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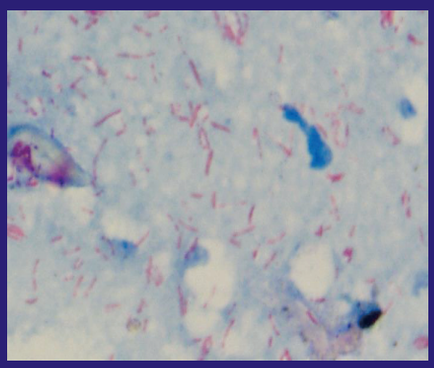

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Research Report

PROPORTION OF HBsAg AND HBeAg POSITIVE IN MATERNAL PATIENTS AND THEIR HBsAg POSITIVES BABIES WITH IMMUNOPROPHYLAXIS OF HBV IMMUNIZATION IN Dr. SOETOMO GENERAL HOSPITAL, SURABAYA

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ABSTRACT

Hepatitis B Virus (HBV) can be transmitted vertically from mother to her baby. Mothers with HBsAg and HBeAg positives have more risk of transmitting HBV to her baby rather than HBsAg positives only. The aim of this study is to determine the proportion of maternal patient with HBsAg and HBeAg positives and their HBsAg positives babies with immunoprophylaxis of HBV immunization. This study was performed by analytical observation using medical records in 2013-2014 at Obstetric and Gyn ecology Department, Dr. Soetomo Hospital. The samples were all maternal patients (3796) during that period and also their babies from HBsAg positives mothers. Unfortunately, several original medical records were not available. Thirty two (0,85%) out of 3781 maternal patients were found to be HBsAg positives, and three (9,37%) of 32 patients with HBsAg positives were HBeAg positives. From 32 mothers who were positive HBsAg, 22 complete medical records of their babies were found and all of them (100%) had been given Hepatitis B Immunoglobulin (HBIG) and hepatitis B vaccine less than twelve hours after birth. In three cases of the babies from HBeAg positives mothers which had been given prophylaxis properly, two cases each of which was with caesarean and spontaneous delivery were HBsAg negatives. Interestingly, the other one which born with spontaneous delivery was found to be HBsAg positives. Further study in this HBsAg positives baby, especially in analyzing its HBV DNA is needed. The epidemiology of hepatitis B in maternal patients, especially that with complete and neat data needs further research.

Keywords: *HBsAg, HBeAg, hepatitis B, maternal patient, vertical transmission*

ABSTRAK

Virus Hepatitis B (VHB) dapat ditularkan secara vertikal dari ibu ke anak. Pada ibu dengan HBsAg dan HBeAg positif lebih beresiko menularkan VHB pada anaknya daripada hanya positif HBsAg. Penelitian ini bertujuan untuk mengetahui proporsi ibu bersalin dengan HBsAg dan HBeAg positif dan bayi dengan HBsAg positif yang telah diberi imunoprofilaksis terhadap HBV. Penelitian ini dilakukan dengan teknik observasional analitik menggunakan rekam medis pasien ibu bersalin periode tahun 2013-2014 yang dirawat di Departemen Obstetri dan Ginekologi RSU Dr. Soetomo. Sampel penelitian ini adalah semua ibu bersalin (3796) pada periode tersebut serta bayi dari ibu bersalin yang positif HBsAg. Sayangnya, ada beberapa data asli yang tidak tersedia. Dari 3781 pasien ibu sebanyak 32 (0,85%) pasien ibu positif HBsAg. Dari ibu positif HBsAg ditemukan tiga (9,37%) pasien HBeAg positif. Dari 32 pasien ibu positif HBsAg, dapat dikumpulkan 22 rekam medis bayi yang lengkap dan semuanya (100%) sudah diberi Imunoglobulin Hepatitis B dan vaksin HBV kurang dari 12 jam sejak lahir. Pada tiga kasus anak dari ibu positif HBeAg yang telah diberi profilaksis, ditemukan negatif

HBsAg pada dua kasus dengan tindakan persalinan Caesar dan spontan. Menariknya, satu kasus lainnya dengan tindakan persalinan spontan ditemukan positif HBsAg. Diperlukan penelitian lebih lanjut mengenai analisis DNA VHB pada bayi yang positif HBsAg ini. Perlu pula penelitian lebih lanjut mengenai epidemologi hepatitis B pada ibu bersalin dengan data yang jelas dan lengkap.

Kata kunci: *HBsAg, HBeAg, hepatitis B, pasien bersalin, penularan vertikal*

INTRODUCTION

Hepatitis B Virus (HBV) infection was one of major global health problems because about two billion people in the world have been infected with HBV and more than 350 million people are chronic carriers.[1] In 2013, Indonesian Basic Health Research (Riskesdas) also found the highest cause of the prevalence of hepatitis in Indonesia is by HBV (21,8%).[1]

Hepatitis B virus can be transmitted parenterally, in contact with blood or other body fluids. In endemic area of chronic HBV infection, transmission mainly through vertical transmission, especially during perinatal and early childhood.[2] Approximately 3,9% of pregnant women in Indonesia in 2007 as a carrier of HBV infection.[3] Risk Factors of HBV infection can be divided based on agent, host, and environment. People who are susceptible to the infection of HBV are people who live in Asia, Africa, and other regions with high prevalence of HBV infection, has not been vaccinated, had sexual intercourse with someone who had HBV infection, living with a person infected with HBV, homosexuals, using parenteral drugs, undergoing hemodialysis, and someone who is undergoing chemotherapy or other immunosuppressive treatment.[4] In hemodialysis patients study, the most common HBV agent in East Java, especially in Surabaya had genotype B,[5] whereas the most common subtype in Java was adw.[6]

Hepatitis B virus infection can be diagnosed with a laboratory test. Screening of HBV infection commonly by Hepatitis B Surface Antigen (HBsAg) tests. Hepatitis B surface antigen appears in blood serum after 1-10 weeks of exposure to HBV. Hepatitis B surface Antigen test has a specificity of 99.7% and a sensitivity of 100%.[7] After discovering someone has positive HBsAg, further examination with HBeAg test is also recommended. Hepatitis B envelope Antigen is a test to determine whether there has been virus replication.[8]

Centers for Disease Control and Prevention (CDC) recommends that all pregnant women should be screened with HBsAg marker. If it is found only HBsAg positive, then the risk of perinatal transmission is 10%.[9] While newborns of an HBV carrier woman with HBsAg and HBeAg positive, have 90% risk of becoming infected and carrier.[10] It is because of the baby tolerance to virus antigen. If these babies are not treated properly, it can develop into Hepatocellular Carcinoma (HCC) and leads to death after decades. However, HBV infection can be prevented by providing effective vaccination. Hepatitis B Virus vaccine is effective to 90% adults and children if given in three doses (three injections with a period of 6 months). Injections with hepatitis B immunoglobulin (HBIG) and HBV vaccine may also be granted because HBIG can give immediate but temporary protection against the virus until HBV vaccine is effective.[11]

Infection of HBV is also very likely to transmit in medical personnel who assist the delivery. Prevalence in this group varies between 10%-20%.[9] Therefore, right education and techniques are needed for helping the delivery process in pregnant women and to care the infants who are at risk to be vertically infected by HBV. This study will analyze the proportion of maternal HBsAg and HBeAg positive and HBsAg positives babies with HBV immunoprophylaxis (HBIG and HBV vaccine) which was administrated to the babies of HBsAg positive maternal patients in Dr. Soetomo General Hospital during the period of 2013-2014. This information is expected to be useful as information to the public and government in order to develop the prevention to vertical transmission of HBV infection. This study was conducted in Dr. Soetomo General Hospital because: firstly, it has a lot of patients so that we can get a large number of samples. Secondly, it is a type A hospital in Indonesia which means it is supposed to have the best health care service in Indonesia. Therefore from knowing how is the result of prevention of vertical transmission of HBV in Dr. Soetomo General Hospital, this study will show how far Indonesia has been handling the prevention of HBV transmission.

MATERIAL AND METHOD

This study used a cross-sectional design. Materials used in this research were secondary data: medical records documents of maternal patients who were checked using HBsAg and HBeAg test, and her babies were born during the period of 2013-2014 in the Departement of Obstetric and Gynecology Dr. Soetomo General Hospital Surabaya. The samples of this study were chosen by total sampling technique.

Variables examined in this study were HBsAg and HBeAg positives status of maternal patients and immunization status (passive and active) of her babies from HBsAg positive mothers. Maternal patients that HBsAg positive, will be tested with HBeAg marker. At that time, the examination of HBsAg and HBeAg were done in Laboratory of Clinical Pathology at Dr. Soetomo General Hospital using ELISA technique. Data from the observation that had been collected, then it was processed and analyzed descriptively using simple statistics (percentages).

RESULT AND DISCUSSION

From the data in the period of January 2013-December 2014 that had been collected, 47 out of 3796 (1,24%) maternal patients were valid and found to be HBsAg positives, but unfortunately only 32 patients out of 3781 (0,85%) that were valid and met HBsAg and HBeAg data. This was because medical records are used for another research by another medical personnel.

This proportion of maternal patients who were HBsAg positives (0,85%) is smaller compared with the results in 2007 which prevalence of maternal patients with hepatitis B in Indonesia was 3,9%.[3] The result of this study does not represent the population because this study took samples from a refer center hospital. Therefore, only rare and complicated cases are handled here. Besides that, many cases of maternal patients with HBsAg positives can be handled in the local hospital.

The proportion of HBsAg negative was 99,15%. Out of 32 HBsAg positive maternal patients, there were 3 (9,37%) HBsAg and HBeAg positive maternal patients. From 32 positive HBsAg maternal patients, there were 22 medical records of the babies that successfully collected.

In Table 1, if the frequency of HBsAg positive maternal patients compared between 2013 and 2014, the higher proportion was in 2014 (0,97%) although there were the fewer number of maternal patients than in 2013.

In Table 2, from the maternal patients with complete HBsAg and HBeAg data, the highest frequency of HBsAg positive maternal patients in 2013 was in May, while in year the 2014 were in February, March, May, and October which had a same number of patients (two patients). Bhatt (2000) study showed that no seasonal distribution for HBV infection.[12] There was no incidence of HBV infection that had drastic increase in a particular season, only hepatitis A virus (HAV) which has a certain seasonal cycle. Its peaks in March and September.[13] Hepatitis A virus infection is an acute disease and can be cured and all of the transmission of HAV through the fecal-oral route.

Hepatitis B virus and hepatitis C virus (HCV) infection can develop to be a chronic disease so that the carrier of HBV and HCV infection always transmit the virus every year and month. According to Soemoharjo,[6] the transmission of HBV can pass through contact with the not intact body with blood, mucus of infected persons and also through sexual intercourse to infected persons. Therefore, this allows HBV infect anyone who had not infected and contact with blood on body fluid of HBV patients.

Based on delivery techniques in HBsAg positive maternal patients, the most frequent technique used was Caesarean (62%). Based on Chang *et al* (2014) research, were found a decrease of HBV transmission in caesarean delivery technique,[14] because the doctors who helped childbirth process knew that there were higher risks for spontaneous delivery for increasing the transmission of HBV. Hepatitis B virus from amniotic fluid can enter the body through the wound in the baby's skin that happened because of entering the birth canal or accidentally swallowed if there was a contraction of the uterus (materno fetal micro infusion).[6] If there is no

Table 1. The frequency of positive HBsAg and HBeAg maternal patients in 2013 and 2014

Year	N		HBsAg Positives	%
	Incomplete Data	Complete Data		
2013	7	2541	20/2541	0.79%
2014	8	1240	12/1240	0.97%
Total	**15**	**3781**	**32/3781**	**0.85%**

Table 3. Delivery techniques in HBsAg positives maternal patients

Delivery Techniques	HBsAg Positives (%)	HBsAg and HBeAg Positives (%)
Spontan	11/29 (38%)	2/3 (67%)
Caesar	18/29 (62%)	1/3 (33%)
Total	**29 (100%)**	**3 (100%)**

Table 2. Frequency of maternal patients with HBsAg positive, and HBsAg, HBeAg positive

Month	2013		2014	
	HBsAg Positives	HBsAg Positives HBeAg Positives	HBsAg Positives	HBsAg Positives HBeAg Positives
Jan	3	1	1	0
Feb	0	0	2	0
Mar	1	0	2	0
Apr	1	0	0	0
May	5	0	2	0
Jun	1	1	0	0
Jul	0	1	1	0
Agt	0	0	1	0
Sept	0	0	1	0
Oct	1	0	2	0
Nov	1	0	0	0
Dec	4	0	0	0
Total	**17**	**3**	**12**	**0**

Table 4. Gestation age distribution in HBsAg positives and HBsAg, HBeAg positives patients

Gestation Age	HBsAg Positives (%)	HBsAg and HBeAg Positives (%)
Preterm	3/29 (10,34%)	0/3 (0%)
Aterm	26/29 (89,65%)	3/3 (100%)
Total	**29/29 (100%)**	**3/3 (100%)**

contraction during caesarean delivery then it will decrease the risk of HBV transmission.

In this study out of three maternal patients who had HBsAg and HBeAg positive, there were two patients who had spontaneous birth. It cannot be a reference because a small number of patients cannot represent the overall picture of delivery techniques that often used in HBeAg positive maternal patients.

In Table 4, both HBsAg positive and HBsAg, HBeAg positive maternal patients had the significant difference in the gestation age distribution, which term gestation had the higher frequency. Incidence of low birth weight and prematurity increased in women with acute hepatitis B.[15] Another study found that carrier HBsAg women had higher risk to become preterm labor.[16]

However, Wong et al research didn't find any association between HBV infection in maternal patients with preterm labor, same as this research results in Dr. Soetomo General Hospital.[17]

Based on the occupation as seen in figure 1, the most frequent occupation of maternal patients was housewife (13 patients), followed by private employees (10 patients). In 2014, a study in Nigeria at a University of Medicine, the highest number of jobs in the antenatal women who were HBsAg positive was unemployment (13 among 42 women).[18] The result study in Nigeria is similar to the result study in Dr. Soetomo.

General Hospital which is the most frequent occupation was housewife or unemployment. The previous result's study showed that low socioeconomic status that initiates multi-sexual partners, unprotected sexual intercourse was more susceptible to get Sexually Transmitted Disease.[18]

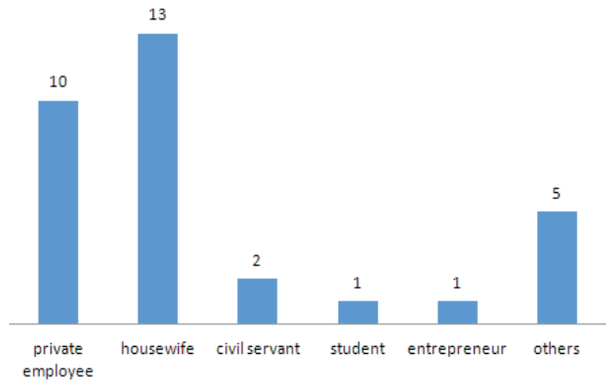

Figure 1. Bar Diagram of HBsAg positive maternal patiens based on Occupation distribution

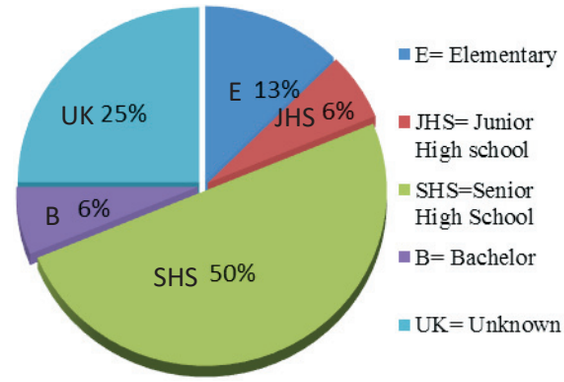

Figure 2. Circles Diagram of HBsAg positive maternal patients based on the education

Based on their education level, highest frequency in HBsAg positive maternal patients were Senior High School Graduate (50%).

The raw education data showed that the population with lower education status (Elementary, Junior High school, and Senior high school) were more prone to be infected with hepatitis B. It was because of lack of education in promotive prevention of the disease given in early grade school (Elementary, Junior High School, and Senior High School).

This study found only 22 complete medical record documents of the babies from 32 HBsAg positive maternal patients.

The remaining 10 files were not found in the medical records storage. All of 22 babies were given HBIG and HBV vaccine less than 12 hours after birth (100%). This result suggests that the prevention of transmission of HBV infection in Dr. Soetomo General Hospital had been done properly.

Table 5. Frequency of prophylactic administration of the baby from HBsAg positive maternal patients

Prophylaxis Action	Quantity (n=22)	Percentage
Hepatitis B vaccine	22	100%
HBIG	22	100%

Table 6. Data of babies from HBeAg positive maternal patients

	Delivery Technique	Gravida	Administration of Hepatitis B Vaccine and HBIG	Complete Vaccination (3 x)	HBsAg Status of the Child at This Time
Mother A	Spontaneous	Multigravida	yes	Complete	Positive
Mother B	Spontaneous	Multigravida	yes	Complete	Negative
Mother C	Caesarean	Multigravida	yes	Complete	Negative

As seen in table 6, all children from HBeAg positive maternal patients had been given immunoprophylaxis (HBV vaccine and HBIG) less than 12 hours after birth and had undergone complete vaccination three times.

Two children from two maternal patients (one mother who delivered with caesarean and another mother with spontaneous delivery) were found to be negative HBsAg. But interestingly, the other child of the mother who had spontaneous delivery was HBsAg positive even though her child had been given phrophylaxis properly. This positive HBsAg of the child may be caused by the presence of escape mutant HBsAg in infants who had been given HBIG and HBV vaccination. Hepatitis B surface antigen (HBsAg) with arginine replacement for glycine at amino acid 145 is the most common escape mutant HBsAg found in several clinical samples.[19] All infants who fail to respond to immunophrophylaxis were born from HBeAg positive mothers who had HBV DNA levels ≥6 $\log_{10}$ copy/mL. The existence of HBV DNA in cord blood also reflects the failure to respond passive and active immunization.[20]

From the result in this study, there was no tendency of transmission of HBV through certain delivery technique, same as in Hu et al (2013) study which stated that if hepatitis B vaccine and HBIG were given immediately after birth, the choice of labor procedure didn't determine the tendency of HBV transmission.

CONCLUSION

The proportion of maternal patients who was HBsAg positive in Dr. Soetomo General Hospital in period of 2013-2014 was 0,85%. Some missing data should take into consideration. Out of 32 HBsAg positive maternal patients, there were three maternal patients who were positive HBeAg (9,37%). All of the children with complete medical record documents in this study (22 children) had been given HBIG and HBV vaccine properly (100%). A good management of the medical records is needed in every hospital so that the practitioner who used data for research will get correct and complete data, therefore the results will be more accurate.

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Research Report

ANTIVIRAL ACTIVITY OF COPPER(II)CHLORIDE DIHYDRATE AGAINST DENGUE VIRUS TYPE-2 IN VERO CELL

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ABSTRACT

Infection of dengue virus (DENV) was number of globally significant emerging pathogen. Antiviral dengue therapies are importantly needed to control emerging dengue. Dengue virus (DENV) is mosquito-borne arboviruses responsible for causing acute systemic diseases and grievous health conditions in humans. To date, there is no clinically approved dengue vaccine or antiviral for humans, even though there have been great efforts towards this end. Copper and copper compounds have more effective in inactivation viruses, likes an influenza virus and human immunodeficiency virus (HIV). Purpose in this project was investigated of Copper(II) chloride Dihydrate antiviral compound were further tested for inhibitory effect on the replication of DENV-2 in cell culture. DENV replication was measures by Enzyme-linked Immunosorbent Assay (ELISA) with selectivity index value (SI) was determined as the ratio of cytotoxic concentration 50 (CC_{50}) to inhibitory concentration 50 (IC_{50}) for compound. The maximal inhibitory concentration (IC_{50}) of Copper(II)chloride Dihydrate against dengue virus type-2 was 0.13 µg/ml. The cytotoxic concentration (CC_{50}) of compound against Vero cell was 5.03 µg/ml. The SI values for Copper(II)chloride Dihydrate 38.69. Result of this study suggest that Copper(II) chloride Dihydrate demonstated significant anti-DENV-2 inhibitory activities and not toxic in the Vero cells. Copper mechanisms play an important role in the prevention of copper toxicity, exposure to excessive levels of copper can result in a number of adverse health effects, as a result increased reactive oxygen species and oxidative damage to lipid, DNA, and proteins have been observed in human cell culture models or clinical syndromes of severe copper deficiency and inhibition was attributed to released cupric ions which react with cysteine residues on the surface of the protease.

Keywords: *antiviral, dengue virus type-2, Copper(II)chloride Dihydrate, inhibitory, cytotoxity*

ABSTRAK

Infeksi virus dengue (DENV) adalah patogen yang muncul secara global. Terapi antivirus dengue penting diperlukan untuk mengontrol muncul dengue. Dengue virus (DENV) disebabkan oleh mosquito-borne arboviruses yang menyebabkan penyakit sistemik akut dan kondisi kesehatan pada manusia. Sampai saat ini, tidak ada vaksin dengue klinis disetujui atau antivirus bagi manusia, meskipun telah ada upaya besar menjelang akhir ini. Tembaga dan senyawa tembaga memiliki efektivitas dalam inaktivasi virus, seperti virus influenza dan human immunodeficiency virus (HIV). Tujuan dalam proyek ini adalah menyelidiki senyawa antiviral Copper (II) klorida Dihidrat yang kemudian diuji lebih lanjut untuk efek penghambatan pada replikasi DENV-2 dalam kultur sel. Replikasi DENV diukur dengan Enzyme-linked Immunosorbent Assay (ELISA) dengan nilai indeks selektivitas (SI) ditentukan sebagai rasio konsentrasi toksisitas 50 (CC_{50}) ke konsentrasi penghambatan 50 (IC_{50}) senyawa. Maksimal konsentrasi penghambatan (IC_{50}) Tembaga(II)klorida Dihidrat terhadap virus dengue tipe 2 adalah 0,13 µg/ml. Konsentrasi toksisitas (CC_{50}) senyawa terhadap sel Vero adalah 5.03 µg/ml. SI nilai untuk Tembaga(II)klorida Dihidrat 38.69. Hasil penelitian ini menunjukkan bahwa Tembaga(II) klorida Dihidrat signifikan anti-DENV-2 dan tidak toksik dengan sel Vero. Mekanisme tembaga berperan penting dalam pencegahan toksisitas tembaga, paparan kadar tembaga yang berlebihan dapat mengakibatkan sejumlah efek kesehatan yang merugikan, akibatnya

peningkatan spesies oksigen reaktif dan kerusakan oksidatif pada lipid, DNA, dan protein telah diamati pada Model kultur sel manusia atau sindrom klinis defisiensi tembaga berat dan penghambatan dikaitkan dengan ion cuprik yang dikeluarkan yang bereaksi dengan residu sistein pada permukaan protease.

Kata kunci: *antivirus, virus dengue tipe-2, Tembaga(II)klorida Dihidrat, penghambatan, toksisitas*

INTRODUCTION

Infection of dengue virus (DENV) was number of globally significant emerging pathogen. It is member of Flaviviridae family, with the genus Flavivirus. DENVs were distributed in the tropical and sub-tropical areas and transmitted to humans by Aedes agypty and Aedes albopictus.[1] Dengue virus (DENV) is mosquito-borne arboviruses responsible for causing acute systemic diseases and grievous health conditions in humans. More than 2.5 billion cases of dengue infection occurred in the worldwide.[2] Indonesia is one of the largest counties in the dengue endemic region worldwide. Dengue was occurred for the first time as an outbreak in Surabaya and Jakarta in 1968.[3] To date there are not effective vaccine and antiviral treatment for DENV, patient supportively-treated without any specific treatment measures.[4] Antiviral dengue therapies are importantly needed to control emerging dengue. Effective antiviral therapies, currently unavailable for any type of DENV, are urgently needed to ameliorate the disease burden by DENV.[5] Ribavirin has shown activity against all flaviviruses tested in a broad array of cell types in vitro but efficacy in vivo has generally been poor, ribavirin can be toxic in vivo.[6] A compound that exhibited a lower effective dose and toxicity than ribavirin while retaining its broad spectrum of activity would be particularly desirable as a candidate flavivirus therapy.[5]

Copper and copper compounds have been used as important antiviral material.[7] Recently, group found that Cu^{+} species in the related compounds is much more effective in inactivation of bacterial and viruses than copper metal and copper(II) compounds.[8] On the other hand, copper has long been used as an antibacterial material,[9] and several copper compounds have been reported to exhibit viral inactivation. More recently, the inactivation of avian influenza virus by copper metal[10] and divalent ions (Cu^{2+})[11] and the inactivation of human immunodeficiency virus (HIV) by copper ions[12] and copper oxide have been reported.[13] Copper iodide nanoparticle against for feline calicivirus (FCV) was demonstrated that the antiviral behaviors of CuI nanoparticles against FCV were identified to detect Cu^{+} ions, hydroxyl radicals, and capsid protein oxidation. Copper iodide nanoparticles showed high antiviral activity against FCV was attributed to Cu^{+} ions, followed by ROS (O_2• or •OH) generation and subsequent capsid protein oxidation.[14] The antivirus properties of the $CuFeO_2$ crystals achieved about 8 log inactivation of the phage after 4 h of contact time in the dark, $CuFeO_2$ are good chemical stability in a weak acid condition.[7] Copper is a bio-essential element and copper complexes have been extensively utilized in metal mediated DNA cleavage for generation of activated oxygen species, was reported that teraaza macrocyclic copper coordination compounds have anti-HIV activity. Macrocyclic complexes can react with DNA in different binding fashions and axhibit effective nucleus activities.[15]

Copper monodispersed nanoparticles (2-5 nm) in submicron particles of sepiolite, structure of sepiolite is $Mg_8Si_{12}O_{30}(OH)_4(H_2O)_4.8H_2O$, have revealed as a strong bactericide so that they were able to decrease the starting microorganism concentrations of Staphylococcus aureus or Escherichia coli by 99.9%.[16] The antibacterial stainless steels included a copper-bearing austenitic antibacterial showed excellent with antibacterial rate to E.coli over 99.99%, copper ions play the dominant role in the antibacterial effect of antibacterial stainless steels acted with E. coli.[17]

Previous result, ribavirin exerts its toxicity through an inhibiton of intracellular energy metabolism and axidative membrane damage, leading to an accelerated extravascular hemolysis by the reticulo-endothelial. But not significant inhibiton of level, ribavirin was more to toxic to replicating cells than to stationary cell monolayers in Vero cells.[18] Currently, there is no published data on the possible anti-DENV activities of Copper and copper compounds. In the present study, we investigated of Copper(II)chloride Dihydrate antiviral compound were futher tested for inhibitory effect on the replication of DENV-2 in cell culture.

MATERIAL AND METHOD

Material

Chemical reagents used in this research is the Copper(II) chloride Dihydrate ($CuCl_2.2H_2O$) (Merck 99.0%), Dimethyl Sulfoxide (DMSO) (Merck 99.98%), Minimum Essential Medium Eagle (MEM Media) (Sigma-Aldrich), DENV-2 Surabaya Isolate, Vero cell (African green monkey kidney), Cell Proliferation Reagent WST-1 (Roche Applied Science), and Dengue Virus Antibody (4G2) for ELISA.

Method

Antiviral activity assay

Confluent monolayers of Vero cells were prepared in 96 wells cell culture microplate. The numbers of DENV-2 were counted using a Hemocytometer and the titer of virus was expressed as Foci-Forming-Unit (FFU). Seed Vero cells in a 96-well plate ($1x10^6$ cells/10 ml), add serially diluted

test compounds to Vero cells, add DENV-2 solution ($2x10^4$ FFU/well) and incubate 37°C for 2 days. The percentage of inhibition concentration (IC_{50}) compared with controls was calculated as follows: IC_{50} (%) = (NC-AC) x 100/ NC. Where, NC is the mean of the number for negative control and AC is the number absorbance of compound. Inhibition of compound to DENV-2 was further verified using quantitative Enzyme-linked Immunosorbent Assay (ELISA).

Cytotoxicity assay

Cytotoxicity used WST-1 cell proliferation reagent by Roche Applied Science, Mannheim, Germany.[19] The dye of WST-1 reagent has a larger linear range and increased stability compared to other tetrazolium salt based assays. The WST-1 assay is suitable for use with adherent and suspension cells. The assay is very sensitive, it can detect 500 to 50,000 cells in a single well of a 96-well plate. Vero cells ($1x10^5$ cells/ml) were seeded in 96-well plate at 37 °C in 5% CO_2 overnight. A total of 100 µl of serial delusion compound were incubated with Vero cells for 24 h. A total of 10 µl of Cell Proliferation Reagent WST-1 was added into each well, incubated for 1 hour at 37 °C. The plate was read at 450 nm (main filter) and 655 nm (reference filter) using an ELISA reader (iMarkTM Microplate Absorbance Reader).

RESULT AND DISSCUSSION

Inhibitory Effect of Copper(II)chloride Dihydrate

Copper(II)chloride Dihydrate were futher studied for their inhibitory effect on replication of the DENV-2 in Vero cells. The IC_{50} (inhibitory concentration 50) was determined from the dose response curves. This compound proved to be effective as inhibitor of replication of DENV-2, the IC_{50} value was 0.13 µg/ml and R^2 value was 0.9812 with selectivity indices (SI) was 38.69. The selectivity indices of these antiviral compounds appeared to be moderately influenced by the strain of DENV tested.

The mechanisms of how Copper(II)chloride Dihydrate exerts it is anti DENV-2 effects are not known. However, the effects of other compounds against cellular RNA polymerases and formation of the complex with RNA have been reported suggesting that Copper(II)chloride Dihydrate could also affect the similar replication enzymes. Viral replication was inhibited during a simultaneous treatment assay, indicating that the entry of the virus was impeded by peptide.

Previous research was reported protease inhibitory activity on DENV2V NS3 target, Palmatine has active concentration 26.4 µM, this compound was subsequently analyzed for antiviral activity in cell-based replication assays in cell culture.[20] The neutral red assay mean EC_{50} of ribavirin was only 106 µg/ml with SI of 9.4 against West Nile Virus (WNV) New York isolate and EC_{50} of

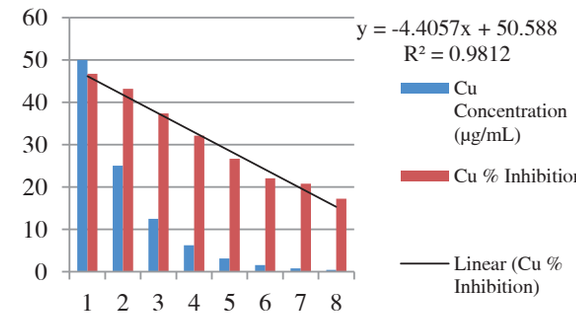

Figure 1. Inhibitory chart of Copper(II)chloride Dihydrate for Vero cells by ELISA

6-Azauridine was 1.5 µg/ml with SI of WNV New York isolate. This result confirm by virus yield reduction assay when the assay when the assay was performed 2 days after initial infection in Vero cells.[21]

Copper homeostatic mechanisms play an important role in the prevention of copper toxicity, exposure to excessive levels of copper can result in a number of adverse health effects. Similar to Cu toxicity, Cu deficiency also affects, directly or indirectly, the components of the oxidant defense system and as a result increased reactive oxygen species and oxidative damage to lipid, DNA, and proteins have been observed in human cell culture models or clinical syndromes of severe copper deficiency.[22] In these cases, the observed inhibition was attributed to released cupric ions which react with cysteine residues on the surface of the protease.[23]

Maximal inhibitory concentration (IC_{50}) of quercetin against DENV-2 was 35.7 µg/ml when it was used after virus absorption to the cells and decreased to 28.9 µg/ml when the cells were treated continuously for 5 h before virus infection and up to 4 days post-infection. A weak effect for prophylactic activity of quercetin however. These findings suggest that the main anti-dengue activity of quercetin is likely due to its activity against the different stages of its replication of DENV-2 instead of early stages of intracellular replication cycle such as virus attachment or entry.[4]

Cytotoxicity of Copper(II)chloride Dihydrate

The cytotoxicity study was carried out for compound of Copper(II)chloride Dihydrate. This extract was screened for its cytotoxicity against Vero cells at different concentrations to determine the CC_{50} by WST-1 assay.

The percentage growth cytotoxicity was found to be increasing with increasing concentration of test compound, and that show in figure 4. Copper(II)chloride Dihydrate effect on Vero cells (CC_{50}) up to 5.03 µg/ml and R^2 value was 0.9174. In this work, we have examined the relationship between the concentration in the culture medium of Vero cells and the cytotoxic potency of Copper(II)chloride Dihydrate.

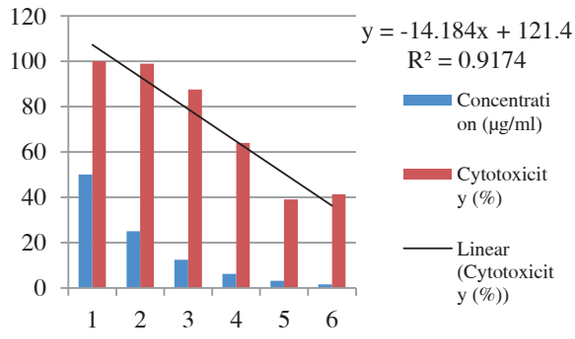

Figure 2. Cytotoxicity chart of Copper(II)chloride Dihydrate for Vero cells by WST-1 assay

CONCLUSION

As the conclusion, the study demonstrated that the Copper(II)chloride Dihydrate exhibited significant anti DENV-2 replication properties. These results suggest that these Copper(II)chloride Dihydrate could be further investigated IC_{50} was 0.13 µg/ml, CC_{50} was 5.03 µg/ml, and SI was 38.69.

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Research Report

THE ANTIBACTERIAL EFFECT OF ROSELLE (*Hibiscus sabdariffa*) EXTRACT AGAINST *Staphylococcus epidermidis* IN VITRO

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ABSTRACT

Infection of Staphylococcus epidermidis is still a common problem in many hospitals. Factor determining biofilm formation makes it harder for atibiotics to cure the infection. Roselle (Hibiscus sabdariffa), a well known traditional medicine plant, is a potential candidate as a drug againts infectious disease. The purpose of this research is to investigate the antibacterial effect of ethanol extract from Roselle (Hibiscus sabdariffa) calyx againts the growth of Staphylococcus epidermidis. Assessment for antibacterial effect is performed using broth diffusion method. The extract is made by maceration of the calyx of Roselle in 96% ethanol. Extracts with concentration of 125, 62.5, 31.25, 15.63, 7.81, 3.90, 1.95, 0.97, 0.48, 0.24 mg/mL are added into separated Mueller-Hinton broths (MHB), which have already been inoculated by Staphylococcus epidermidis. As for bacterial growth control, we used MHB with bacterial inoculation, while sterility control we used mixture of extract and MHB. Then from each broth, the solutions are added into separated nutrition agar plates. Replications are done three times. Clarity and bacterial growth are observed after 24 hours of incubation. However, clarity cannot be observed in 36 broth, but bacterial growth is observed on the plate for concentration 0.97, 0.48, and 0.24 mg/mL. Therefore Minimum inhibitory concentration (MIC) cannot be determined because the extract's color interfere the observation. While minimum bactericidal concentration (MBC), the last concentration before the concentration where the bacteria are still viable, is 1.95 mg/mL. Based on the result of the research, the Roselle calyx ethanol extract (Hibiscus sabdariffa) through dilution method with a concentration of 1.95 mg / mL can kill Staphylococcus epidermidis and in order to find MIC in collored and turbid solution (before being incubated in incubator), we can consider using agar dilution methode or microdilution methode.

Keywords: *Hibiscus sabdariffa, antibacterial, Staphylococcus epidermidis, biofilm, flavonoids*

ABSTRAK

Infeksi Staphylococcus epidermidis masih merupakan masalah umum yang ditemukan di banyak rumah sakit. Kemampuan bakteri untuk membuat biofilm mempersulit atibiotik untuk menyembuhkan infeksi. Rosella (Hibiscus sabdariffa), tanaman obat tradisional yang umum beredar di masyarakat, adalah bahan yang berpotensial untuk dikembangkan menjadi obat untuk mengatasi infeksi. Tujuan dari penelitian ini adalah untuk mengetahui efek antibakteri dari ekstrak etanol kelopak Rosella (Hibiscus sabdariffa) terhadap pertumbuhan Staphylococcus epidermidis. Penelitian ini merupakan penelitian eksperimental laboratorik dengan metode dilusi. Ekstrak Rosella dibuat dengan cara maserasi dari tampuk Rosella dengan menggunakan etanol 96%. Kemudian dilakukan pengenceran sebanyak 10 kali didalam 10 tabung. Konsentrasi yang didapatkan adalah 125, 62.5, 31.25, 15.63, 7.81, 3.90, 1.95, 0.97, 0.48, 0.24 mg/mL. Masing-masing tabung sudah berisi bakteri. Sebagai kontrol tumbuh bakteri digunakan campuran bakteri dengan Mueller-Hinton broth dan kontrol sterilitas menggunakan cairan ekstrak dengan Mueller-Hinton broth. Kemudian dari ke-12 tabung, dilakukan streaking pada media nutrient agar plate untuk melihat pertumbuhan dari bakteri. Replikasi percobaan dilakukan sebanyak 3 kali hasil percobaan diamati setelah inkubasi selama 24 jam. Hasil penelitian yang didapat, dari 36 tabung tidak dapat diamati kejernihan dari tabung. Hal ini disebabkan warna dari ekstrak mengganggu dari kejernihan pengamatan sehingga tidak dapat ditentukan nilai dari konsentrasi hambat minimal (KHM). Kemudian dari nutrient agar plate, didapatkan pertumbuhan bakteri pada

konsentrasi 0.97, 0.48, dan 0.24 mg/mL. hal ini berarti bahwa konsentrasi bunuh minimal (KBM) dari ekstral Rosella adalah 1.95 mg/mL. Berdasarkan hasil penelitian yang dilakukan, ekstrak tampuk Rosella dengan menggunakan metode dilusi dapat membunuh bakteri Staphylococcus epidermidis pada konsentrasi1.95 mg / mL dan untuk memperoleh hasil KHM pada larutan yang berwarna dan keruh (sebelum diinkubasi dalam inkubator). dapat dipertimbangkan untuk menggunakan metode dilusi agar atau mikrodilusi. Sehingga Rosella memiliki efek antibakterial dan memiliki potensi yang besar sebagai bahan antimikroba.

Kata kunci: *Hibiscus sabdariffa, antibacterial, Staphylococcus epidermidis, biofilm, flavonoids*

INTRODUCTION

Bacterium is the microorganism that is most frequently found in human body. This microorganism often causes infection and medical problems. One of them is *Staphylococcus* epidermidis. Normally, this bacterium is present in healthy people and doesn't cause infectious disease, however in certain condition like immunodeficiency syndrome, it can cause infectious disases.[1] Because of this condition, *Staphylococcus epidermidis* is a common cause of nosocomial infections. As many as 40-90% of nosocomial infections associated with hospital tools are caused by this bacterium.[2] This increases the patient's health expenditure and duratiuon of staying in hospital. In America, 50-70% of the 16,000 cases of bacteremia by catheter infection in ICU are caused by *S. epidermidis* with an additional cost of 37.000-39.000 US$ for each person.[3] Besides the high incidence rate, many strains of *Staphylococcus* have antibiotics resistance like methicillin and vancomycin. This resistance is also associated with the bacteria's ability to make biofilms.[4] Biofilm is an ability possessed by a certain kind of bacteria to bind and create a complex structure which is formed by its colonization. It has the ability to allow bacteria to develop resistance to host immune responce and antibiotics.[5] Therefore, the discovery of treatments to infectious diseases, in particular caused by *S. epidermidis* is very important.

In addition to drug development, the use of medical plants as natural antimicrobial agents is gaining popularity. Roselle (*Hibiscus sabdariffa*) is a tropical and sub-tropical plant with a potential candidate in herbal medicine. It is commonly used to make form drink and pickle and it is used in folk medicine infor treatment of hypertention, liver disease and fever.[6] Several studies have been conducted on Rosella and have shown various benefits for medical purpose. A research was conducted by Majorie *et al*. Bioactive substances in Hibiscus like alkaloids, flavonoids, phenolics and biterpenoids) may have antibacterial effect agains *Esherichia coli.*[7] Anthocyanin and protocatechuic acid compounds also isolated from dried flower of Hibiscus sabdariffa demonstrate protective effects against oxidative agents.[6] Moreover, a research also conducted found that the protocatecuic acid also inhibited the growth of methicillin-resistant *S. aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, and Acinetobacter baumanniliu, tsao, yin).*[8]

With the increase in *Staphylococcus* resistance and Roselle medical potential, we need further research regarding the antibacterial effect of this plant. In this study, researcher aims to investigate the antibacterial effect of Roselle extract on the growth of *Staphylococcus epidermidis* in vitro.

MATERIAL AND METHOD

Plant Material Extraction

The flowers of *Hibiscus sabdariffa* were purchased from UPT Materia Medica in Batu small town, east java. The plant materials were taxonomically identified by a botanist at the same location. Calyces of the plant were separated and ground to a fine powder. About 250 g dried powder was taken and soaked with 96% ethanol. The wet powder is put in a jar and as much as 1500 mL 96% ethanol was poured into the jar. The jar was then closed tightly for 24 hours and placed on the shaker with 50 rpm. After that the suspension was filtered and was placed into erlenmeyer. The sediment from the filtration was re-maceration with the same technique (1500 mL 96% ethanol). The ethanolic extract was evaporated with rotary evaporator for 2 hours and water bath for 1 hour. The final result from the extraction was 77 mL extract with concentration 250 g/mL.

Antibacterial Assay

Prior to the experiment, a colony of *S. epidermidis* was subcultured in Mueller Hinton Agar (MHA) and incubated for 24 hours at 37 ºC. The bacteria were then adjusted by adding normal saline to be equivalent to 0.5 McFarland standard which comprised 1.0 x 10^8.

Susceptibility Testing Procedure

The experiment was repeated 3x. The extract was dispensed in 1 mL volume in each sterile tube with decreasing concentration starting from 125 g/mL. Each tube was then inoculated by 1 mL volume of diluted *S. epidermidis*. The growth control tube consists of 1 mL inoculum and extract free medium while the sterility control contains 1 ml extract and 1 ml medium. All tubes were incubated at 37 ºC and MICs were read after 24 hours of incubation.

RESULT

A total of 30 tubes were tested for MIC. The result of the MIC is shown in Table 1.

Overall, by using macrodilution methode the MIC couldn't be determined. This result happens because the extract's red color and turbidity interfere with the observation and the assessment. The same conditon could also be seen in sterility conttrol tube because of that it couldn't be used as a comparation to determine sterility. so in order to determine the extract's efficacy, each of the 30 tubes was streaked into nutrient agar plate to determind MBC value which was shown in Table 2.

From the experiment, it can be determind that the MBC of Roselle extract against *S. epidermidis* is 1.95 mg/mL (1.56%).

DISCUSSION

Roselle is a great plant to be used for medical purpose. First, it is easily grown in tropical country like in Indonesia and has many properties. The time required to grow is around 4 to 8 months with the lowest temperature 20°C, 13 hours lighting, and 130 to 250 mm of rainfall for each month.[9] With these condition, people can easily get the plant and cultivate it. Second, it is known to have many good effects. Roselle has antimicrobial, anti-parasite, anti-cancer, anti-pyretic, anti-inflamation, anti-oxidant, nephro-protective, hepato-protective, diuretic, anti-cholesterol, antidiabetic, and antihypertensive.[6, 10-11]

From the result, the MIC in the experiment couldn't be indentified because of the extract's red colour and turbidity. To find out the result of the MIC, extract Roselle can be tested with another dilution methode. In Nigeria, research was conducted by Mary[12]. She did MIC testing by agar dilution methode. She examined the antimicrobial effect of Roselle against *Staphylococcus aureus, Bacillus stearothermophilus, Micrococcus luteus, Serratia mascences, Clostridium sporogenes, Escherichia coli, Klebsiella pneumoniae, Bacillus cereus,* and *Pseudomonas fluorescence* with MIC 0.30 ± 0.2-1.30 ± 0.2 mg/mL. A similar research was conducted by Sulistyani[13] and her research group with microdilution methode. They tested antimicrobial activity against mouth pathological bacteria that could make biofilm. These bacteria were *Streptococcus mutans, Streptococcus sanguinis, Lactobacillus casei, Actinomyces naeslundii, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Porphyromonas gingivalis* and *Prevotella intermedia* with MIC and MBC 7.2 mg/mL to 28.8 mg/mL and 14.4 to >57.6 mg/mL. Interestingly, Roselle extract also has the ability to inhibit biofilm formation on the concentration of the MIC.[13] The formation of biofilm is also found in *S. epidermidis.*[4]

The overal mechanism how Roselle extract has antibacterial effects is still not completely comprehanded. In USA, Marjorie, Janak, Jacqueline, Shurrita, and Leonardo were conducted a research about the antimicrobial activity of *Hibiscus sabdariffa* against *E. coli.* By using disk diffusion method, they concludedgain conclusion that all concentration (10%, 5%, and 2.5%) of *H. sabdariffa* could

Table 1. Roselle extract's MIC

	Extract Concentration											
	100% (1)	**50% (2)**	**25% (3)**	**12.5% (4)**	**6.25% (5)**	**3.12% (6)**	**1.56% (7)**	**0.78% (8)**	**0.39% (9)**	**0.19% (10)**	**G+**	**S-**
1	X	X	X	X	X	X	X	X	X	X	X	X
2	X	X	X	X	X	X	X	X	X	X	X	X
3	X	X	X	X	X	X	X	X	X	X	X	X

G+: Growth control S-: Sterility control X: Can't be assessed

Table 2. Roselle extract's MBC

	Extract Concentration											
	100% (1)	**50% (2)**	**25% (3)**	**12.5% (4)**	**6.25% (5)**	**3.12% (6)**	**1.56% (7)**	**0.78% (8)**	**0.39% (9)**	**0.19% (10)**	**G+**	**S-**
1	-	-	-	-	-	-	-	+	+	+	+	-
2	-	-	-	-	-	-	-	+	+	+	+	-
3	-	-	-	-	-	-	-	+	+	+	+	-

G+: Growth control S-: Sterility control +: Viable bacteria -: No viable bacteria

inhibit *E. coli* activity in from food, veterinary, and clinical samples and showed that the most effective concentration was at 10%, whereas the least effective concentration was at 2.5%. They were stated that the antimicrobial effect of *H. sabdariffa* might come from its flavonoids chemical. The structure of flavonoids have the ability to form combined complex with bacterial walls. Besides that, the number of hydroxyl groups present on the phenolic ring helps the antimicrobial activity of the extract. Due to the increase of hydroxyl group, the hydroxylation would accelerate and cause the increase of antimicrobial activity.[7] Issam and Ahmed alsowere stated a similar discussion that phenolic compounds including flavonoids and cyaniding contribute to antimicrobial activity. They added that flavonoid with its phenolic chain could decreases iron level and increases hydrogen level, which deactivates bacterial enzymes.[14] Moreover Sulistyani et al. reported that Flavonoids are also thought to have the ability to inhibit the formation of bacterial biofilms. This capability is possible because the phenolic group in the extract capable to bind strongly to proteins and enzymes from the bacteria. This makes the bacteria unable to produce biofilms.[13] This effect is important considering *S. epidermidis* and some gram-positive and gram-negative bacteria capable of producing biofilms.

Based on the research that has been conducted, Roselle calyx extract can be used as a alternative treatment for infections caused by *Staphylococcus epidermidis*.

CONCLUSION

The Roselle calyx ethanol extract (*Hibiscus sabdariffa*) through dilution method with a concentration of 1.95 mg / mL can kill *Staphylococcus epidermidis* and in order to find MIC in collored and turbid solution (before being incubated in incubator), we can consider using agar dilution methode or microdilution methode.

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Research Report

PLASMA LEAKAGE PROFILES OF DENGUE HEMORRHAGIC FEVER PATIENTS IN RSUD Dr. SOETOMO, SURABAYA, EAST JAVA, INDONESIA JANUARY – JUNE 2014

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ABSTRACT

Plasma leakage is one crucial point of dengue hemorrhagic fever (DHF) that differentiates it from dengue fever (DF). DHF has to meet 4 criteria which are 2 – 7 days of acute fever, hemorrhagic manifestation, thrombocytopenia (≤100.000 cells/mm^3) and evidence of plasma leakage. Plasma leakage consists of increasing hematocrit ≥20%, hypoalbuminemia or evidence of pleural effusion or ascites. Often doctors only base their DHF diagnosis on the presence of thrombocytopenia. This study analyzed the presence of plasma leakage between adult and pediatric patients with a DHF diagnosis in RSUD Dr. Soetomo in order to make the diagnosis and healthcare services better in the future. This was a retrospective study which used medical records of DHF patients admitted from January to June 2014. 78 cases were included, 24 adult patients (31%) and 54 pediatric patients (69%). 29/78 (37%) patients had no evidence of plasma leakage. No adult patients had ascites whereas 11/54 (20%) pediatric patients presented with ascites. No adult patients had pleural effusion whereas 25/54 (53%) pediatric patients did. Most adult patients that had serum albumin checked had normal albumin levels (12/14 [86%]) while only 14/28 (52%) pediatric patients had normal albumin level. 5/22 (23%) adult patients versus 32/53 (60%) pediatric patients showed hematocrit increments ≥20%. Patients admitted with dengue virus infection may currently be often misclassified as DHF because there are no plasma leakage manifestation in some patients.. There are significant differences in plasma leakage manifestations between adult and pediatric patients which poses a theory that pediatric patients are more susceptible to have plasma leakage manifestations than adult patients.

Keywords: *plasma leakage, dengue hemorrhagic fever, dengue fever, pediatric patients, adult patients*

ABSTRAK

Kebocoran plasma adalah salah satu gejala penting demam berdarah dengue (DBD) yang membedakan dengan demam dengue (DD). Ada 4 kriteria dalam penegakan diagnosis DBD yaitu demam tinggi mendadak 2 – 7 hari, manifestasi perdarahan, trombositopenia (≤100.000 sel/mm^3) dan bukti dari kebocoran plasma. Kebocoran plasma terdiri dari peningkatan hematokrit ≥20%, hipoalbuminemia, bukti dari efusi pleura atau asites. Masih banyak dokter yang hanya berpatokan pada kriteria trombositopenia saja pada penegakan diagnosis DBD. Penelitian ini bertujuan untuk menganalisis profil dari kebocoran plasma dari pasien DBD dewasa dan anak di RSUD Dr. Soetomo pada periode Januari – Juni 2014 sehingga bisa didapatkan diagnosis dan pelayanan kesehatan yang lebih baik ke depannya. Penelitian ini adalah penelitian studi retrospektif dengan menggunakan rekam medik pasien DBD. 78 rekam medik pasien DBD ditemukan, terdiri dari 24 pasien dewasa (31%) dan 54 pasien pediatri (69%). 29/78 (37%) pasien ditemukan tanpa adanya manifestasi kebocoran plasma. Sama sekali tidak ditemukan pasien dewasa dengan asites tetapi ditemukan 11/54 (20%) pasien pediatric dengan asites. Tidak ada pasien dewasa dengan manifestasi efusi pleura sama sekali sedangkan pada pasien pediatri ditemukan 25/54 (53%) pasien dengan efusi pleura. Mayoritas pasien dewasa yang telah dicek serum albumin memiliki kadar albumin normal (12/14 [86%]), di sisi lain hanya 14/28 pasien pediatri yang telah dicek serum albumin memiliki kadar albumin normal. 5/22 (23%) pasien dewasa berbanding 32/53 (60%) pasien anak menunjukkan kenaikan hematokrit ≥20%. Dalam penelitian ini dapat disimpulkan bahwa masih cukup banyak terjadi misdiagnosis dari DBD karena dapat ditemukan beberapa pasien yang tidak memiliki manifestasi kebocoran

plasma. Pasien anak dan pasien dewasa memiliki perbedaan yang signifikan sehingga dapat memunculkan teori bahwa pasien anak lebih rentan untuk memiliki manifestasi kebocoran plasma.

Kata kunci: *kebocoran plasma, demam berdarah dengue, demam dengue, pasien pediatri, pasien dewasa*

INTRODUCTION

WHO classifies symptomatic dengue virus infection into four groups which are undifferentiated fever, dengue fever (DF), dengue hemorrhagic fever (DHF), and expanded dengue syndrome. Dengue Hemorrhagic Fever (DHF) remains one of the tropical diseases with an enormous worldwide caseload. Around 50 million dengue virus infections happen annually of which 500,000 people are diagnosed with DHF and need hospitalization. Approximately 90% of DHF patients are children aged less than five years and 2.5% die.[1] Indonesia in 2014 had 100,347 cases of DHF of which 907 (0.9%) were lethal.[2]

DF and DHF mostly have similar symptoms. The requirement for DHF diagnosis is acute onset of fever lasting 2 – 7 days, hemorrhagic manifestation, a platelet count ≤100,000 platelets/mm^3 and evidence of plasma leakage.

Plasma leakage is the main hallmarks of DHF and it differentiates DHF from DF. DHF and DF nearly have a similar sign & symptom (like thrombocytopenia, hemorrhagic manifestation, fever etc.). Plasma leakage manifestation is essential for the diagnosis making of DHF because any signs and symptoms of dengue viral infections without plasma leakage manifestations is considered to be DF.

Plasma leakage is considered to have occurred in case of a suddenly rising hematocrit to ≥20% from baseline or decrease in convalescence, the presence of ascites, a new pleural effusion on lateral decubitus chest x-ray (CXR), or low serum albumin or protein for age and sex.[1]

Many doctors base their diagnosis of DHF only on the presence of thrombocytopenia.[3] WHO changed the classification of dengue in 2011 which refers back to the classification from 1997 with some changes. Many doctors were confused about the 2009 WHO dengue classification since the WHO 2009 classification created about twice the workload for health care personnel and required a dengue confirmatory test.[4] The changes made in 2009 created confusion among healthcare personnel and may have had unwanted consequences for healthcare services.

By the latest 2011 dengue classification, most patients did not fulfill the DHF case definition: evidence of plasma leakage in the presence of thrombocytopenia.[4] In the early phase of mild cases of DHF one might not find evidence of plasma leakage by physical examination.[1] This phenomenon makes it easier misclassify patients with dengue virus infection.

This goal of study was to analyze the presence of plasma leakage between adult and pediatric patients with a DHF diagnosis in RSUD Dr. Soetomo Surabaya in the period of January – June 2014.

MATERIAL AND METHOD

This study was a retrospective study using medical record of patients that had had a DHF diagnosis in RSUD Dr. Soetomo, Surabaya between January – June 2014. DHF patients which had unusual manifestations or complications that were not correlated with dengue virus infection were excluded. These exclusions were based on the 'Comprehensive Guidelines for Prevention and Control of Dengue and Dengue Hemorrhagic Fever' published by WHO in 2011. These exclusions would minimalize plasma leakage manifestations that caused by non - dengue virus infection.

Variables recorded in this study consisted data regarding plasma leakage, i.e. increasing hematocrit ≥20%, hypoalbuminemia, and evidence of pleural effusion and ascites. There were no data available regarding the baseline levels of hematocrit in Indonesia, instead we therefore subtract the lowest hematocrit level from the highest hematocrit level, then dividing that value by the lowest hematocrit level and multiplying by 100.[5] As normal albumin levels in this study we used 3.4 – 5 g/dl. Pleural effusion were checked by Chest X-Ray (CXR) and the presence of ascites was ascertained by physical examination only.

Table 1. Grading of disease severity among patients admitted to RSUD Dr. Soetomo academic hospital with a diagnosis of Dengue Hemorrhagic Fever.

Diagnosis	Adult Patients		Pediatric Patients	
	Frequency	Percentage (%)	Frequency	Percentage (%)
DHF Grade I	13	54.2	16	29.6
DHF Grade II	11	45.8	19	35.2
DHF Grade III	0	0	16	29.6
DHF Grade IV	0	0	3	5.6
Total	**24**	**100**	**54**	**100**

p value calculated by Mann – Whitney test = 0.002

Adult data are the data of patients treated in Internal Medicine department and pediatric data are from cases treated in Pediatric Medicine department.

RESULT AND DISCUSSION

48 patients were excluded based on exclusion criteria. There were 78 DHF patients who fulfilled this study's criteria in RSUD Dr. Soetomo between January – June 2014 that consisted of 24 adult patients (31%) and 54 pediatric patients (69%). 76% patients enter the hospital in critical phase while 6% and 18% enter the hospital in febrile and recovery phase.

No dengue shock syndrome (DSS) happened in adult patients while in pediatric patients there were 19 DSS patients. The clinical presentation of adult and pediatric with DHF differed significantly (Table 1).

Overall, 29/78 patients (37%) did not have any evidence of plasma leakage. These 29 patients consisted of 17 adult patients and 12 pediatric patients.

None of the adult patients had ascites on physical examination while 11 pediatric patients did have ascites. Among the 11 pediatric patients with ascites, 9 patients had developed ascites before entering the hospital and 2 patients developed ascites during their hospital stay.

None of the adult patients had radiological evidence of pleural effusion while pleural effusions were detected in 25/34 (74%) pediatric patients that had CXR taken. 11 adult patients and 20 pediatric patients did not have a CXR taken during their hospital stay.

From the albumin level data presented in Table 3 it is become evident that there were more patients which had normal albumin level than patients that had hypoalbuminemia. 12/14 (86%) adult patients had normal albumin levels while only 14/27 (52%) pediatric patients

Table 2. Distribution of clinical and laboratory manifestations of plasma leakage in patients admitted with a Dengue Hemorrhagic Fever diagnosis.

Manifestation	Frequency	Percentage (%)	Percentage with No Manifestations (%)
No evidence of plasma leakage	29	37.2	-
Ascites only	1	1.3	2.0
Pleural effusion only	4	5.1	8.2
Hypoalbuminemia only	2	2.6	4.1
Hematocrit increase š20% only	19	24.4	38.8
Pleural effusion + Hypoalbuminemia	1	1.3	2.0
Pleural effusion + Hematocrit increase š20%	7	9.0	14.3
Hypoalbuminemia + Hematocrit increase š20%	1	1.3	2.0
Ascites + Hematocrit increase š20%	1	1.3	2.0
Ascites + Pleural effusion + Hematocrit increase š20%	3	3.8	6.1
Pleural effusion + Hypoalbuminemia + Hematocrit increase š20%	4	5.1	8.2
Ascites + Pleural effusion + Hypoalbuminemia	3	3.8	6.1
Ascites + Pleural effusion + Hypoalbuminemia + Hematocrit increase š20%	3	3.8	6.1
Total	**78**	**100**	**100**

Table 3. Albumin level distribution of DHF patients

Albumin	Adult			Pediatric		
	Frequency	Total	Percentage (%)	Frequency	Total	Percentage (%)
Normal	12	14	85.7	14	27	51.9
Hypoalbuminemia	2		14.3	13		48.1
No Data	10			27		
Total	**24**		**100**	**54**		**100**

p value calculated by Chi – square test = 0.03

Table 4. Changes in hematocrit in adult and pediatric patients with a Dengue Hemorrhagic Fever diagnosis.

Hematocrit	Adult			Pediatric		
	Frequency	Total	Percentage (%)	Frequency	Total	Percentage (%)
Increased š20 %	5	22	22.7	32	53	60.4
Negative	17		77.3	21		39.6
Only tested once	2			1		
Total	**24**		**100**	**54**		**100**

p value calculated by Chi – square test = 0.003

had normal albumin level (Table 3, p = 0.03). However, in a large proportion of patients suspected of DHF, their serum albumin level was not determined.

37/75 (49%) patients had hematocrit increments ≥20 %. More so among pediatric patients (32/53 [60%]) than among adult patients (only 5 out of 22 patients [22.7%]), a highly significant difference (Table 4, p = 0.003. In three patients their increments could not be determined because they had their hematocrit level determined only once during their hospital stay.

DISCUSSION

Plasma leakage is a crucial point that differentiates DHF from DF. The possibility to misdiagnose DF into DHF is high because plasma leakage manifestations are difficult to observe and ascertain. Evidence of plasma leakage may not be detectable by physical examination alone, especially in the early phase of plasma leakage or in mild cases of DHF.

29 out of 78 patients (37.2%) had no evidence of plasma leakage. However, many of those did not receive a full clinical and laboratory work-up. This poses the question how did doctors make the DHF diagnosis in these cases if there was no plasma leakage evidence? These 29 patients included 17 adult patients and 12 pediatric patients.

For the first manifestation of plasma leakage, ascites, there were no cases among the adult patients while there were 11 cases among the pediatric patients, nine had already developed ascites before entering the hospital. Patients with ascites in RSUD Dr. Soetomo, Surabaya received a full physical examination. Other studies showed varied results. Srikiatkhachorn et al.[6] (2011) in his research in pediatric patients with DHF found that 34% had ascites, while Navarrete-Espinosa et al.[7] (2005) shows that there were only 4 ascites cases among a cohort of 898 DHF patients. Another study by Balasubramanian et al.[8] (2005) showed that by using ultrasonography, 91% of their DHF patients had ascites. This big gap between physical examination and USG results showed that in many, possibly in the majority of them, ascites cannot be detected by physical examination alone.

Ascites developed during the patient's stay in the hospital may not be the direct consequence of the virus infection but rather should be considered as an early symptom of fluid therapy overload.[1] Routine usage of ultrasonography is thus recommended as the method of choice in detecting cases with ascites which couldn't be detected by physical examination alone. Presence of fluid leakage by ultrasound might differentiate cases with borderline hematocrit levels (10 – 20%).[9]

Classification of patient's data based on the course of dengue illness in febrile phase, critical phase and recovery phase based on WHO 2009 guidelines.[10] Mostly, patients enter the hospital in the critical phase. The most important things to do in the critical phase is give fluid therapy as fast as possible. It is better to use ultrasound but it is overrated because we could use other sign and examination which cheaper and easier in critical phase like temperature, potential clinical issues, and laboratory changes to make a dengue viral infection suspect. Because of fluid therapy importance, probably there were patients with possible over fluid therapy.

The other plasma leakage manifestation, pleural effusions did not occur in adult patients with DHF. In contrast, a large majority, approximately 74% of pediatric patients had radiological evidence of pleural effusions. In the study by Navarrete-Espinosa et al.[7] (2005), 3/898 DHF patients had pleural effusions whereas Srikiatkhachorn et al.[6] (2011) reported that 78% of their cases had pleural effusions. The study by Balasubramanian et al.[8] (2005) in 89% of DHF patients checked by ultrasonography had pleural effusions. There were no significant difference in the presence of pleural effusions when examination by CXR and ultrasonography were compared. However, 31 patients in our retrospective were not checked by CXR.

Transudates pleural effusion could be caused by hypoalbuminemia.[11] Minimum volume of pleural effusion that become visible in CXR is 50 ml.[12] Mild pleural effusion/less than 50 ml couldn't be analyzed by CXR. This number is probably relative based on thoracic volume. 74% of pediatric patients had radiological evidence of pleural effusion while none of adult patients had radiological evidence of pleural effusion. Smaller thoracic volume in pediatric patients make the ratio between fluid volume and thoracic volume bigger than the same fluid volume in adult thoracic volume.

Hypoalbuminemia was found in only a minority of the cases enrolled in this study, especially among adult patients whereas in pediatric patients, hypoalbuminemia was detected in close to half of the patients tested. However, not all of the patients got their albumin level tested (37/78 patients had not been tested). This study is somewhat contradictive with a study by Villar-Centeno et al.[13] (2008) reported that 57% of their DHF patients presented with hypoalbuminemia.

Albumin levels less than 4 g/dL may be an early indicator of vascular permeability alteration.[13] Lower albumin levels could be caused by liver involvement in DENV infections which makes severe liver damage that leading to decreased production of albumin.[14] Mostly DHF patients in this study have normal albumin level.

The final plasma leakage manifestation was the hematocrit. Nearly half number patients had increases of their hematocrit ≥20%. Only few adult patients have developed increasing hematocrit ≥20% while 60% of pediatric patients have increasing hematocrits of ≥20%. This finding corresponds with Srikiatkhachorn et al.[6] (2011) and Guilarde et al.[15] (2008) who showed that among pediatric patients, increasing hematocrit ≥20% is the rule, whereas other studies found that among adult patients with hematocrits increasing ≥20% constituted a minority, albeit a sizable minority of 49%.

Increasing hematocrit levels is a consequence of plasma leakage. Extravasations of plasma without RBC makes the ratio between RBCs and total volume of blood is higher. A rising hematocrit, e.g. 10% to 15% above baseline, is the earliest evidence of plasma leakage[1] but DHF would be misdiagnosed if only based on hematocrit as a diagnostic criterion.[9] Highly significant difference of hematocrit level and albumin level between adult and pediatric patients poses a theory which pediatric patients are more susceptible to have a plasma leakage than adult patients.

CONCLUSION

Plasma leakage is the most important signs / symptoms for the diagnosis making of DHF. Signs and symptoms of dengue viral infection without any of plasma leakage manifestations is not classified as DHF but is classified as DF. This study found that patients admitted with dengue virus infection may currently be often misclassified as DHF because there are no evidences of plasma leakage manifestations. There are significant differences in plasma leakage manifestations between adult and pediatric patients which poses a theory that pediatric patients are more susceptible to have plasma leakage manifestations than adult patients.

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Research Report

MODEL OF *LOCAL CAPACITY DEVELOPMENT* FOR THE *TROPICAL DISEASES HANDLING* IN EAST JAVA

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ABSTRACT

Indonesia is a tropical country with its all potential for tropical diseases that are vulnerable to attack its population. This study aims to identify the mechanisms of the tropical disease handling and the potentials that can be done to increase the capacity of tropical disease handling itself. The focus of this research is to increase the capacity of the tropical diseases handling existing in East Java, more specifically in some regencies or cities, among others are Bojonegoro, Sampang and Pacitan. The approach of the study was the qualitative approach which was characterized by the existence of an actual setting, researchers as a key instrument, emphasizing the process, and the data analysis is inductive. Data were collected using in-depth interview has well as secondary data from health care institution and the internet. A focused group discussion was also occupied to enrich the results, the cases were illustrated and the models were structured more comprehensively in the handling of tropical diseases. Participants of this study were health care workers who work at the health institutions including the Health Department, Hospitals, the and Public Health Centers. The findings were all analyzed qualitatively. The results of this study indicated that there are four dimensions of capacity, namely the capacity of the human resource, the capacity of the institution, the capacity of the system and the capacity of the community or the community itself.

Keywords: *tropical diseases, capacity, health workers, community development*

ABSTRAK

Indonesia adalah Negara tropis dengan segala potensi penyakit tropis yang rentan sekali menyerang penduduknya. Menyadari resiko yang ditimbulkan, maka mekanisme penanganan penyakit tropis perlu menjadi perhatian serius oleh pemerintah dan masyarakat. Penelitian ini bertujuan untuk mengidentifikasi mekanisme penanganan penyakit tropis dan potensi yang dapat dilakukan untuk peningkatan kapasitas penanganan penyakit tropis itu sendiri. Fokus dari penelitian ini adalah peningkatan kapasitas penanganan penyakit tropis yang ada di Provinsi Jawa Timur, lebih spesifiknya di beberapa kabupaten atau kota yang menjadi focus penelitian antara lain Bojonegoro, Sampang dan Pacitan. Dengan menggunakan pendekatan kualitatif yang bercirikan adanya setting yang aktual, peneliti sebagai instrumen kunci, data yang ditampilkan adalah data yang bersifat deskriptif, menekankan kepada proses, dan analisis datanya bersifat induktif. Focused group discussion juga digunakan untuk memperkaya hasil penelitian, kasus lebih tergambarkan dengan jelas dan model penanganan penyakit tropis dapat disusun dengan lebih komprehensif. Partisipan dalam penelitian ini adalah tenaga kesehatan yang bekerja pada departemen kesehatan, rumah sakit dan puskesmas. Penelitian ini dianalisis secara kualitatif. Hasil penelitian ini menunjukkan adanya empat dimensi kapasitas dalam penanganan penyakit tropis yaitu kapasitas SDM, kapasitas institusi, kapasitas sistem dan kapasitas komunitas atau masyarakat itu sendiri.

Kata kunci: *penyakit tropis, kapasitas, tenaga kesehatan, pengembangan masyarakat*

INTRODUCTION

As a tropical country, Indonesia is vulnerable to tropical diseases which are very specific, such as dengue fever, tuberculosis (TB) and leprosy. In East Java, these diseases remain a serious problem. Sampang is one of the regencies that is still considered to have many lepers, of which approximately 607 patients who have leprosy.[1] Besides, there are also many other tropical diseases that become the Extraordinary Events or outbreaks in East Java, such as dengue fever and Tuberculosis (TB). Therefore, some concentration should be paid for developing mechanism for handling the diseases using some systematic approach, such as improving capacity of human resources, capacity of institution, some systems, and procedures and increasing community engagements.[2,3]

Unfortunately, the efforts to improve the handling are still necessary even harder, since there are many common problems in health care, for example, the community assessment which only reached the criteria of "enough" for hospital services and noncompliance health workers in carrying out standard operations procedure (SOP) on leprosy.[1] The problems that are quite complete are also described as follows (1) the great number of health workers do not certainly improve the services, but there are still many "dual practices" without any adequate supervision, contributing to the weakness of the system to be applied; (2) the decentralization system of authority has not shown its potential to fix health care issues; (3) Infrastructure and medical equipment are inadequate and not evenly distributed; (4) Inefficiency makes less optimal health care, especially in the context of the utilization of medical equipment; (5) Utilization of inpatient services is still low, because the aspects of cost, especially for the poor.[4]

One of the government's efforts to reform the health sector is the decentralization of authority. However, the decentralization itself also still has many weaknesses.[4] *First*, the difficulty of managing the fiscal decentralization in the beginning of the decentralization. When a transfer of budget allocation has been done to an area via the General Fund Allocation, the occurrence problem the failure of the health sector to get funding in the area happens. It has been responded by the central government by providing a great concentration fund. However, the limited ability of the central government's financial and technical difficulties of the concentration fund distribution have caused a great difficulty in central government funding in 2006-2007 and early 2008. *Second*, the implementation of the *Askeskin (Health Insurance for the Poor) program* has indicated a failure of central government to understand the meaning of decentralization in financing. In the beginning of the *Askeskin program*, there was a tendency that the Ministry of Health did not pay attention to the area in funding and implementing the *Askeskin program*. *Third*, in the third year of decentralization, the Health Department issued a Decree of the Minister of Health about surveillance, but it has not run. The local government has ignored those important technical guidelines.

Based on the problems, this research tries to create a model for the development of local capacity in tropical diseases handling. The area mentioned here is East Java, which is also still known as a prone area of tropical diseases such as tuberculosis, dengue fever, and so on. The goals to be achieved from this research are: (1) to understand the problems faced in the tropical diseases handling; (2) to know specifically the scope of the policy and institution to implement and handle the tropical diseases in East Java; (3) to know specifically the harmonization of policies and interaction among institutions in the tropical disease handling efforts; and (4) to arrange a model of action plan to improve services in the tropical diseases handling in East Java.

The Capacity Building

In a simple way, the concept of capacity building is defined as the process of improving the ability of people, organizations, and systems to achieve the goals of the organization that have been set.[5–9] Although this definition is very simple, actually it contains extensive and very important meaning. Specifically, the capacity can be seen as something that is specific to a particular task, and the limits of the capacity are specific as related to factors within an organization or a particular system for a particular period.[10] The capacity building explains how far the staffs are able to show the real contribution to the development of personal, organization and community.[11] The meaning has been extended and linked to the role of the civil/regional institutions, in which the capacity building is interpreted as an effort to improve the ability of people in developing nations to develop management skills and essential policies needed to build the cultural, social, politic, economic and human resources structures so that they are able to exist in the global world.[12]

An increase of the capacity-building in developing countries will also be able to affect changes in the cultural community, although the transformation of the changes is not so easy and not so quick to do. In fact, the development process of the capacity building can be seen as a political process, because it affects the elite decision-makers to create a policy based on adequate evidence. The development and capacity buildings are not only meant as an individual effort but also an institutional one.[13]

The capacity building does not only mean as an individual and an institutional effort but also as an effort to improve the community. Six main domains to assess community capacity, namely: (1) a partnership in networking concerning the existence and functionality of a leadership role within the networking community; (2) the ability to formulate goals and to act collectively together with other community members; (3) the ability to identify and mobilize the organization and resources (both human and material), to implement a program, a knowledge

transfer related to the ability to develop programs; (4) the ability to transfer the information/ knowledge to other members, the ability to integrate those programs into the main agenda of the group, and problem solving to identify the key actor that influences for problem solving; (5) the ability to discuss and negotiate in problem solving with a good process; (6) the ability to identify problems followed by the correct solving.[14]

The Capacity of Decentralization System

The implementation of decentralization requires the changing transformation towards the increasing of the local government ability in the aspects of the system, the management of the institution and the increasing of the quality and capacity of the personnel in the implementation of the development and public service process.[15,16] Osborne and Gaebler has offered the idea of 'reinventing government', as an effort to carry out an entrepreneur transformation into a bureaucratic organization that has two goals at once, namely to improve the performance of the bureaucracy in running the role of public service and to create a bureaucratic efficiency aiming at overcoming the resources crisis faced by the government.[17]

Meanwhile, the transformation will bring a change in the cultural aspects of the bureaucracy itself, namely from the bureaucratic to a governance model that involves community participation, from the command and control to the accountability of results achieved, from the reliance on internal systems to be competitive and innovative, from which are closed to the open, and from which does not tolerate the risk becomes open to the risks of success or failure.[18] The main objective of the renewal is to do a planned change towards a better condition. On that ground, the renewal is called effective if, within the planned period, the better condition really happens. On the contrary, the renewal is called a failure if within the planned period the condition remains as usual and or even gets worse than before. In line with the view that it can be seen that the transformation dynamics of changes or renewals still need to be run within the scope of bureaucratic capacity building to improve the performance effectiveness of the bureaucracy itself.[17]

Along with a number of policies that have been issued by the government in the health sector, there are still many health problems that cannot be handled optimally. Indonesia, a country located in the tropics, has a positive or negative access to its people's health, especially the rise and growth of tropical diseases, such as malaria, *leishmaniasis*, *schistosomiasis*, *onchocerciasis*, *lymphatic filariasis*, Chagas disease, dengue fever, *framboesia,* and *vector*. Those diseases are the major health problem in almost all developing countries because of the morbidity and death that are relatively high in a relatively short time.[19]

RESEARCH METHOD

The approach of study is qualitative which is characterized by the existence of an actual setting, researchers as a key instrument, emphasizing the process, and the data analysis is inductive. Researchers conducted the data exploration related to tropical diseases in three areas, namely Bojonegoro, Sampang and Pacitan and the findings of both quantitative and qualitative data that are all analyzed qualitatively.[20–22] There are several reasons for the selection of the research locations, including: *first*, Pacitan is a relatively remote district from the central government of East Java; *second*, Bojonegoro is a region experiencing significant social changes in the presence of oil exploration; *third*, Sampang has the lowest human development index in East Java and the low category in Indonesia. The location of research is health institutions including the Health Department, Hospitals, the Public Health Centers.

The data collection in this study done was in 3 ways, namely *in-depth interviews* by using *guided interview;* collecting document data in Hospital, Health Department and the Internet; and *focus group discussion* with subjects of research. The process of inference interpretation of research associated with two dimensions: the text and the social contexts combined as a single unit of analysis. The next step is reconstructing the results of text analysis, the social cognition and social context with the theory framework within categorizations in order to obtain a new understanding of the phenomenon.

RESULT AND DISCUSSION

The Capacity of Human Resources

The discussion of this study is focused on how far the implementation of capacity-building efforts is. Human resources in the health sector include Health Workers and Non- Health Workers under the Health Act No. 36 of 2009 Section 1, stated that: "Health Worker is any person who is devoted to the health sector and owns knowledge and/or skills through education in the health field that for certain types require an authority to do health efforts".

This study related to the problems encountered in the management of human resources in the scope of Health Department in several regencies and cities in East Java. Problems faced by the Health Department in Pacitan, relate to the number of inadequate human resources, especially the non-medical personnel. As stated by the Head of Public Health Center (Puskesmas) of Nawangan, Pacitan,

> *"Functional officer concurs treasurer, one person does six programs, although only a few but all diseases exist. Cooperation with the midwife, and*

HIV, never isolates the HIV. The system of early awareness has been done in this Public Health Center. The number of public health resources is more than the doctors. The Health Department that also has a shortage of human resources with administrative competence (for making Letter of Responsibility, handling financial affairs) should be added as well. There are only two accountants in the Health Department who control 24 Public Health Centers. In almost every year, we propose it to the Labor Agency (BKD)"

The fundamental problem of the analysis of human resources need is the insufficient amount of that, particularly in certain competencies. Unfortunately, the need precisely is on the non-medical personnel. As a consequence, some medical personnel also concurrently function as financial staff. This will inevitably have an impact on the motivation of health workers themselves, because they are given the workload that is not in accordance with their competence, and in the end it will also have an impact on the services provided to the public because health professionals are less focused on their tasks; meanwhile, public have already got their protection on their rights over public services, as stipulated in Act No. 25 of 2009 on Public Service.

Health personnel is the responsibility of the government, either the central government or the local one, as set in Act No. 36 /2009 on Health, Article 25, paragraph 1, 2 and 3. The capacity building of human resources through various activities such as training, workshop, and education that hold a degree or not, should be facilitated by the institution, that is the Health Department either at the regency or provincial levels. The efforts for the development of individuals are facilitated through workshops on diseases handling such as HIV. The proportion of the budget for the capacity building covering 12% of the Net-Operational Costs and it is focused on tropical diseases. In addition, the budgetary resources for the capacity building of human resources are also derived from General Fund Allocation (*DAU*) in every field, according to the needs.

An interesting mechanism of evaluation which becomes a model has been carried out by Bojonegoro Regency, in which the Local Regional Head enforces an open communication to the public and Local Government Unit to get input on the performance of the subordination, as stated, "then like handling, actually we have been helped by direct messages (Short Message System) to the regent, that was actually very helpful, there may be regarded as the disruption.."

Regent allowed people to send their complaints, including problems related to health care. Furthermore, those messages will also be forwarded to the Local Government Unit and personnel related, then hopefully it can improve their service. Besides, Bojonegoro Regency also has regular meetings among Local Government Unit personnel to improve their coordination, considering that the handling of cross-cutting issues related to tropical diseases is really needed. Of the two earlier evaluation mechanisms, the positive impact is being felt by officers in the field. It becomes a boost for the officer to provide the best service for the people and also becomes a form of attention of the chief executive to the subordinates.

The Capacity of Institutions

The structure of organizations and procedures remain an important parameter in viewing the capacity of the institution. The emergence of a variety of health policy in Indonesia will have implications on the development of various duty and authority of the district. Surely, the presence of various regulations such as Law, Decree of Health Minister, District Regulation and Regent Regulation becomes a lever for health service personnel to improve the program of their activities.

In the tropical diseases handling, it has been structured in detail about the authority, duty and coordination flow among institutions through Standard Operating Procedure (SOP). It becomes the basis for the institution to act in the tropical diseases handling and has been provided by the Health Department.

Both the Standard Operating Procedures (SOP) and SOP implementation mechanism have been structured, especially when the government would declare the conditions of Extraordinary Events (*Kejadian Luar Biasa/ KLB*) in a region. In the case of the extraordinary events, the referral mechanisms go from doctors, clinics, hospitals and the Health Department. Preliminary examinations of a patient done by a doctor (public or private) to get the referral and from the examination the patient will be directed to a hospital depending on the grade of the patient. When the patient reaches the third grade, the patient will be referred to the hospital. Then, based on the hospital examination results, the patient will be delivered by the Public Health Center where the patient comes to be declared as an extraordinary events in a particular grade. The statement of the extraordinary events will be followed by the Epidemiology Research (ER) with a random sample in the region. The observation was done at a distance of 100 meters from the houses surrounding the patient. Finally, the government takes action in the form of *fogging*, as said by an informant that:

"We face a case when sometimes the patients do not come to us, they may firstly check up his health toa private doctor. After being tested that they are positive, it shows clinically that the dengue fever is on grade 3. They are immediately referred to the hospital that the platelets counted drop significantly though not until 150.000. I have even experienced it when I was in Ngumpak Dalem, there was a feedback when a patient with dengue fever corrected by Viper, Epidemology Evaluation (EE) on grade 3 or grade 4 or grade 2 and after that we do an Epidemiology Research (ER) around the 100 homes of patients who have symptoms of fever or perhaps they show the same symptoms. We do an ER, so if there are such cases they have

automatically followed the SOP, they have known what they should do"

The capacity of the institution is also determined by the coordination between health institution such as the Public Health Center and the health department. One determination in coordination among institutions in the tropical diseases handling is the availability of health resources. Often the availability of resources becomes an obstacle to meet the health services primarily. A few numbers of qualified doctors in handling HIV/AIDS has caused slow treatments to patients. An example is the incidence of a late handling of HIV disease despite the availability of two nurses and one specialist and one general practitioner who have the expertise to handle HIV/AIDS; but, it is not possible to always stay in the HIV room because they have to carry out their duties in other places as an additional task.The three districts under study (Bojonegoro, Sampang, and Pacitan) have had a standard organizational structure, such as the existence of the General Hospital and Public Health Center and the institutions below it. Nevertheless, only a few units of work has been certified.

Not all the financial managements use the pattern like Public Service Agency. Several legal institutions that shade health programs in five regencies are Constitution of the Republic of Indonesia Year 1945, Presidential Decree, Health Minister Decree, Decentralization Laws, and Regent Regulation, also several articles such as: Article 20, Article 28H paragraph (1), and Article 34 paragraph (3) of the Constitution of the Republic of Indonesia Year 1945 and the Law of the Republic of Indonesia Number 36 Year 2009 on Health. See the organizational structure in the Health Department.

The Capacity of Systems

The system capacity refers to the regulations issued by the ministry of health which involves the health sectors, local regulations as the implementation of national health policy, the decisions of governor and regent. The local government should synchronize the national policies and local policies. Long before the Decree of the Health Minister and the Handbill has existed in East Java, the East Java Provincial Regulation No. 5 of 2004 on the Prevention and Control of HIV - AIDS has been published in East Java. Referring to the handbill, East Java provincial government has made efforts on health. The principal efforts expected to run well is strengthening a health promotion of prevention and expanding the HIV counseling and testing, care, support, and treatment. However, all the efforts that are written in this policy document are only as an appeal, not an obligation. It means that the Health Ministry cannot insist and ensure that the efforts will be done by local governments and hospitals.

To support the health policy to achieve the Millennium Development Goals (MDGs), the Bojonegoro Government tries to improve the health infrastructure so that it is able to provide a better health degree. In 2013, the Health Department of Bojonegoro prioritized the improvement and repair of the infrastructure of Public Health Centers in 28 districts. The increase includes the improvement of infrastructure, equipment and human resources of medical

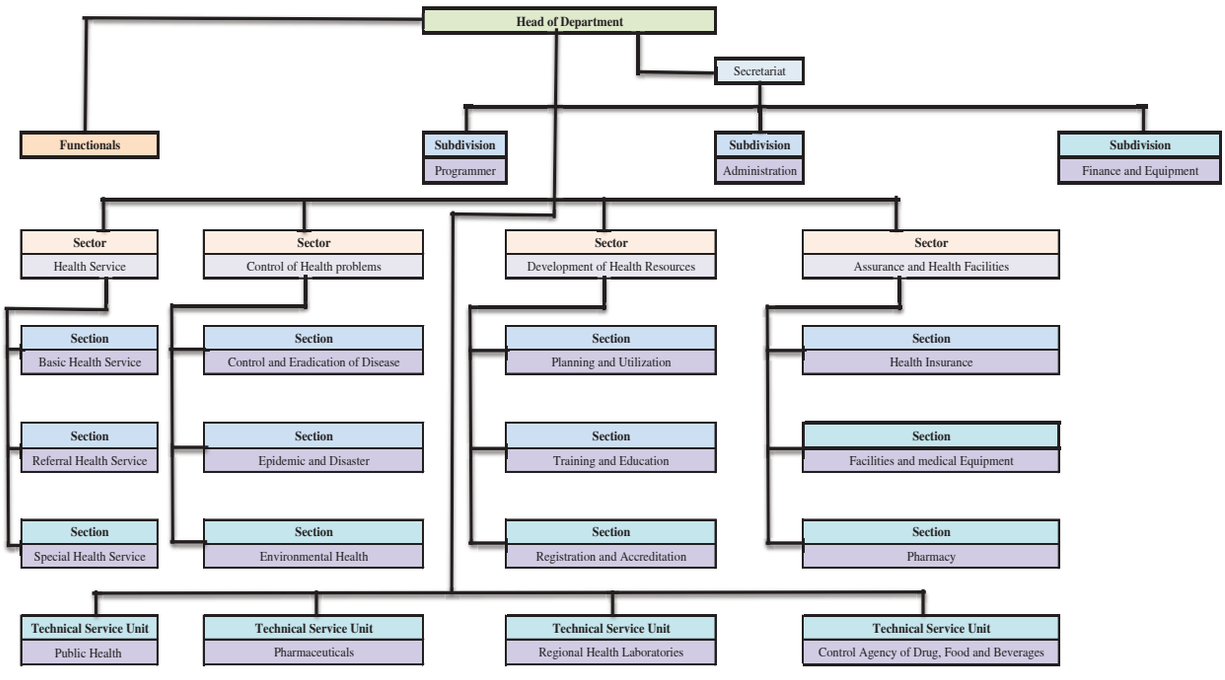

Figure 1. Organizational structure of provincial health department

personnel. This activity was budgeted at IDR.16.2 billion, coming from the budget of Bojonegoro Governance. It is not only for the Public Health Center (*Pusat Kesehatan Masyarakat*), but for Basic Essential Obstetric Neonatal Clinics/BEONC (*Pondok kesehatan*) and Rural Birthing Clinics (*Poliklinik Desa/ Polindes*).

In Indonesia, dengue fever was first discovered in 1958 in Surabaya and now is spreading throughout the provinces in Indonesia. The incidence of dengue fever was suspected by the existence of a correlation between strain and genetics, but recently there has been a tendency of different causing-agents of dengue in each area. The cases related to the epidemic of dengue fever that attacked East Java during 2013 increased to 80 percent when compared to the previous year, i.e.8.257 cases increasing to be 14.837 cases. The data of the Health Department showed that the mortality rate declined. It means that the Health Department succeeded to reduce the mortality rate of patients due to dengue although cases found increased. The areas which the mortality rate increased include Sampang and Bojonegoro. Bojonegoro Government intends to carry out a program of preventing and overcoming infectious diseases, as informant said that,

"There are 10 types of the diseases to be suppressed including Acute Respiratory Infections/ARI (ISPA) attacking an 100.524 people (7.95%), diseases of the muscular system and connective tissue attacking 81.868 people (14.62%), gastric ulcer attacking 46.605 people (8.32%), high blood pressure attacking 46.099 (8.23%).Then an observation on febricity attacking 28.212 people (5.04%), diarrhea attacking as many as 24.951 people (4.45%), skin diseases as many as 20 016 people allergic (3.57%), allergic skin disease attacking 14.469 people (2.94%), other diseases in the upper bronchial tube attacking 13.831 people (2.47%), asthma attacking 12.964 people (2, 31%)".

The Capacity of Community

The public health efforts to involve the community are the establishment of Rural Health Post (*Pos kesehatan desa/ Poskesdes*), The Vigilant Village (*Desa Siaga,* the Health Efforts on Community Based (Usaha Kesehatan Bersama Masyarakat/UKMB). In order to develop community participation, the government has encouraged the formation of *Poskesdes* with the support of the Social Assistance of Fund Operational.

The Vigilant Village (*Desa Siaga)* is one of the breakthroughs of the health development in empowering the community in East Java. It is a village whose inhabitants have the readiness of resources, ability and willingness to prevent and solve health problems, disasters, and health emergencies independently. The *Desa Siaga* is developed through the preparation of the community, the introduction of the problem, the formulation of the achievement follow-up especially the agreement of *Poskesdes* formation and the resources support.

The government also develops the Health Efforts on Community Based (Usaha Kesehatan Bersama Masyarakat/UKMB) that has been formed in a village in order to bring/provide basic health services for rural communities that include activities of increasing healthy life (promotion), disease prevention, and treatment done by health workers (especially midwives) by involving cadre or other volunteers. The form of *UKBM* in a village includes *Posyandu* involving community participation, and *Tiwisada Cadre* at school, who are scout members that care about health and are ready to provide assistance.

This joint movement is built in the form of solidarity when encountering extraordinary events. Various efforts for preventing diseases are carried out in a community movement, namely cooperation among Local Government Unit (*Satuan Kerja Pemerintah Daerah/SKPD*), Police/ Army and Non-Government Organization/NGO (*Lembaga Swadaya Masyarakat/LSM*). This cooperation fosters a spirit of solidarity to tackle tropical diseases such as in Sampang.In the tropical disease handling, people are given an understanding of diseases such as *Lectospyrosis* through socialization by giving pictures to all staff in Public Health Centers (*Puskesmas*) to be delivered to the public. To promote tropical disease prevention, a special program is made through a variety of media, as said by an informant from Sampang:

"The socialization has been done through the radio broadcast, mobile broadcast, spread pamphlets about how the symptoms and treatment. It has been spread to almost all districts, to rural areas, teenagers, schools; through cross-sector cooperation, collected to local government by involving the chairman of Neighborhood Association (Rukun Tetangga/RT) and Administration Unit (Rukun Warga/RW),and NGO agencies. They did the voluntary work, including in the traditional market, since it is the habitation of rats"

These joint movements of community in Bojonegoro start from a village, district and Public Health Center, and school. One of the movements is in the form of implementation of Communication Information Education (CIE) and Mosquito Eradication, as stated:

"Nggayam shade 23 villages whose 3 villages are certainly dengue endemic. In those 3 dengue endemics, almost all in habitants, one or two of them, were certainly infected although not to die. We have triedto do CIE to our community by cross-sector cooperation, all villages and sub-districts completely moved together so that this mosquito problem could be fought. So before they become adults, they would later become our fekton to voluntary work together. We selected those three endemic villages, we all did the voluntary work and asked the head of sub-district for help and his officials to suppress the dengue fever, and we do

this attempt this year, so that one village was not affected by dengue fever".

The development of another community involvement is the formation of the association community. As occurred in Sampang, there is an establishment of the association of former leprosy patients aimed to provide a reinforcement to the lepers and to empower leprosy patients. They become volunteers for other lepers. Those volunteers are given the task as Supervisors of Swallowing Drugs (*Pengawas Menelan Obat/PMO*) besides giving skill assistance to lepers, so that they can be more independent and economically productive.

Another development of social networking is searching donation from corporate institutions. In tropical diseases handling, Bojonegoro government received contributions from several institutions or mining investors such as PT.Petrocina and PT. Exxon Mobile. Those corporations provided infrastructure supports as a form of social responsibility (charity) for their existence. Facilities provided by those corporations in the form of the building, training for health personnel and fund.

CONCLUSION

To deal with tropical diseases, human resources such as health workers become an important dimension. It is not only supported by human resources that are adequate or have an appropriate professional field but also strengthened by experience in handling emergency situations of disease incidence.

The good governance achievement in tropical diseases handling can only be done when the framework of the development of institutional networking between health care units is implemented properly. The cooperation ability within a working unit and among working units is a condition that sustains the success of diseases handling. Coordination, communication, and synchronization are important prerequisites in strengthening the health institutional capacity. The pattern of vertical coordination between levels of government, namely between regency and province levels, and pattern of horizontal coordination among levels of government, namely the Health Department, Hospitals and Public Health Center become the key to success.

The capacity of the system also becomes a dimension in the development of local capacities. The local policy in Indonesian refers to the national policy. Indonesia's health policy is directed to one goal that is the achievement of the MDGs which has become an international agreement. Various policy ratifications have been established by the Indonesian government either in the form of laws or ministry regulations. In the context of preparing regulations at the local level, it still refers to the higher level's regulations, it means that the regulations made by the local government do not disapprove the regulations of above level, but they are made in detail for implementation such as Guidelines, Regent Regulations or Local Government Regulations. A regulation consistency is a prerequisite for the implementation of the policy so that there is no debate in the implementation of disease handling.

The involvement of health stakeholders contributes to the success of the capacity building. The development of community capacity on the incidence and prevention of health is one of the solutions to overcome limitations of health resources. The involvement of stakeholders in the search for donations either from corporations or foreign country in the tropical diseases handling is necessarily done by local governments in the concept of helping to resolve an incident. The reinforcement of non-institutional network, that is community resource development through volunteers or health cadres both of family or community, helps in the tropical diseases handling.

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Notes to authors

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- **FIGURES.** Provide figures embedded in page. Figures should be drawn professionally. Photographs should be sharp (contrast). Provide footnotes and other information (e.g., source/copyright data, explanation of boldface) in figure legend. Submit image files (e.g., electromicrograph) without text content as high-resolution (300 dpi/ppi minimum) TIFF or JPG files.
- **PLAGIARISM CHECK.** Please kindly tell us if you already use plagiarism check (Turnitin, etc.).

II. TYPES OF ARTICLES

- **PERSPECTIVES.** Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary of the conclusions, and a brief biographical sketch. Articles in this section should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may also address factors know to influence the emergence of diseases, including microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.
- **SYNOPSES.** Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text in recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words) a one-sentence summary of the conclusions. This section comprises concise reviews of infectious diseases or closely related topics. Preference is given to reviews of emerging and reemerging diseases; however, timely updates of other diseases or topics are also welcome. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.
- **RESEARCH STUDIES AND SCIENTIFIC REVIEW.** Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text in recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words) a one-sentence summary of the conclusions. Report laboratory and epidemiologic results within a public health perspective. Explain the value of the research in public health terms and place the findings in a larger perspective.
- **POLICY AND HISTORICAL REVIEWS.** Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary of the conclusions. Articles in this section include public health policy or historical report that are based on research and analysis of emerging and reemerging disease issues.
- **DISPATCHES.** Articles should no more than 1,200 words and need not be divided into sections. If subheadings are used, they should be general, e.g., "The study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed 2). Dispatches are updates on infectious disease trends and research. The articles include descriptions of new methods for detecting, characterizing, or sub typing emerging or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination program are appropriate. Case reports are also welcome.
- **COMMENTARIES.** Thoughtful discussions (500-1,000 words) of current topics. Commentaries may contain references but not figures or tables.
- **ANOTHER DIMENSION.** Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit.
- **LETTERS.** Letters commenting on recent articles as well as latters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 figure or table and should not be divided into sections. All latters should contain material not previously published and include a word count.
- **BOOKS, OTHER MEDIA.** Reviews (250-500 words) of new books or other media on emerging and reemerging disease issues are welcome. Name, publisher, number of pages, other pertinent details should be included.
- **ANNOUNCEMENTS.** We welcome brief announcements (50-150 words) of timely events of interest to our readers. (Announcements may be posted online only, depending on the event date).
- **CONFERENCE SUMMARIES.** Summaries of emerging and reemerging infectious disease conference activities are published online only. Summaries, which should contain 500-1,000 words, should focus on content rather than process and may provide illustrations, references, and links to full reports of conference activities.