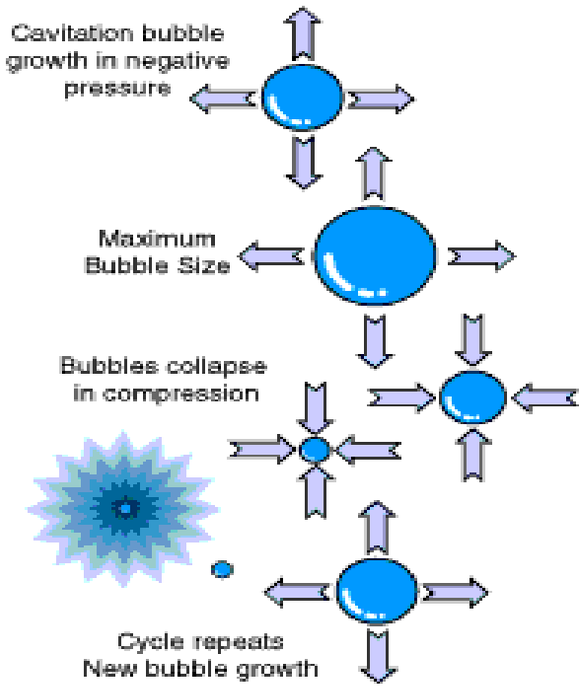

Figure 1. The mechanism of cavitation.[3]

ρ is the acoustic pressure, P is the total pressure, and P0 is the average local pressure. P value is always positive in the gas medium so that the amplitude of the acoustic pressure must be less than atmospheric pressure. While the liquid has a specific volume and can withstand the negative pressure. When the pressure is very low, the liquid will break up and form small cavities such as the ball is called cavity.[11]

Based on the characteristic of its formation, cavitation is distinguished on acoustic cavitation caused by ultrasonic wave and hydrodynamic cavitation caused by variations in fluid pressure.[10] The mechanisms of cavitation started by the formation of bubbles which get pressure from the outside so that the bubbles are unstable and eventually rupture.

Cavitation causes free radicals because of molecular bonds damage. For example H_2O molecule breaks into H^-, OH^-, and HO_2^- and eventually form H_2O_2 which can damage the chemical structure of the bacterial cell wall so the cell wall is weak and broken and the liquid from the outside enter the cell and lyse is occurred resulting in death of the bacterial.[8,11]

Besides cavitation, ultrasonic wave exposure can also increase temperature of the fluid due to acoustic energy imposed on a medium will be released back into heat. It causes temperature rising.

EXPERIMENTAL

Research Sample

The sample of *Salmonella typhi* was obtained from Institute of Tropical Desease, Airlangga University, Surabaya. The sample was grown in Nutrient Broth sterile medium for treatment and Salmonella Shigella Agar for TPC.

Expossure Equipment

Ultrasonic wave generator was a piezoelectric tweeter with 2 cm of diameter were fitted with 10 ohm resistor and generated by function generator (FG-350 IWATSU). Voltage and frequency issued by function generator were detected by using an oscilloscope type Protex 20 MHz (Oscilloscope 6502A). Scheme of the ultrasonic wave instrument is shown in Figure 2.

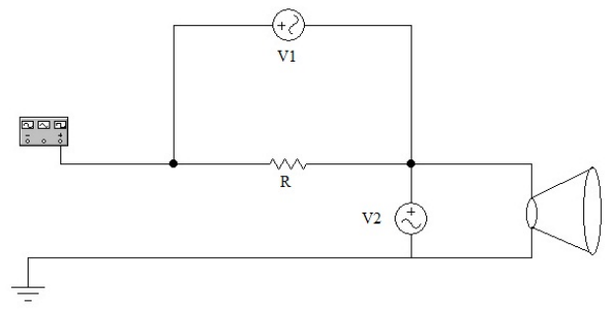

Figure 2. Scheme of ultrasonic wave instrument.

This research used square wave with 48 kHz of frequency and AC current.[4] The circuit of V_2 in Figure 1 is RC integrator circuit. RC integrator circuit is shown in Figure 3 below:

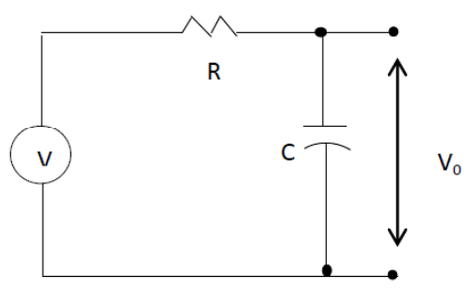

Figure 3. RC integrator circuit.[12]

Performance test which consist of calibration and measurement of fluid temperature rising were done before giving treatment on bacterial suspension. Calibration was done to calculate the voltage. That voltage was used to determine the electrical power which was converted into ultrasonic power by the transducer. The power output was calculated by Equation 2.

$$P = \frac{V_{rms_1} \times V_{rms_2}}{R} \quad (2)$$

Measurement of liquid temperature rising was performed to determine the effect of rising temperatures on the viability of Salmonella typhi because the bacterial could be killed at certain temperature. This method was used to determine whether the bacterial actually killed caused by the mechanical vibrations of ultrasonic waves or due to the heating effect. If the number of live bacterial colonies derived from ultrasonic wave exposure is fewer than heating, it means that bacterial kill caused by mechanical vibrations of ultrasonic waves.

The exposure of ultrasonic waves in liquids can increase the liquid temperature because acoustic energy imposed on a medium will be released back into heat, causing an increase in temperature of the fluid.

Besides heating, the exposure of ultrasonic waves in the sample can also cause mechanical vibration as the effects of cavitation. Ultrasonic wave's exposure on bacterial with causes mechanical stress on the bacterial cell wall so the cell wall stretches beyond the limits of its elasticity. Stretching of cell wall can lead to rupture of the cell wall, lyse, and ended in the death of the bacterial.[7]

Research Methods

This research conducted using completely randomized design. The first experiment was conducted using exposure time variation, these were 15 minutes, 20 minutes, 25 minutes, and 30 minutes[12] with a fixed volume was 2 ml to determine the optimum time with 100% of bacterial kill[7] at a fixed frequency that was 48 kHz.[4,7,13] Each treatment was accompanied by the control group using 5 times replication.

The percentage of bacterial kill was plotted in a graph to obtain the optimum time with 100% of bacterial kill by using linear regression equation (Equation 3).

$$y = m\,x + c \quad (3)$$

Where y was the percentage of bacterial kill up to 100%, x was the optimum time (minutes) required to kill the bacterial up to 100%.

This optimum time used to do the second experiment to determine the optimum dose for the 100% of bacterial kill. The second experiments were performed using a variation of the volume; these were 2 ml, 4 ml, 6 ml, and 8 ml with a fixed exposure time.

The Bacterial Growth

Salmonella typhi were cultured at Luria Bertani Broth sterile (Miller M1245-500 G). The bacterial cultures were incubated at 37°C of temperature for 18 hours. The dilution factor was qualify if the number of bacterial colonies that grew as much as 30–300 colonies, so this culture was incubated until OD600nm = 0.7 and the value of dilution up to 10^{-6} dilution (30–300 colonies). Bacterial dilution which was exposed by ultrasonic waves was cultured on sterile agar medium called Salmonella Shigella Agar (OXOID CM 0099) and incubated at 37°C of temperature for 18–24 hours.

Ultrasonic Wave Exposure on Bacterial

2 ml of bacterial suspension with 10^{-6} bacterial concentration was poured into a glass with 3 cm diameter and 4 cm of height and exposed by ultrasonic waves with a variation of exposure time 15, 20, 25, 30 minutes. The height of that suspension was approximately 3 mm. Exposure was done by dipping the piezoelectric tweeter into the bacterial suspension. The second experiments were performed using a variation of the volume, these were 2 ml, 4 ml, 6 ml, and 8 ml with a fixed exposure time which giving lethal dose 100% on bacterial. The bacterial in the treatment group and the control were grown on Salmonella Shigella Agar medium.

Counting the number of bacterial colonies

The bacterial colonies were counted by Total Plate Count Method using a Quebec Colony Counter. The next was calculating the percentage of bacterial kill by using Equation 4.

$$\%\ of\ bacterial\ kill = \left| \frac{\overline{control\ colony} - \overline{treatment\ colony}}{\overline{control\ colony}} \right| \times 100 \quad (4)$$

Statistical Analysis

This research data were analyzed by using SPSS (Statistical Package For Social Science) 20 that was one

way ANOVA for determining the effect of each factor. Multiple Comparison Post Hoc was used to determine the factors that most influence the percentage of bacterial kill.

RESULTS AND DISCUSSION

Design and Assembly Tool

The set up of experiment tool in this research is an integrator circuit which processes of charging and discharging capacitor were happened. The circuit has a time constant $\tau = RC \gg T$ so that when the capacitor was not fully charged, the voltage Vs has changed the sign to be negative. That leads to discharge the capacitor. The capacitor was charged by using negative charge up to -Vp. Before it was fully charged, the voltage Vs changed the sign. The process occurred repeatedly and forms a triangular output signal.[12] The results of electrical voltage measurements which were converted into an ultrasonic voltage by each of Piezoelectric Tweeter tabulated in a table (Appendix 1). The next step was combining the entire of Piezoelectric Tweeters into one so that the distribution of the power supplied by each transducer was the same. The results tabulated in a table (Appendix 2).

Based on the results of electrical voltage measurements which were converted into ultrasonic voltage by the entire of Piezoelectric Tweeters obtained an average power value in Appendix 2 was 0.191 Watt.

Measurement of liquid temperature rising

These measurements were performed by means of ultrasonic wave's exposure to the bacterial suspension and the rising of temperature occurred were measured. The ultrasonic exposure in 8 ml of bacterial suspension for 30 minutes increased temperature from 25°C to 27°C. Results of liquid temperature rising was plotted in a graph (Figure 4).

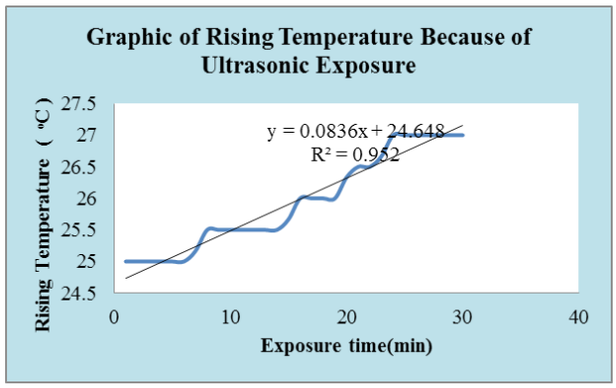

Figure 4. Graphic of Rising Temperature Because of Ultrasonic Exposure.

Bacterial exposure process using ultrasonic waves in that process has the potential to kill the bacterial *Salmonella typhi* up to 55.9%. The next step was to warm the bacterial suspension up to 27°C and obtained 0% of percentage killing of bacterial (didn't cause killing effect on bacterial). Based on these results, it was certain that the death of the bacterial was not due to the effect of rising temperature as a result of ultrasonic wave's exposure but due to the cavitation effect caused by the ultrasonic waves.

Ultrasonic Wave Exposure on *Salmonella typhi*

The results of this study is the decrease of *Salmonella typhi* colony due to exposure time variations of ultrasonic waves (15, 20, 25, and 30) min, volume variations (2, 4, 6, 8) ml, fixed frequency (48 kHz), and fixed power (0.191 W). The results of the study are shown in Figure 5 below.

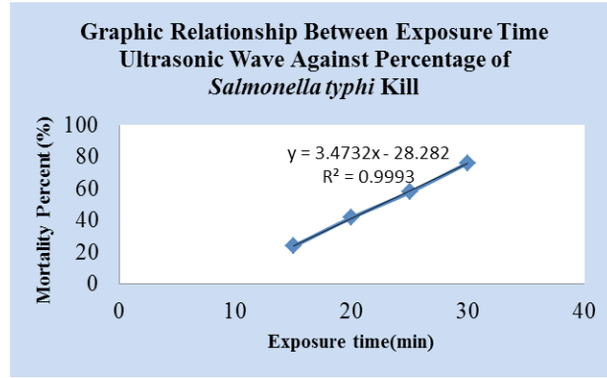

Figure 5. Graphic Relationship of Exposure Time Ultrasonic Wave against Percentage of *Salmonella typhi* Kill.

Equation 5 below is the linear regression equation obtained from Figure 5:

$$y = 3{,}4732\,x - 28{,}282 \qquad (5)$$

Percentage of bacterial kill (y%) obtained for exposure time x minutes with a gradient of 3.4732 and a constant of 28.282. Based on the linear regression equation, Lethal Dose 100% could be obtained by exposing for 36.94 minutes or 36 minutes 56 seconds. This time variation was the time that would be used in all subsequent experiments. The next experiment used variations of volume (2 ml, 4 ml, 6 ml, and 8 ml) with a fixed time (36 minutes 56 seconds). The results of the study are shown in Figure 6.

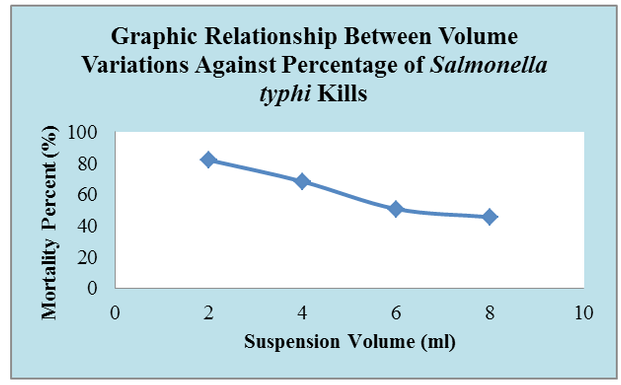

Figure 6. Graphic Relationship between Volume Variations against Percentage of *Salmonella typhi* kills.

The results were analyzed by using One Way ANOVA test to determine the effect of each factor. Terms of ANOVA test is interval and ratio scale data and normally distributed. Normality test performed using the Kolmogorov-Smirnov 1 sample. The test is used to compare the distribution of the data of the study sample with a theoretical distribution.

Results of Kolmogorov-Smirnov test showed a significance value P = 0.809 for time variation and P = 1.14 for volume variations. The test results showed that the data were normally distributed as P > α (0.05). Levene test results generated significant value for P = 0.101 and P = the time variation of 0.309 for variations in the volume so that it could be concluded that the variance of the data was homogeny, which means that the population had the same variance (uniform).

Summary of ANOVA test in Table 1 indicate that the time factor and the volume has a significance level of P = 0.000 is < 0.05, which means that the time factor and the volume effect on the decrease in the number of bacterial colonies.

$$D = \frac{E}{v} = \frac{P\ x\ t}{v} \quad (6)$$

Explanations:

D = dose (J/ml)
E = energy (J)
v = volume (ml)
p = power (W)
t = exposure time (s)

Based on *Salmonella typhi* research data obtained the percentage of bacterial kill at various energy doses which are tabulated in Table 2.

Table 1. Summary of One Way Anova test of ultrasonic exposure to percentage of *Salmonella typhi* kill

Factor	Group	N	Mortality Percent (%)		Anova	
			Average	SD	Significance	Summary
Time	15 min[a]	5	24	2.58135	0.000	There is a significant difference
	20 min[b]	5	42	6.47927		
	25 min[c]	5	59	7.46598		
	30 min[d]	5	76	1.11437		
Volume	8 ml[a]	5	46	0.61409	0.000	There is a significant difference
	6 ml[b]	5	51	0.24234		
	4 ml[c]	5	70	0.83264		
	2 ml[d]	5	82	0.76731		

(6)

Dose of Energy

Dose of energy is the energy of ultrasonic waves exposure which absorbed by the bacterial suspension. Basically, the emitted energy is electrical energy which is converted into mechanical vibration by the transducer. But there is proportionality between the electrical energy emitted by the ultrasonic energy received by a medium with a constant of proportionality *k*. Thus, ultrasonic energy received by the medium approaches the electrical energy emitted.

Mathematically, dose of energy is the result of power (P) times exposure time (t) divided by the volume of the bacterial suspension (v). The optimum dose exposure of ultrasonic wave on *Salmonella typhi* inactivation obtained from Equation 6 below.

Table 2. Results of the percentage of *Salmonella typhi* kills on the variation of ultrasonic wave exposure time and volume, frequency of 48 kHz, and a power of 0.191 W

Time (min)	Volume (ml)	Energy (J)	Dose (J/ml)	Percentage of bacterial kill (%)
15,00	2	171,90	85,95	23,68
20,00	2	229,20	114,60	41,76
25,00	2	286,50	143,25	57,80
30,00	2	343,80	171,90	76,22
36,94	2	423,33	211,67	81,98
36,94	4	423,33	105,83	68,27
36,94	6	423,33	70,56	50,72
36,94	8	423,33	52,92	45,52

The relationship between ultrasonic wave's doses exposures with the percentage of *Salmonella typhi* kill clarified by Figures 7 and 8 below.

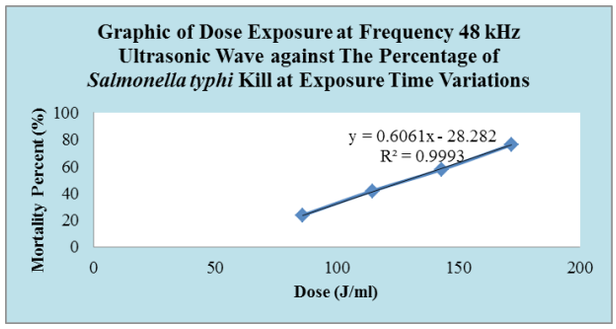

Figure 7. Graphic of Dose Exposure at Frequency 48 kHz Ultrasonic Wave against The Percentage of *Salmonella typhi* kill at Exposure Time Variations.

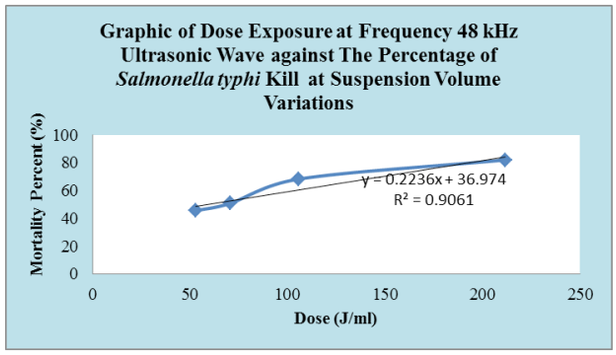

Figure 8. Graphic of Dose Exposure at Frequency 48 kHz Ultrasonic Wave against The Percentage of *Salmonella typhi* Kill at Suspension Volume Variations.

The percentage of *Salmonella typhi* kill at different doses ultrasonic waves with the volume variation plotted in linear regression to determine the dose with 100% bacterial kill. Figure 8 gives the linear regression equation on Equation 7 below.

$$y = 0{,}2236\,x + 36{,}974 \qquad (7)$$

If *y* is the percentage of bacterial kill up to 100% and x is the desired dose, the dose can be used to kill bacterial up to 100% is 281.87 J/ml.

CONCLUSION

The research results show that piezoelectric tweeter produces ultrasonic waves and the voltage generated by the signal generator. The optimum time exposure of ultrasonic waves which effectively decrease the viability of *Salmonella typhi* up to 100% is 36.94 minutes. The optimum volume of bacterial suspension is 2 ml with 81.78% of bacterial kill. The optimum dose exposure of ultrasonic waves which effectively decrease the viability of the bacterial *Salmonella typhi* is 281.87 J/ml with 100% of bacterial kill. The relationship between the dose of ultrasonic energy to the ultrasonic energy which produces mechanical vibration; ultrasonic power; and ultrasonic voltage are proportional. Dose of ultrasonic energy is proportional to ultrasonic energy where ultrasonic energy is a product of ultrasonic power with long time exposure. Ultrasonic power itself is the product of voltages generated by the transducer with long time exposure.

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Research Report

LYMPHOCYTE RESPONSE TO *Mycobacterium leprae* ANTIGENS IN REVERSAL REACTION STATE OF LEPROSY

An in vitro study of Lymphocyte Stimulation Index using MTT method

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ABSTRACT

Reversal Reaction (RR) in Leprosy is a sudden inflammatory episode in the chronic course of the disease due to rapid change of cellular immunological status. The aim of the study is to measure the in vitro results of Lymphocyte Stimulation Index (LSI) RR leprosy derived lymphocytes after challenged with M.leprae antigens. Twenty three Borderline Leprosy with RR and 11 Borderline Leprosy patients without RR were included in the study. Peripheral Blood Mononuclear Cells (PBMC) were separated from peripheral blood of these patients using Ficol-Hypaque column and cultured in laboratory. Using the colorimetric tetrazole (MTT) method these lymphocytes were challenged with PHA, Dharmendra antigen (1/100 and 1/10 dilutions), LAM (50 and 100 nanograms). Stimulation Index were calculated and superanatans were collected for measuring the IFN-γ and IL-10 production (ELISA). All of lymphocytes from RR patients showed higher Stimulation Index after challenged with the five M.leprae antigens compared to lymphocytes from non RR patients ($p < 0.05$) . IFN-γ and IL-10 also increased but not significant ($p > 0.05$). It is concluded that lymphocytes of leprosy patients during RR state are more sensitive to antigenic stimuli compared to non-RR leprosy patients. Further extended studies are needed to determine the "cut off" value of lymphocyte Stimulation Index that is useful for clinicians in the field in the prediction of RR before starting anti leprotic treatment.

Key words: Leprosy, reversal reaction, lymphocyte stimulation index, MTT, borderline type

ABSTRAK

Reversal reaction (RR) dalam kusta adalah periode inflamasi mendadak pada jalur kronis penyakit akibat perubahan status imunologi selular yang cepat. Tujuan penelitian ini untuk mengukur secara in vitro hasil indeks stimulasi limfosit (ISL) RR kusta berasal dari limfosit setelah menantang dengan M.leprae antigen. Dua puluh tiga batas kusta dengan RR dan sebelas batas penderita kusta tanpa RR dimasukkan dalam studi. Sel mononuklear darah perifer (SLDP) dipisahkan dari darah perifer dari para pasien menggunakan kolom ficol-hypaque dan dibiakkan di laboratorium. Menggunakan metode colorimetric tetrazole (mtt), limfosit ini ditantang dengan PHA, antigen dharmendra (1/100 dan 1/10 pengenceran), LAM (50 dan 100 nanograms). Indeks stimulasi dihitung dan supernatan dikumpulkan untuk mengukur IFN-γ dan produksi IL-10 (ELISA). Semua limfosit dari RR pasien menunjukkan indeks stimulasi lebih tinggi setelah ditantang dengan lima antigen M.leprae dibandingkan dengan limfosit yang berasal dari pasien non RR ($p < 0.05$). IFN–γ dan IL-10 juga meningkat tapi tidak signifikan ($p > 0.05$). Hal ini disimpulkan bahwa limfosit pada pasien lepra selama masa RR lebih sensitive terhadap rangsangan antigen dibandingkan dengan pasien lepra non RR. Penelitian selanjutnya diperlukan untuk menentukan nilai cut off dari indeks stimulasi limfosityang berguna untuk klinisi dalam memprediksi RR sebelum memulai perawatan anti lepra.

Kata Kunci: *Lepra, reversal reaction, indeks stimulasi limfosit, MTT, batas kusta*

INTRODUCTION

Leprosy is still a public health problem in Indonesia, especially in the eastern part of the country.[1] One of the problems in the field is the Reversal Reaction (RR), which often occurred during the Multi-drugs Therapy (MDT) course for leprosy. It is an acute inflammatory episode that occurred during the chronic course of the disease and sometimes cause disability to the patient. Clinically it is manifested by acute inflammatory skin lesions that previously relative "silent" and acute neuritis can occurred that led to disability.[2] By histopathological examination of the skin lesions in RR, inflammatory process could be found in the granuloma with more lymphocytic cells due to influx of lymphocytes from surrounding tissue came to the granuloma with its inflammatory mediators.[3] Activation of these lymphocytes could be a result of lymphocyte stimulation by many substances, including several antigens originated from *Mycobacterium leprae,* the cause of the disease. The aim of this study is to conduct *an in vitro* study on the response of lymphocytes from leprosy patients during the RR episode.

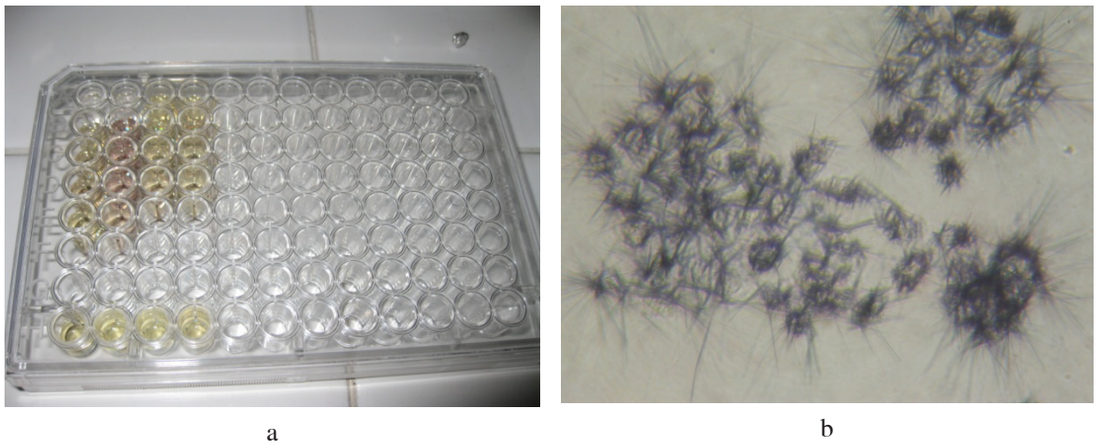
Figure 1. Type 1 Leprosy Reaction (Reversal Reaction)

MATERIAL AND METHODS

Thirty four blood samples obtained from 23 Borderline Leprosy patients with RR and 11 blood samples from Borderline Leprosy patients without RR were included for in *vitro study*. The Peripheral Blood Mono Nuclear Cells (PBMC) were separated using Ficoll Hypaque column and lymphocyte culture were performed.[4] Phyto-haemagglutinine (PHA) were used as nonspecific stimulans, Dharmendra 1/10 and Dharmendra 1/100 as specific protein stimuli from *M.leprae* , Lipoarabinomannan (LAM) 50 nanogram and 100 nanogram as specific carbohydrates stimuli were also used to the lymphocyte cultures. Lymphocyte Proliferation Test (LTT) were performed using the the colorimetric tetrazole (MTT) procedures as recommended by Mosmann in 1983.[5] Stimulation Index (SI) is ratio between stimulation index result and threshold. SI was read by computer and regarded as positive results if the value >1. The level of IFN-gamma and IL-10 from supernatant were measured by ELISA procedure using appropriate kits. Statistical Significant differences between the RR and non RR group were analyzed using Mann Whitney U test and Fisher's Exact test. Spearmans rho test was also used for calculating Correlation Coefficient.

RESULTS & DISCUSSION

Stimulation with non-specific mitogen (PHA) resulted SI positive in 20/23 RR patients compared to 7/11 in non RR patients ($p<0.05$). All of specific *M.leprae* antigens using for lymphocytes stimulation showed significant statistical differences between the the RR and non RR group.



a b

Figure 2. (a) Lymphocyte culture, (b) Formazan coated lymphocytes

Table 1. Results of Stimulation Index with non-specific and specific antigens of *M.leprae*

Antigen	Positive SI in RR patients	Positive SI in non-RR patients	p
PHA	20 / 23	7 / 11	0.013
Dharmendra 1/100	22 / 23	6 / 11	0.002
Dharmendra 1/10	22 / 23	3 / 11	0.000
LAM 50	22 / 23	5 / 11	0.000
LAM 100	21 / 23	5 / 11	0.000

Table 2. Mean of IFN-gamma level in supernatans after stimulation with non- specific and specific antigens of *M.leprae*

Antigen	Mean level of IFN-^{1} in RR patients	Mean level of IFN-^{1} in non-RR patients	p
PHA	22.98*	24.27	0.699
Dharmendra 1/100	23.97	21.18	0.299
Dharmendra 1/10	25.86	23.40	0.522
LAM 50	20.26	20.46	0.839
LAM 100	28.05	24.06	0.368

*Unit/ml (ELISA)

Table 3. Mean of IL-10 level in the supernatan after stimulation with non- specific and specific antigens of *M.leprae*

Antigen	Mean level of IL-10 in RR patients	Mean level of IL-10 in non-RR patients	p
PHA	29.41	29.70	0.593
Dharmendra 1/100	30.28	30.26	0.974
Dharmendra 1/10	29.59	29.55	0.934
LAM 50	29.44	29.47	0.942
LAM 100	29.36	29.91	0.259

*Unit/ml (ELISA)

Acute inflammatory process in reaction state in leprosy is a result of sudden changes in immunological response stability during the chronic course of the disease. Treatment with MDT drugs will kill a big amount of lepra bacilli inside the host body and many new antigens including protein and carbohydrates antigens from dead *M.leprae* spread to the surrounding tissues and circulation. These antigens will stimulate T-lymphocytes, especially in areas surrounding the location of lepra bacilli. Some of these lymphocytes are already sensitized previously by the same antigen (T-memory cells).[6] The result of lymphocyte re-activation is the proliferation, differentiation and production of interleukin as IL-2, IFN-gamma, IL-10 and other interleukins. In this study the proliferation of lymphocytes was performed in-vitro using the MTT method that requiring a fresh peripheral blood from patients. Previously Lymphocyte Transformation Test (LTT) with radioactive labeled Thymidine were often used for measuring lymphocyte activation. Recently this procedure has been changed to MTT technique that relatively save and accurate. This colorimetric technique is based on the enzyme utilities that needed during proliferation of lymphocytes, which can be labeled by certain dyes.[7] The amount of labeled enzyme used by cells indicated the amount of cell's proliferation. During the reaction state in Reversal Reaction of leprosy, many T-memory cells are activated and proliferation occurred. As a result of proliferation, the number lymphocytes increase including the T-memory cells. Subsequent stimulations by antigens from lepra bacilli will stimulate memory T-lymphocytes that already accumulate during granuloma formation in the pathogenesis of leprosy.[8] Stimulation Index in this study showed that lymphocytes from RR patients gave a higher proliferation process compared to non-RR patients. Not only stimulation by protein antigens, carbohydrate antigens were also showed higher results. Proliferation of lymphocytes is always followed by release of many inflammatory mediators and resulted an acute inflammatory reaction of the skin lesions.[9,10] In this study, the production of interleukins were not significant difference between RR and non-RR patients. This results need to be studied further to find the reason, it might be due to technical or time of harvesting the lymphocytes after stimulation. The results of this study showed that not only a single antigen involved in RR process, but many antigens can stimulate lymphocytes of leprosy patients. How the lymphocytes can be stimulated by carbohydrate antigens from *M.leprae* need to be investigated and which antigen is predominated the lymphocyte stimulation in RR still a question.

CONCLUSION

Lymphocytes from leprosy with RR patients showed significant higher Stimulation Index compared with non RR patients. Both protein and carbohydrate antigens from *M.leprae* can stimulate lymphocytes in leprosy patients. More investigations are needed to clarify the true mechanism of RR.

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Literature Review

PATHOGENESIS, DIAGNOSTIC AND MANAGEMENT OF TOXOPLASMOSIS

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ABSTRACT

Toxoplasma gondii is an obligate intracellular parasite of protozoa groups, can infect humans and all warm-blooded animals, are found in almost all locations around the world. Infection generally occurs orally through the consumption of animal products that are not perfectly cooked infected oocyst, parasite containing foods in the form of bradyzoite, contact with cat's feces containing oocysts or vertical transmission occurring through hematogenous placenta. Toxoplamosis can occur in acute or chronic. It divided into five categories, namely, toxoplasmosis in patients immunocompetent, toxoplasmosis in pregnancy, congenital toxoplasmosis, toxoplasmosis in immunocompromised patients and ocular toxoplasmosis. In each category of clinical manifestations of toxoplasmosis are often non-specific. Methods of diagnosis and interpretation are often different for each category. Toxoplasmosis can be diagnosed through a series of tests such as serology, PCR, histology parasites and parasite isolation. Treatment management of this disease requires a long time. Therapy depends on the category of infections as well as individual therapeutic response. The combination of pyrimethamine with sulfadiazine is the drug choice for toxoplasmosis.

Key words: Toxoplasma gondii, toxoplasmosis diagnostic, toxoplasmosis management, PCR, parasite

ABSTRAK

Toxoplasma gondii merupakan parasit intraseluler obligat dari kelompok protozoa yang dapat menginfeksi manusia dan seluruh hewan berdarah panas yang ditemukan hampir di seluruh dunia. Pada umumnya infeksi tersebar secara oral melalui konsumsi produk hewani terinfeksi ookista yang tidak dimasak sempurna, makanan mengandung parasit dalam bentuk bradizoit, kontak secara langsung dengan kotoran kucing mengandung ookista ataupun terjadi transmisi vertikal melalui plasenta hematogen. Toksoplasma dapat terjadi secara akut maupun kronik. Toksoplasma terbagi menjadi 5 kategori yaitu toksoplasmosis pada pasien imunokompeten, toksoplasma pada masa kehamilan, toksoplasma kongenital, toksoplasma pada pasien imunokompromais dan toksoplasma okuler. Pada setiap kategori manifestasi klinik toksoplasma sering tidak spesifik. Metode diagnosa dan interpretasi seringkali berbeda untuk setiap kategori. Diagnosa toksoplasma dapat dirumuskan melalui beberapa seri pengujian seperti serologi, PCR, parasit histologi dan isolasi parasit. Penatalaksanaan perlakuan terhadap penyakit ini membutuhkan waktu yang lama. Proses terapi bergantung pada kategori infeksi seperti halnya terapi respon individual. Kombinasi pyrimethamine dengan sulfadiazine adalah pilihan obat untuk toksoplasma.

Kata kunci: *Toxoplasma gondii, diagnosa toksoplasma, penatalaksanaan toksoplasma, PCR, parasit*

INTRODUCTION

Toxoplasmosis is a zoonosis disease causing by *Toxoplasma gondii.*[1,2] *Toxoplasma gondii* was founded by Nicola and Manceaux in 1908 on lymphatic and liver of *Ctenodactylus gondii* in Tunisia Africa and in a rabbit in Brazil.[3] Toxoplamosis spread around the worldwide and mostly without symptoms. Generally, infection happen orally from consume animal product that infected oocyst and not cook properly, food that contain parasit like bradyzoite, contact with cat's feces that contain oocyst or vertical spreading in a hematogen from placenta.[4,5,6]

Immunocompromised condition such as AIDS object, ferocity and tissue transplanted resipien have high risk of Toxoplasma infection. Build this disease diagnostic in clinic and laboratory is very important to determine therapy and prognosis plan. It is depend on the knowledge about epidemiology, pathogenesis and clinical manifestation.[3]

EPIDEMIOLOGI

Toxoplasma gondii almost can found in worldwide and has been infected more than 50% human population in the world.[2,4] About 10–15% inhabitant in United States shown the positive result in serology check up.[7] Seropositif in HIV-Aids patients estimate about 10–45%.[1,2] Checkup result of IgM and IgG anti *Toxoplasma* in Indonesia, human about 2–63%, cat 35–73%, pig 11–36%, goat 11–61%, dog 75% and the other livestock under 10%.[1]

ETHIOLOGY

Toxoplasma gondii is a parasite obligate intracellular, there are three type, tachyzoite (proliferative form), cyst (contain bradyzoite) and oocyst (contain spozoite).[4,6] Tachyzoite form look like sickle moon with pointed point, and the other point about rounded. Length 4–8 micron, width 2–4 micron, has membrane cell and one nucleus in center.

Cyst formed in host cell if tachyzoite who splits have formed a wall. A cyst has varying size, there is a small that only contain some bradyzoite and there is a 200 micron contain about 3000 bradyzoite. Cyst in host body can found in lifetime especially in brain, heart muscle and striated muscle. Constitute rested stage from *T. gondii.*[1]

Oocyst has the shape ovale, 11–14 9–11 micron. Oocyst has a wall, contain one sporoblast that split into two sporoblast. In the next development, both sporoblast forming wall and being sporocyst. Every sporocyst contain four spozoite that having size about 82 micron.[1,4]

LIFE CYCLE AND THE TRANSMISSION WAY

Toxoplasma gondii has two life cycles. Sexual cycle happen on cat as definitive host, while asexual cycle happen in other mamalia (include in human) and various bird strain.[1,2] This life cycle consist of three forms, tachyzoite and bradyzoite that forming in host mediator and oocyst stage that forming in definitive host epithelial gut cell. Parasite invades erythrocytes then forming microgamete and macrogamete. Zygot or oocyst that produced then come out with feces. Oocyst undergo meiosis outside cat's body. Oocyst endure for many years in moist condition.[2] Then oocyst consumed by host mediator and forming tachyzoite inside digestion track that causing acute infection.[4]

Acute infection can be cronic if tachyzoite change into bradyzoite. Bradyzoite go into host tissue (brain, heart, muscle and retina) and stay in there for host lifetime in dorman condition.[2,4] The changes of tachyzoite stage into bradyzoite depend on multiplication speed, pH, area temperature and the existence of anti mitochondria *Nitric Oxide* (NO) in host body. If human consume meat or dringking water that contaminate with oocyst so bradiizoit or spozoite that resistance with acid pH and enzyme digestive will reach gut, invaded epithelial cell and after several hours change into tachyzoite.[4]

PATHOGENESIS AND IMMUNE RESPONSE

Toxoplasmosis can take an acute or chronic. Acute infection is associated with proliferative forms (tachyzoite), whereas chronic infections associated with tissue cyst forms. During the acute process, tachyzoite invades all cells in the body except host nucleated cells such as red blood cells.[4,6] Tachyzoite enters the host cell via active penetration into the host plasmalemma or by phagocytosis. Parasites adhere to micronema are able to recognize and target cells, produce enzymes to mature rhoptries parasitophorus vacuoles.[5] In vitro replication of intracellular tachyzoite occur every 6-9 hours. Having collected 64–128 parasites in each cell the parasite will be out to infect neighboring cells. With the host immune system, can turn into a subpopulation tachyzoite bradyzoite.[4]

Macrophages, NK cells, fibroblasts, epithelial cells and endothelial cells become activated by *T.gondii* infection in the host body, so it can be inhibited parasite proliferation. Non-specific immune response depends on the ability of IL - 12 produced by macrophages and dendritic cells to stimulate NK cells produce IFN - γ. TNF - α also increases the ability of IL - 12 to induce NK cells to produce IFN - γ. IFN -γ inhibit the replication of the parasite because it induces macrophages to release nitric oxide (NO), which kills the parasite. IFN -γ also increases the activity of indoleamine 2,3 dioxygenase that destroys tryptophan which is a substance necessary for the growth of the parasite.[6]

These parasites will induce immunity 4 types of T cells, namely cell-mediated immune response as *T.gondii* are intracellular parasites.[6] IL - 12 produced by macrophages also strengthen the work of CD4 + cells producing IFN - γ in. CD8 + cells also induces the release of IFN - γ, interferon γ (IFN - γ) plays a role in cyst formation by inhibiting replication in macrophages tachyzoite mice and induce antigen specific for bradyzoite. The humoral immune system has a small role in the fight against toxoplasmosis but is of significant importance in the diagnosis of toxoplasmosis in humans. Antibodies produced by the humoral immune system is able to kill extracellular *T.gondii* in and through the activities of its complement can inhibit parasite multiplication.[6]

Pathogenesis of toxoplasmosis in the immunocompromised host such as HIV - AIDS patients is influenced by many things, among others, a decrease in CD4 + cell count, the failure of production of IL - 12, IL - 2 and IFN - γ and cytotoxic activity of T – Limphocyte is declining. Cells infected with the HIV virus to inhibit the formation of IL - 12 and IFN - γ, leaving them vulnerable to infection toxoplasmosis.[8] Levels of IFN -γ usually decrease in patients with AIDS and it could lead to reactivation of chronic toxoplasmosis.[4]

THE DIAGNOSIS OF TOXOPLASMOSIS

The diagnosis of toxoplasmosis can be established through a series of tests such as serology, polymerase chain reaction (PCR), histological examination of the parasite (imunoperoksidase) and the isolation of the parasite.[9]

Serology Test

The combination of serology is often necessary to determine whether the patient is really infected or not and to determine the acute or chronic infection lasts. The panel of serological tests or toxoplasma serological profile (TSP) includes sabin - Fieldman dye test (DT), Double sandwich IgM enzyme linked immunosorbent assay (ELISA), ELISA IgA, IgE ELISA and aglutination test (AC/HS test).[9]

IgG can be checked by engineering sabin Fieldman DT (Gold standard), indirect fluorescent antibody (IFA) or ELISA. IgG appeared in the first 1–2 weeks of infection and usually can last for years or a lifetime.[9] However, in immunocompromised patients IgG levels can not be detected.[10] IgG positive indicates that the patient has been exposed by *T.gondii* but can not indicate whether the newly infected patients or long-term infection.[11] IgG Avidity has been widely used as additional tests to determine if ongoing infection is acute or chronic. High avidity IgG titer indicates that the infection lasts approximately 4 months earlier while a low titer indicates acute infection.[11,12]

IgM can be examined by the technique of double sandwich ELISA, IFA and immunosorbent agglutination assay (ISAGA). IgM appeared soon after infection and disappears within a few months.[11] In some cases IgM can be detected for> 12 years, therefore the serum IgM positive results still need other tests to determine whether the infection is acute or chronic lasted[9,13]. The sensitivity and specificity of serology varies greatly depending on the lab and the techniques used. A study comparing 6 of tests IgM ELISA found that sensitivity ranged from 93–100 %, and a specificity of 99.1 % 77,5.[14]

IgA was detected in acute infection in adults and congenital infection. IgA can exist for approximately 1 year. In the examination of congenital toxoplasmosis infection is more sensitive IgA. IgE was detected by ELISA in acute infections in adults and congenital infection and serve as additional tests to identify acute infection.[9,13]

Tests AC / HS uses two antigen preparations, namely methanol -fixed tachyzoites (AC antigen) indicating acute infection and formalin -fixed tachyzoites (HS antigen) that indicates chronic infection. The ratio of the AC and HS ratio may indicate acute results, equivalence or non- reactive.[9]

Serologic tests for toxoplasmosis in immunocompromised patients often do not provide a diagnosis for IgG levels in these patients is often low or even undetectable, whereas for the IgM test is often negative. Examination of antigen in the circulation of patients with AIDS have been investigated but have low sensitivity.[10,14] A definitive diagnosis can be established if the formation tachyzoite obtained on biopsy results.[15]

PCR

PCR could detect DNA *T.gondii* in brain tissue, cerebrospinal fluid, amniotic fluid, aqueous humor and vitreous fluid and Bronchoalveolar Lavage (BAL).[9] In patients with toxoplasmic encephalitis sensitivity of PCR in the CSF of approximately 50-60 %, a specificity of approximately 100 %. PCR on blood samples had a low sensitivity.[8]

Histology Examination

Immunoperoxsidase staining technique can show tachyzoite formation in tissue sections or infected body fluids. Multiple tissue cysts with necrotic inflammation surrounding areas can indicate the presence of an acute infection or reactivation of latent infection. This examination is not routinely performed.[2,9]

Isolation of *T. gondii*

A definitive diagnosis of toxoplasmosis can be established by isolation of the parasite from the body fluids (blood, CSF, BAL) or tissue biopsy. This examination is not practical because of the culture of the sample takes approximately 6 months.[8]

CATEGORIES OF TOXOPLASMOSIS

For clinical purposes, toxoplasmosis is divided into five categories, namely (1) toxoplasmosis in patients immunocompetent, (2) toxoplasmosis in pregnancy, (3) congenital toxoplasmosis, (4) toxoplasmosis in immunocompromised patients, (5) ocular toxoplasmosis.[9]

1) Toxoplasmosis in Immunocompetent Patients

1. Clinical Manifestation
   Only 10–20% of toxoplasmosis in children and adults who have symptoms.[2] In immunocompetent patients with toxoplasmosis often without symptoms or only mild symptoms and provide non-specific as fever, enlarged lymph nodes, myalgia, stiff neck, painful swallowing or abdominal pain.[6,9]
2. Examination Supporting
   Examination of IgM and IgG performed for initial evaluation on suspicion of toxoplasmosis. Parallel examination performed 3–4 weeks after the first examination. Results of IgM and IgG were negative excluding the diagnosis of toxoplasmosis. Acute

infection occurs when there is an increase in titer of more than 4 -fold compared to titers at baseline examination. Examination of the panel such as Toxoplasma Serological Profile (TSP) or IgG avidity to distinguish whether the infection to occur acute or chronic.[9]

3. Management
   Treatment is not necessary in cases of asymptomatic except in children < 5 years.[2] Only immunocompetent patients who have symptoms are treated. Pyrimethamine were given 100 mg loading dose, then 25–50 mg / day in combination with sulfadiazine 2–4 g / day in divided doses 4 times / day for 2–3 weeks or can also be combined with clindamycin 300 mg 4 times / day for 6 weeks. Sulfadiazine and clindamycin can be replaced with azithromycin 500 mg / day or 750 mg atovaquone 2 times / day. Another alternative that can be given is Trimethoprim (TMP) of 10 mg / kg / day, sulfamethoxazole (SMX) 50 mg / kg / day for 4 weeks.[7]

2) Toxoplasmosis in Immunocompromised Patient

1. Clinical Manifestation
   In the immunocompromised host such as patients with AIDS, hematologic malignancies, bone marrow transplant recipients, solid organ transplant (including the heart, liver, liver, kidney), toxoplasmosis can cause encephalitis, meningoencephalitis, myocarditis, and pneumonitis.[6,9,17] The incidence of toxoplasmosis in allogenic transplant recipients was 40%, the mortality rate reaches 60–90%. CNS infections occur in 5–10% of transplant recipients.[15,17] *Toxoplasmic encephalitis* (TE) is the most frequent manifestations in immunocompromised patients.[9] In 58–89% of cases occur in sub-acute clinical manifestations in the form of focal neurologic abnormalities, in 15–25% of cases with more severe clinical manifestations of seizures and cerebral hemorrhage. Other clinical manifestations such as loss of consciousness, meningismus, cerebellar signs, neuropsychiatric disorders, dementia, agitation.[2]
   In HIV patients the risk of CNS infection associated with CD4 levels, higher risk in those who only have the number of CD4 + < 200 cells / mm^3.[15,18] In some studies noted that for every decrease in CD4 + cells by 50 cells will increase the risk of TE by 30%, but in the era of HAART (Highly Active Antiretroviral Therapy) as the current risk and mortality TE decreased due to the improvement of the immune system.[18] Toxoplasmosis in AIDS patients can also attack the lungs, eyes and other organs. Pulmonary toxoplasmosis (pneumonitis) occurred mainly in patients with advanced AIDS clinical manifestesi include fever, dyspnea, and cough and is often difficult to distinguish from jeroveci pneumocystic pneumonia. The mortality rate ranges from 35%.[19]
2. Examination Supporting
   Reactivation of chronic infection is the most frequent cause of toxoplasmosis in immunocompromised patients. IgM and IgG titer increased in reactivation.[8] Nonetheless serum anti- Toxoplasma IgM and IgG were negative does not automatically exclude the diagnosis of toxoplasmosis.[15] Isolation of parasites from the blood, infected body fluids, BAL fluid is a definite diagnosis of toxoplasmosis infection. Other tests that may be done include PCR assay to detect DNA *T.gondii* in the blood or body fluids.[2,9] CT scan or MRI should be performed on suspicion of CNS involvement in *T.gondii* infection. Overview lesions of multiple ring -Enhance support the diagnosis of toxoplasmosis.[9]
3. Management
   Toxoplasmosis therapy in HIV - AIDS patients were divided into 2 acute treatment and maintenance therapy. Acute therapy is given for at least 3 weeks and can be given for 6 weeks if complete response does not occur, the next required maintenance therapy to prevent relapse.[8]
   Primary prophylaxis is recommended in HIV-seropositive AIDS where the number of CD4 + < 100 / mm^3 or patients with CD4 < 200 / mm^3 were accompanied by opportunistic infections and malignancies. Regimens used can be given TMP - SMX (trimethoprim - sulfamethoxazole).[8] The dose of TMP - SMX is one double strength tablet (DS) (160 mg trimethoprim, 800 mg sulfamethoxazole) 2 times / day (14 DS tablets / week).[20]
   In acute infections may be given a combination of pyrimethamine and sulfadiazine. This regimen is the standard regimen for the treatment of TE. Pyrimethamine initial dose of 200 mg / day next 50-75 mg / day plus sulfadiazine 4–8 g / day for 6 weeks then referred to a lifelong suppressive therapy or to improve the immune system.[7,8] In some of the studies mentioned combination of pyrimethamine - clindamycin and trimethoprim - sulfamethoxazole as effective as the use of a combination of pyrimethamine – sulfadiazine.[7] Clindamycin can be given at a dose of 600 mg PO / IV, 4 times / day for 3–6 weeks. The dosage for suppressive therapy 300–450 mg PO every 6–8 hours.[2,21] The combination of atovaquone with pyrimethamine or sulfadiazine also provide high effectiveness. These drugs are able to eliminate bradyzoite in experimental animals. Can be administered at a dose of 750 mg (5 mL) PO when eating for 21 days.[2,21] In some studies this regimen gives good results on the clinical and radiological picture of 77% within 6 weeks of treatment and recurrence rate of 5% in the maintenance period.[8] Maintenance therapy (secondary prophylaxis) can be started after completion of therapy in the acute phase is given, which used the same regimen as in the acute phase but with a half dose.[8]
   Primary prophylaxis can be stopped if the CD4 count after the use of antiretroviral (ARV) increased > 200 / mm^3 were settled for approximately 3 months, with an examination of the amount of virus negative.[8,22] Secondary prophylaxis was stopped if the patient had

undergone treatment of acute and showed clinical improvement is characterized by loss of the signs and symptoms of toxoplasmosis and improvement of the immune system after treatment with HAART are characterized by increased CD4 + > 200 / mm^3 were settled for for about 6 months.[8,22]

3) Congenital Toxoplasmosis

1. Clinical Manifestation
   Cases of congenital toxoplasmosis have been reported in Indonesia. Lazuardi et al (1989) reported *T.gondii* antibodies in 44.6% of children with mental retardation, 44.6 % in children with ocular lesions and 9.5% in children with common symptoms.[1] The risk and severity of congenital toxoplasmosis symptoms more severe if infection occurs early in pregnancy.[23] Classic triad of congenital toxoplasmosis is chorioretinitis, hydrocephalus, and intracranial calcification. The involvement of neurological and ocular systems often arise later if not found at the time of birth. Seizures, mental retardation, and rigidity is the common sequelae.[2]
2. Examination Improving
   IgM positive is strong evidence of congenital infection, but a negative IgM does not exclude the diagnosis. Serum IgA is more sensitive for detecting congenital toxoplasmosis than IgM.[9]
   When symptoms and serological evidence of toxoplasmosis is detected during pregnancy, infection of the fetus can already be enforced by IgM detection and isolation of parasites from fetal blood or amniotic fluid at 18 weeks of gestation. Examination before 20 weeks gestation is difficult to enforce because of the immunological response of the fetus is still low. PCR on amniotic fluid can more accurately diagnose infection in the fetus before 20 weeks gestation.[9] The sensitivity of this test is 64% with a negative predictive value of 87.8%, specificity and positive predictive value of 100%.[9]
   Antenatal Ultrasound can identify abnormalities in the fetus is infected. Approximately 36% of fetuses with abnormalities can be identified. Abnormalities that can be found are bilaterally symmetrical ventricular dilatation, intracranial calcification, increased placental thickness, hepatomegaly and ascites.[9]
3. Management
   In newborns with toxoplasmosis, can be given a combination of pyrimethamine 1 mg / kg per day for 2 months followed by 1 mg / kg every 2 days for 10 months, sulfadiazine 50 mg / kg body weight per day, as well as folic acid 5–10 mg 3 times week to prevent the side effects of pyrimethamine[2]. In addition to the provision of drugs are also required regular follow-up. A complete blood count 1–2 times per week to daily dosing of pyrimethamine and 1–2 times per month for the dosing of pyrimethamine performed every 2 days to monitor the toxic effects of the drug. Also required a complete pediatric examination, including ophthalmologic examination every 3 months until the age of 18 months and then once a year, as well as neurological examination every 3–6 months to 1 year of age.[2]

4) Ocular Toxoplasmosis

1. Clinical Manifestation
   Toxoplasmic chorioretinitis can occur because of congenital or postnatally acquired infection. Infection occurs in 2/1000 pregnancies America, with an average of transplacental infection Ý50%.[25] Seventy percent of infants with congenital infection showed a scar on korioretina.[24] Symptoms include blurred vision, scotoma, fotofobi and pain. Of ophthalmology examination obtained focal necrotizing retinitis formation that resembles a yellowish white cotton, with unclear boundaries. In congenital infection are often bilateral lesions in infections acquired while generally unilateral.[2]
2. Examination Improving
   Serologic tests are often unhelpful because the diagnosis is often obtained with the IgG titers were low, often undetectable IgM. Increased levels of IgG 4 times the initial levels within 4 weeks showed primary infection. Other tests that can be done is the amplification of parasite DNA from the aqueous or vitreous humor.[9]
3. Management
   Treatment depends on several factors such as the location of lesions, degree of inflammation, the threat of blindness and immune status of the patient. If the infection is not on the optic disc and macula and is only accompanied by mild inflammation, treatment is not required.[10] Pyrimethamine most effective for this infection, given the loading dose of 25 mg 3 times / day followed by 25 mg / day. This drug should be combined with sulfadiazine with further loading dose of 2 g 1 g 4 times / day. Therapy is done for 6-12 weeks. Treatment response was indicated by the disappearance of a yellowish white spot on the retina, the vitreous becomes clear and atrophic scars korioretina being demarcated. Another drug option is clindamycin 300 mg 3-4 times / day for 3-4 weeks, then 150 mg four times / day for the next 3-4 weeks. Spiramycin is the drug most commonly used and has the least amount of side effects among other drug options, can be administered in a dose of 1 g 2 times / day.[11]

5) Toxoplasmosis in Pregnancy

1. Clinical Manifestation
   Most pregnant women with acute acquired infection do not experience specific symptoms. Some have symptoms of malaise, subfebris, lymphadenopathy. The frequency of vertical transmission to the fetus increased with increasing gestational age.[25]
2. Examination Improving
   Examination of IgG and IgM should ideally be done in the first trimester of pregnancy. Serum IgG and IgM

negative by showing that pregnant women not infected, face further investigation performed during pregnancy to anticipate the occurrence of seroconversion.[24]

On the positive results of IgG but negative IgM in pregnancy < 18 weeks showed an infection occurred in the past, while in gestation > 18 weeks of this result is difficult to interpret whether the infection is acute or chronic lasted so avidity required examination. In the results were negative but IgG positive IgM examination should be repeated in 1–3 weeks later, if the result remains the same mean positive IgM has no clinical significance, whereas in case of seroconversion of IgG becomes positive which indicates that the infection occurs during pregnancy so that the fetus is at high risk affected by congenital toxoplasmosis.[24]

On examination of the IgG and IgM positive follow-up examination to confirm acute or chronic infections such indispensable avidity test.[24] high avidity IgG indicates that infection occurred > 16 weeks in advance, so that the examination in the first trimester of pregnancy showed an infection occurs before conception reduces the risk of transmission and the risk of fetal defects is low.[23]

3. Management

Spiramycin is Drug Of Choice for maternal toxoplasmosis. Dose of 3 g / day PO in divided doses 24 times / day for 3 weeks, stopped for 2 weeks and then repeated the cycle of 5 weekly during pregnancy.[2,24] If PCR positive amniotic fluid regimens should be replaced with pyrimethamine 50 mg / day and sulfadiazine 3 g / day in 2–3 divided doses for 3 weeks interspersed with the provision of spiramycin 1 g 3 times / day for 3 weeks or can be given pyrimethamine 25 mg / day and sulfadiazine 4 g / day in divided doses 2-4 times / day was given until delivery.[7]

PREVENTION

Prevention of toxoplasmosis can be made by cooking the meat until done, wash your hands thoroughly after handling raw meat, wash vegetables and fruits before eating, wash clean kitchen equipment after use, pregnant women should wear gloves when gardening and wash hands afterwards, avoid contact with cat feces, the primary and secondary prophylaxis should be administered to patients with AIDS.[7]

PROGNOSIS

In immunocompromised patients reactivation of chronic toxoplasmosis are common. Suppressive therapy and improving the immune system may reduce the risk of recurrent infection. Infants with ocular toxoplasmosis acquired have a good prognosis and in the next four years have the same development as uninfected infants. Immunocompetent patients have a good prognosis, lymphadenopathy and other symptoms disappear within a few weeks after infection.[7]

SUMMARY

Methods of diagnosis and interpretation are often different for each category. The diagnosis of toxoplasmosis can be established through a series of tests such as serology, PCR, histology parasites and parasite isolation. Management the treatment of this disease requires a long time. Therapy depends on the category of infections as well as individual therapeutic response. The combination of pyrimethamine with sulfadiazine is the drug of choice for toxoplasmosis.

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Literature Review

PATHOGENESIS OF HEMORRHAGIC DUE TO DENGUE VIRUS

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ABSTRACT

Dengue is a viral disease that is mediated by a mosquito, which causes morbidity and mortality. Viruses can increase vascular permeability which can lead to hemorrhagic diathesis or disseminated intravascular coagulation (DIC) known as dengue hemorrhagic fever (DHF). In Indonesia, dengue hemorrhagic fever (DHF) are caused by dengue virus infection which was found to be endemic accompanied by an explosion of extraordinary events that appear at various specified period. The diagnosis of dengue is determined based on the criteria of the World Health Organization (WHO, 1999), which are sudden high fever accompanied by a marked tendency to hemorrhage positive tourniquet test, petechiae, ecchymosis, purpura, mucosal hemorrhagic, hematemesis or melena and thrombocytopenia. The problem that still exists today is the mechanism of thrombocytopenia in patients with varying degrees of dengue involving levels of vWF (von Willebrand factor) and prostaglandin I2 (PGI2) can not be explained. The mechanism of hemorrhagic in dengue virus infections acquired as a result of thrombocytopenia, platelet disfunction decreased coagulation factors, vasculopathy with endothelial injury and disseminated intravascular coagulation (DIC).

Key words: Hemorrhagic, dengue virus, DHF, criteria, WHO

ABSTRAK

Dengue adalah penyakit viral yang diperantarai oleh nyamuk, yang menyebabkan morbiditas dan mortalitas. Virus dapat meningkatkan permeabilitas vaskular yang dapat memicu hemorrhagic diathesis *atau* disseminated intravascular coagulation *(DIC) yang lebih dikenal dengan demam berdarah dengue (DBD). Di Indonesia, DBD disebabkan karena infeksi virus dengue yang ditemukan untuk menjadi endemik disertai dengan suatu ledakan luar biasa dari peristiwa yang muncul di berbagai periode tertentu. Diagnosis dengue ditunjukkan berdasarkan kriteria World Health Organization (WHO, 1999), yaitu demam tinggi secara tiba-tiba yang ditandai dengan tes hemorrhage positive tourniquet, petechiae, ecchymosis, purpura, mucosal, berdarah hematemesis atau melena dan trombositopenia. Masalah yang masih ada ialah mekanisme trombositopenia di pasien dengan variasi derajat level dengue vWF (*von Willebrand factor*) dan prostaglandin I2 (PGI2) tidak bisa dijelaskan. Mekanisme demam berdarah dengue (DBD) pada infeksi virus dengue yang diperoleh sebagai hasil trombositopenia, platelet disfungsi, faktor koagulasi menurun, endotel vasculopathy dengan cedera serta* disseminated intravascular coagulation *(DIC).*

Kata kunci: *Hemorrhagic, virus dengue, DBD, kriteria, WHO*

INTRODUCTION

Dengue is a viral disease transmitted by mosquitoes that cause morbidity and mortality. In some cases, the virus that causes can lead to an increase in vascular permeability and can cause hemorrhagic diathesis or disseminated intravascular coagulation (DIC) known as dengue hemorrhagic fever (DHF). Of the 20–30% of patients will experience a shock dengue, called dengue shock syndrome/dengue shock syndrome.[1]

Dengue hemorrhagic fever (DHF) in Indonesia is still found to be endemic accompanied by an explosion of outbreaks that appear at various specified period. The results of epidemiological observations also show that the number of dengue fever is increasing from year to year with widespread deployment.[2]

The diagnosis of dengue is determined based on the criteria of the World Health Organization (WHO, 1999)[3] which in essence is found sudden high fever accompanied by a marked tendency to hemorrhage positive tourniquet test, petechiae, echymosis, purpura, mucosal hemorrhagic, hematemesis/melena and thrombocytopenia (platelet count of blood edge of less than 100,000/mm^3) beginning on day 5–8. Determination of the diagnosis requires further confirmation by serology, antigen detection or isolation of dengue virus.[2,4,5,6]

Research in the 20s on the new man can prove that dengue viruses can create pain. Pathogenesis is unclear. It was still considered a theory malignancy virus and the number of viruses that infect the body. This theory developed viral virulence theory, to study genotype, phenotype and molecular epidemiology of dengue. Since the '50s developing immunological theories have much effect on the current. From epidemiological observations, clinical and laboratory appears theories of secondary infection by other viruses sequenced, and activation of antigen-antibody theory complement.[5]

From this developed into the theory of infection enhanching antibodies, which then appears endotoxemia role and the role of T lymphocytes Then came the theory and theoretical mediators of apoptosis. So far no one theory can completely explain the pathogenesis of DHF.[5]

Hemorrhagic and fever are characteristic of dengue disease. Over 30 years the researchers concluded that the occurrence of hemorrhagic may occur due to vascular disruption/vasculopathy, thrombocytopenia and impaired platelet function and clotting disorders/ coagulopathies.[2,7,8] The problem that still exists today is the mechanism of thrombocytopenia in patients with varying degrees of dengue involving levels of vWF (von Willbrand factor) and prostaglandin I2 (PGI2) can not be explained.[2,6]

EPIDEMIOLOGY

According to history, the beginning of dengue fever from Egypt and then spread throughout the world. Mosquitoes live in the fertile parts of the world that has a tropical climate and subtropics like Asia, Africa, Australia and America.[8,9] In Indonesia, the first case of dengue fever reported in the Jakarta and Surabaya in 1968. The following years the number of cases of dengue fever each year fluctuated and tended to increase.[6,10]

Data from the Ministry of Health were recorded in 1998 dengue cases from January 2004 to April 2004, there were a total of 58 301 cases of dengue fever in which 658 cases of dengue fever were fatal, especially in the provinces on the island of Java with more than 35% of cases are in the provinces of Jakarta. It seems that the highest outbreak in the province of Jakarta, Central Java, East Nusa Tenggara. However, in the province of West Java, Bali, South Sumatra, Lampung, East Kalimantan, South Sulawesi and West Nusa Tenggara are the trend of increased cases. The most frequent serotypes are circulating dengue-3 (Den-3) that is 37% even though the three other serotypes (Den-4 (19%), Den-2, Den-1) also exist.[11]

In the 1998 pandemic, WHO reported more than 1.2 million cases of dengue fever and DHF from 56 countries in Indonesia where there are 72 133 cases and cases of death by 1414 with a Case Fatality Rate (CFR) 2.0%.[9,11]

Since 1993–1997 the majority of DHF patients 5–14 years age group by 60%, the highest in the 4–12 years of age and at tahun1996-1997 has shifted at the age of 15 years.[10,12]

PATHOGENESIS HEMORRHAGIC

Hemorrhagic manifestations in DHF is most often found in the form of petechiae on the skin and sometimes in the submucosa. Positive tourniquet test an increase in capillary fragility encountered earlier. Symptoms of severe hemorrhagic that often occurs is in the form of gastrointestinal hemorrhagic or hematemesis and melena. In the case of prolonged shock with massive hemorrhagic can occur in the heart, lungs, liver and brain.[5,6,7,13]

Increased hematocrit value is a manifestation of hemoconcentration that occurs due to leakage of plasma into the extravascular space with an effusion of serous fluid through the damaged capillaries. As a result of this leakage of plasma volume is reduced which may result in hypovolemic shock and circulatory failure. Hemoglobin levels in the first days are usually normal or slightly decreased. But later levels will rise following an increase in hemoconcentration and earliest hematologic abnormalities that can be found in DHF.[14,15]

A. VASCULOPATHY

Characteristics of DBD plasma leakage is the manifestation hemoconcentration, pleural effusion or ascites and. Previous allegedly plasma leakage due to increased vascular permeability in addition to the discovery of two new suspects endothelial cell destruction accompanied by the release of inflammatory mediators (IL-6, IL-8 and RANTES) were released by the dengue virus. Dengue virus also activate complement and induce the expression of adhesion molecules such as ICAM-1, in which the expression of ICAM-1 along with IL-8 and RANTES will also increase vascular permeability.[16]

Vascular disorders due to dengue virus infection simplest can be seen with a positive tourniquet test with ptekie that often appear at the beginning of a fever before the thrombocytopenia. Research by performing a biopsy of the skin surface which berptekie showed infiltration of lymphocytes and macrophages containing dengue antigen. Other studies obtain IgM antidengue, complement and fibrinogen in skin berptikie biopsied. Though not known for certain, the presence of vasculopathy likely the result of a direct effect of dengue virus-mediated immune response.[7]

Endothelium is the inner blood vessel is a single-layered cell (monolayer) influential due to injury. In viral infections, including dengue virus infection, endothelial cell death can occur through the mechanism of apoptosis triggered by TNFα and cytokine products of immune response due to dengue virus infection.[2,8]

Funahara at 1987[17] proved that dengue virus antigen can attack directly without platelet immune respons, the bond between dengue virus antigens and antibodies interact with platelets dengue virus, and dengue virus infection causes modulation of the endothelium. Abbas AK. 2007[18] suggested that individual as a result of an activated immune response of dengue virus can have a positive impact in the form of destruction of the virus or on the contrary, the negative impact that ended with endothelial injury and death through cytokine that plays an important role in the course of disease caused by dengue virus infection is TNFα, IL-1, IL-6 and IFNγ. Various research findings indicate that endothelial injury led to the emergence of a variety of adhesive molecules derived from endothelial cells themselves and from the sub- endothelial triggering platelet aggregation. That is, the process of apoptosis that occurs in endothelial cells by TNF as fasligand cause endothelial cells loose the bonds with which the sub-endothelial molecules obtained vonWillebrand (vWF), which appears on the surface and leads to platelet aggregation. Nawroth at 1986[19] found that endothelial injury followed by an increase in procoagulant activity, while Holvoet at 1998[20] found that endothelial injury followed by a decrease in anticoagulant activity. The research findings if Mauro at 1992 showed that IL-6 has the ability to increase endothelial permeability. This means, IL-6 seems to also cause injury to the endothelium.[21] Endothelial disruption due to injury can be examined by inhibition and plasminogen activator-1 (PAI-1) are increased in the circulation.[6]

New development of endothelial dysfunction is the concept of microparticles. All DHF patients show decreased levels of microparticles during acute illness and increased significantly in the past rekonvalesensi. Further research is needed to address the extent to which the role of microparticles causes increased capillary permeability and in certain circumstances lead to disseminated intravascular coagulation.[6]

B. THROMBOPATI AND THROMBO-CYTOPENIA

Thrombocytopenia is one simple criteria proposed by the WHO as a clinical diagnosis of dengue disease. The cause of thrombocytopenia in DHF is still controversial. Thrombocytopenia and hemoconcentration are two circumstances which almost always appear on the disease caused by dengue virus infection. In patients with DHF, tombositopenia be due to decreased production of platelets by the bone marrow, increased destruction of platelets in the Reticulo Endothelial System (RES) and aggregation of platelets by damaged vascular endothelium.[2,5] And allegedly also due to intravascular coagulation and consumption of clotting factors and platelets are increased.[2,5,15]

Nimmannitya at 1999[7] suggested that the main cause of thrombocytopenia is a decrease in consumption and platelets in peripheral desruksi. Destruction of platelets played by complement activation as the bond between platelets with fragments and dengue virus antigen, or it can occur by direct attack against platelets without dengue virus through immune responses.[2] Mitrakul at 1987[22] concluded that the occurrence of thrombocytopenia due to the shortening of the life of platelets and platelet function decline due to dengue virus infection.[7]

From these studies it can be concluded that in patients with DHF decreased production, increased destruction and excessive use of platelets, resulting in thrombocytopenia. In addition to the quantitative deficit, there is also a platelet function disorder. This is evidenced by the increased secretion of ADP and plasma prostaglandin metabolite (PGI 2), namely 6-keto-PGFIa (6KPGF1).[15]

Several reports from the literature indicating normal platelets do not stick to the vascular endothelium, except when there is activation of such damage or tear the vascular intima layer vascular structure.[8,22]

The platelet response to activation, in general there are four types: (1) changes in platelet shape from flat pieces into a round spiked, (2) adhesion, platelet attachment to the vessel wall subendotelium or on the network of collagen, (3) aggregation, platelet attachment of one each other, (4) secretion, such as ADP, tromboxane A2 (TXA), serotonin, calcium and others. ADP -induced platelet aggregation of platelets that have been attached to the walls of blood vessels are damaged while PGI2 is a platelet aggregation inhibitor. PGI2 effects besides opposed to ADP, is also opposed to tromboxane A2 which is also secreted by dense granules in platelets.[7,8,22]

Inactivation of platelets by PGI2 in endothelial suspected as responsible for the absence of platelet adherence. But this hypothesis is not supported by research and the mechanisms by which PGI2 to prevent platelet adherence remains unclear.[2,8,22]

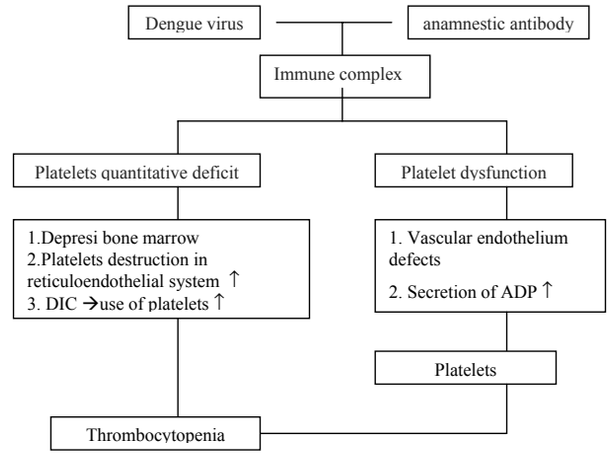

Figure 1. Mechanisms of thrombocytopenia in DHF.[23]

C. COAGULOPATHY

Hemostasis of blood vessels is maintained through a balance between coagulation and fibrinolysis. Coagulation system is activated via the intrinsic and extrinsic pathways to convert fibrinogen to fibrin. While the fibrinolysis system damage tissue degradation products of fibrin to fibrin (FDP).[15]

Many studies have identified the mechanism of occurrence of hemorrhagic in some cases the presence of coagulopathy DHF. Almost all cases of DHF with shock occurs coagulopathy. with prolongation of activated partial thromboplastin time (APTT).[7] In acute infection with dengue virus, which is commonly used coagulation parameters are platelets and (APTT) and the parameters of fibrinolysis is tissue plasminogen activator (tPA) and plasminogen activator inhibitor (PAI-1).[15,16]

Studies that have been conducted in Indonesia noted the thrombocytopenia, a decrease in plasma fibrinogen and factor VIII and increased fibrin degradation product D-dimer (FDP-D).[7]

Mechanisms that might explain the occurrence of coagulopathy is the presence of viral antibody complexes or mediators phagocytes infected with dengue virus. Coagulation is activated sequentially following the cascade that begins with the activation of factor XII into factor XIIa. Subsequently activates Factor XII fibribolisis system with changes of plasminogen to plasmin. Plasmin will break down the fibrin polymer into fragments X and Y. The Y fragment is broken down into two fragments D and one fragment E, known as the D - dimer. The degradation of fibrin (FDP) has the properties as an anti- coagulant, so that considerable amounts will inhibit hemostasis.[6,15]

Activation of coagulation and fibrinolysis system prolonged the resulting decline in various coagulation factors such as fibrinogen, II, V, VII, VIII, IX and X and plasminogen. This situation caused and worsened the hemorrhagic in DHF patients, coupled with the presence of thrombocytopenia.[15]

The complement system and the kinin system plays a role in the inflammatory process is activated by factor XIIa also result ed an by increase in blood vessels which play a role in the occurrence of shock.[15,24]

D. DISSEMINATED INTRAVASCULAR COAGULATION (DIC)

DIC is a clinical syndrome characterized by widespread activation of coagulation system resulting in the formation of intravascular fibrin and eventually thrombosis of blood vessels resulting in decreased blood to organs and cause organ failure. Due to excessive coagulation deficiency of platelets and coagulation factors that can cause heavy hemorrhagic.[25]

The role of DIC in patients with DHF have been widely studied. The occurrence of DIC in patients with DHF is still a question mark.[15,25] The mechanism is based on the possibility of activating factor XII antigen antibody, platelet release reaction or peeling endothelium and exposed subendothelial collagen and basement membrane.[25]

One study shows that in patients with DHF were found to increase the minimum levels of FDP, and not associated with disease severity. In patients with increased FDP, found partial thromboplastin time and prothrombin time were slightly elongated. FDP were increased with thrombocytopenia showed intravascular coagulation process is resulting in hemorrhagic but not prove DIC. However DHF with shock and prolonged acidosis can trigger DIC.[15]

While other researchers say that in all cases of dengue are found manifestations of acute type DIC. So it is clear that the natural course of the disease dengue fever which would cause the complex pathophysiology of the various systems in the body of the patient (Figure 2).[15]

The results of the latest research on the mechanism of hemorrhagic in DHF, states that hemorrhagic due to consumptive coagulopathy that occurs in most cases. Almost all the cases with coagulopathy shock occurs, manifested as prolonged partial thromboplastine time. Changes in liver function and normal prothrombin time/ slightly elongated, support the occurrence of consumptive coagulopathy.[7,22]

It can be concluded that the disturbance of hemostasis in dengue fever can be caused by multifactorial including vasculopathy (capillaries and venules), thrombocytopenia, platelet dysfunction and coagulopathy.[2,5,6,7]

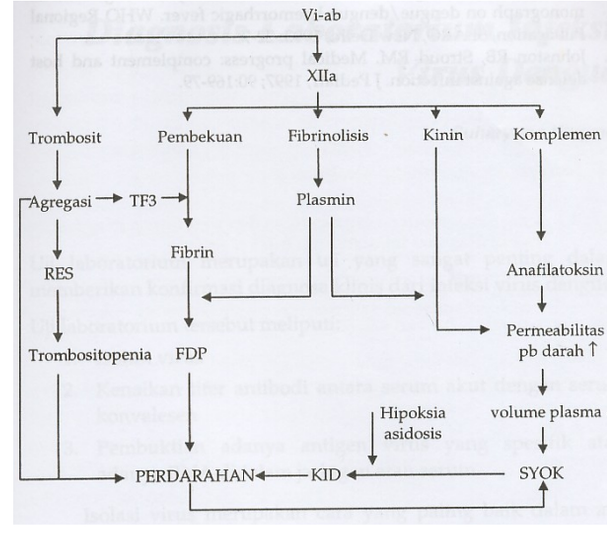

Figure 2. Pathophysiology of Hemorrhagic in DHF.[15]

SUMMARY

The diagnosis of dengue is determined based on the criteria of the World Health Organization (WHO, 1999),[3] the contents of which are found sudden high fever accompanied by a marked tendency to hemorrhage positive tourniquet test, petechiae, ecchymosis, purpura, mucosal hemorrhagic, hematemesis or melena and thrombocytopenia.

The problem that still exists today is the mechanism of thrombocytopenia in patients with varying degrees of dengue involving levels of vWF (von Willebrand factor) and prostaglandin I2 (PGI2) can not be explained.

The mechanism of hemorrhagic in dengue virus infections acquired as a result of thrombocytopenia, platelet dysfunction, decreased coagulation factors, vasculopathy with endothelial injury and disseminated intravascular coagulation (DIC).

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