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CONTENTS

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Notes to authors

INDONESIAN JOURNAL of TROPICAL and INFECTIOUS DISEASE

This journal is a peer-reviewed journal established to promote the recognition of emerging and reemerging diseases spesifically in Indonesia, South East Asia, other tropical countries and around the world, and to improve the understanding of factors involved in disease emergence, prevention, and elimination.

The journal is intended for professionals in infectious diseases and related sciences. We welcome contributions from infectious disease specialists in academia, industry, clinical practice, public health and pharmacy, as well as from specialists in economics, social sciences and other disciplines. For information on manuscript categories and suitability of proposed articles see below and visit www.itd.unair.ac.id. **Indonesian Journal of Tropical and Infectious Disease** is published in English.

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- **Manuscript Preparation**. For word processing, use MS word. The manuscript should be arranged in this order: title page, abstract and keywords, text (Introduction, Material and Methods, Results and Discussion), acknowledgements, references, tables, figure legends, appendixes and figures. Each figure should be in a separate file.
- **Title Page**. Give complete information about each author (i.e., full name, graduete degree (s), affiliation and the name of the institution in which the work was done). Clearly identify the corresponding author and provide that author's mailing address (including phone number, fax number, and email address).
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- **Text**. Double-space everything, including the title page, abstract, references, tables, and figure legends. Indent paragraphs; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use 12-point Times New Roman font and format with ragged right margins (left align). Italicine (rather than underline) scientific names when needed.
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- **References**. Place references numbers in parentheses, not superscripts. Number citations in order of appearance (including intext, figures, and tables). Cite personal communications, unpublished data, and manuscripts in preparation in parentheses in text. Consult List of Journals Indexed in index medicus for accepted journal abbreviations; if a journal is not listed, spell out the journal title. List the first six authors followed by "*et al*". Do not cite references in the abstract.
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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 microbal 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 immediatelly follow the body of the text.
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UPDATE MANAGEMENT OF DENGUE COMPLICATION IN PEDIATRIC

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ABSTRACT

Dengue virus infection is one of the important health problems in Indonesia, although the mortality rate has been decreased but many dengue shock syndrome cases is very difficult to be solving handled. It might be due to nature course of dengue virus infection is very difficult to predict of the earlier time of severity occur. THE AIM *To get idea to make update management of dengue complication in pediatric.* MATERIAL AND METHOD *Data were compiled from Dr. Soetomo Hospital Surabaya in 2009. The diagnosis of all cases was based on criteria WHO 1997 and PCR examination in Institute Tropical Disease for identified serotype of dengue virus infection. The unusual cases of dengue virus infection were treated following the new WHO protocol in 2009. RESULT There were only 3 cases with serotype DEN 1, consisted 2 cases had age 1–4 years and 1 had age 5–14 years. 2 cases showed a severe clinical performance as dengue shock syndrome and 1 case showed as unusual case of dengue virus infection. Three report cases of: a. Dengue hemorrhagic fever grade III which liver involvement and had bilateral pleural effusion; b. Dengue hemorrhagic grade III with liver involvement and encephalopathy; c. Dengue hemorrhagic grade III with liver involvement acute kidney injury, myocardial involvement and encephalopathy. All the patients were treated according to new edition WHO protocol and all of the involving organ recovered along with the improvement of the disease.* CONCLUSION *Update management of dengue complication pediatric should be learned carefully used for helping unusual cases of dengue virus infection.*

Key words: *Dengue, update management, revises criteria diagnosis & treatment*

INTRODUCTION

Dengue virus infection is one of the important health problems in Indonesia, although the mortality rate has been decreased but many dengue shock syndrome cases is very difficult to be solving handled. It might be due to nature course of dengue virus infection is very difficult to predict of the earlier time of severity occur.

Some factor influence this situation such as global warming, increasing sub urban area which have many people don't aware with a bad environment sanitation and have highly dynamic people for getting some money for their life. Beside it many unusually cases were found and need new procedure for making diagnosis and use update management.

Dengue control group of WHO want to revise the criteria WHO 1997 for minimize false diagnostic dengue virus infection and to decrease the mortality rate.

Based on the reason, update management dengue virus infection should be made based on many experiences and followed the protocol WHO in 2009.

This paper will reviewed some unusual cases dengue virus infection that had been found in Dr. Soetomo Hospital Surabaya and promoting update management.

MATERIALS AND METHODS

Data were compiled from Dr. Soetomo Hospital Surabaya in 2009. The diagnoses of all cases were based on criteria WHO 1997 and PCR examination in Institute Tropical Disease for identified serotype of dengue virus infection.

The unusual cases of dengue virus infection were treated based on the new protocol WHO for diagnosis and treatment in 2009.

RESULTS

In 2009 the study Dengue virus infection in patient at Dr. Soetomo hospital found that there were only 3 cases with serotype DEN 1, consisted 2 cases had age 1–4 years and 1 had age 5–14 years. 2 cases showed a severe clinical performance as dengue shock syndrome and 1 case showed as unusual case of dengue virus infection. (See table 1)

Table 1. Distribution of Serotype and Clinical Performance of Dengue Virus Infection in 2009

	Clinical Performance & Diagnostic				
Serotype	**DF**	**DHF**	**DSS**	**UNUSUAL**	**Total**
DEN 1	0	0	**2***	**1**	**3**
DEN 2	30	26	7	2	65
DEN 3	1	0	1	0	2
DEN 4	0	0	0	0	0
Total	31	26	10	3	70

Kruskal-Wallis: p = 0,03*
* = significant (p < 0,05)

Serotype DEN 1 was usually mild case but in this study 1 case showed a severe clinical performance as dengue shock syndrome and identified as primary infection (see table 2).

Table 2. Distribution of Clinical Performance of Dengue Virus Infection in 2009

	Clinical Performance & Diagnostic				
Type of Infection	**DF**	**DHF**	**DSS**	**UNUSUAL**	**Total**
Primary	16	7	1*	2	26
Secondary	15	19	9	1	44
Total	31	26	10	3	70

Mann-Whitney; p = 0,035*
* = significant (p < 0,05)

THREE REPORT CASES IN 2009

1. A seven years old boy was brought by his parent on June 2nd, 2009 to Dr. Soetomo Hospital Emergency Department with the main complaint of fever, shortness of breath, nausea, poor appetite and delirium. Supine chest x-ray showed right pleural effusion. The diagnosis was dengue hemorrhagic fever grade III with encephalopathy and liver involvement.
2. A nine years old boy was brought by his parent on June 25, 2009 to Dr. Soetomo Hospital Emergency Department; the patient was looked dyspnea, abdomen was slight distended, the liver was palpable 3cc below the coastal arc. The supine chest x-ray showed bilateral pleural effusion. The working diagnosis was dengue hemorrhagic fever grade III with liver involvement and had bilateral pleural effusion.
3. a three years old boy was referred from Mojokerto hospital with suspicion of hepatic coma on December 2, 2009 to Dr. Soetomo Hospital Emergency Department he looked as a lethargic boy and extremities were clammy with capillary refill time more than two second, the liver was palpable 4cc below the costal arc with dullness merging laboratory examination revealed hemoglobin level 10,3 g/dl. Leukocyte count 14.600/mm^3, platelet count 15.000/mm^3 hematocrite 31,9% blood glucose 79 mg/dl BUN 48 mg/dl creatinine serum 1,8 mg/dl AST 3154 µ/l ALT. 1274 µ/l; nine hours on admission, the patient had generalized seizure for three minutes. The working diagnosis was dengue hemorrhagic fever grade III with liver involvement Acute Kidney Injury (AKI) and encephalopathy.

Update management is:
The new protocol WHO for diagnosis and treatment in 2009, especially to treat some severe cases which unusual manifestation of dengue virus infection.

DISCUSSION

In 2009 the study dengue virus infection in patient at Dr. Soetomo hospital found that stereotype DEN 1 showed clinical performance of Dengue Shock Syndrome 2 cases and unusual case 1 case and totally 3 cases; DEN 2 were found which had clinical performance of Dengue Fever 30 cases and 26 cases of Dengue Hemorrhagic Fever which had a 7 cases of Dengue Shock Syndrome and 2 unusual cases totally 65 cases; DEN 3 were found clinical performance of Dengue Fever 1 case Dengue Shock Syndrome 1 case and totally 2 cases; Finally DEN 4 virus was not found.

Virus isolation from mosquito bites showed DEN V1 has been isolated and identified on DEN 1 Genotype IV, it was new variant virus that correlated with phylogenetic Dengue Virus came from China which had severe clinical performance of Dengue Virus Infection.

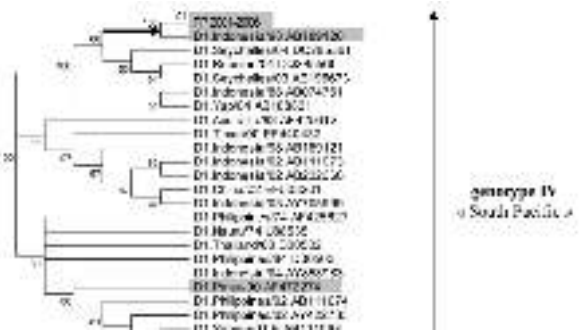

Figure 1. Phylogenetic Dengue Virus in The World

In 2009 we have many experiences to care severe performance of Dengue Virus Infection with unusual

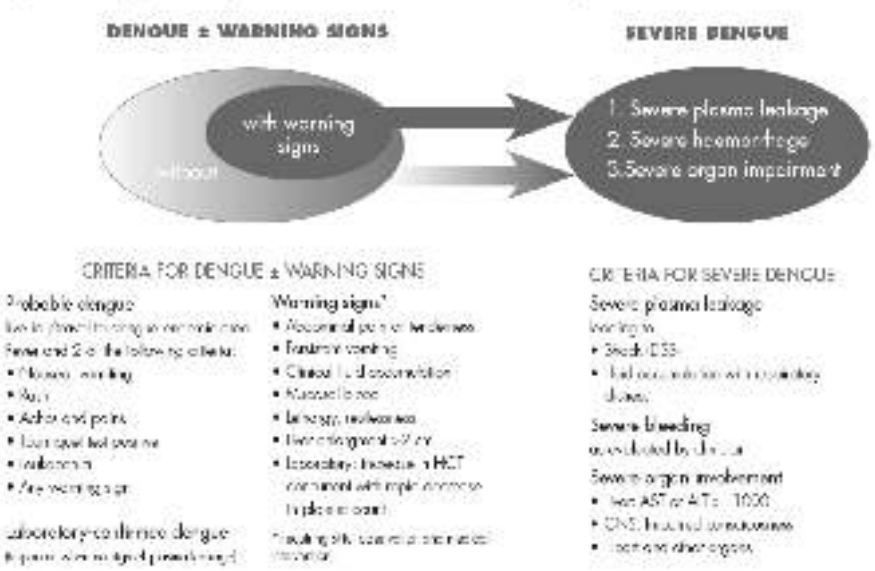

Figure 2. Classification of Dengue Virus Infection cases
(Source: WHO, 2009. Dengue: guidelines of diagnosis,treatment, prevention and control – new edition. Genewa: WHO, p. 23)[3]

manifestation that could not followed WHO criteria 1997. More cases showed criteria for severe dengue virus infection, as followed: Severe plasma leakage (leading to: shock / DSS, Fluid accumulation with respiratory distress), Severe bleeding (as evaluated by clinician), Severe organ involvement (Liver: AST or ALT > = 1000, CNS: Impaired consciousness, Heart and other organ). Therefore in managing the unusual dengue virus infection we should followed new WHO criteria diagnosis and classification of cases as followed:

In 2009, the study found that DEN V1 genotype IV showed a severe clinical performance and also as a primary dengue virus infection. This study support to the Gubler hypothesis which gave information that a new virulent variant DEN V1 can cause a severe clinical performance of dengue virus infection.

During three decades, the World Helath Organization (WHO) has recommended the classification of dengue virus infection in: dengue fever (DF) and dengue hemorrhagic fever (DHF) with or without dengue shock syndrome (DSS). In order to be regarded as a DF (or classical dengue) case, the patient must present fever and two symptoms out of the following: headache, retroocular pain, osteomyoarticular pains, rash, leucopenia, and some kind of bleeding.[6] The DHF requires the presence of the four following criterias: a) acute sudden onset of high fever for 2 to 7 days; b) some kind of spontaneous bleeding, usually petechiaes, or at least having a positive tourniquette test; c) thrombocytopenia lower than 100,000/mm^3; and d) plasma leakage, evidenced by a 20% elevation of the hematocrite, or by a 20% decrease of the hematocrite after the critical stage, or by the verification of pleural leakage, ascites or pericardial leakage by means of image studies.[6] The course of the dengue disease goes through 3 clinical stages: the febrile stage, the critical stage, and the recovery stage (Figure 3).[1,3]

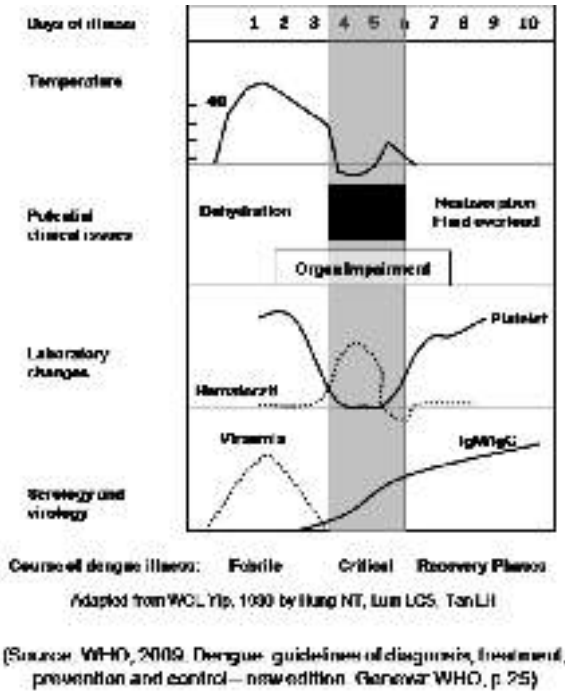

Figure 3. The Course of Dengue Disease

In our cases, all the patients had fever for four to five days on admission, with classic symptoms like aches and pains, nausea and vomiting, and abdominal pain.

They come with clammy extremities, 2 of them with unmeasured blood pressure (case 1 and 2), and 2 with decrease of consciousnesses (case 1 and 3, in case 2 decrease of consciousnesses happened later). They had liver enlargement more than 2 cm and ascites. The signs of bleeding on admission were only present in case 2 as petechiae. In case 1 there was severe gastrointestinal bleeding later (as hematemesis and melena). From the laboratory examination all of them had thrombocytopenia lower than 100,000/mm^3. Two of them had hemoconcentration as shown by the increased hematocrite. The blood coagulation profile tests were performed in case 2 and 3 that revealed abnormal results. From the radiologic examinations all of them had pleural effusions, especially on the right lungs. In all cases there were signs of profound shock that improved after the fluid resuscitation and only in case 2 there was recurrent shock.

The dengue infection may be clinically unapparent and cause an illness with varied intensity, including from febrile forms with body pains to severe pictures of shock and large hemorrhages. The main difference between the classical dengue or dengue fever (DF) and the dengue hemorrhagic fever (DHF) is the leaking of plasma, causing a significant elevation in the hematocrit and an accumulation of fluid in serous cavities.[1] There are also rarer clinical forms that known as "atypical", and result from the especially intense damage to an organ or system: encephalopathy, myocardiopathy or hepatopathy by dengue, as well as kidney dysfunction with acute kidney insufficiency and other that are also associated to mortality.[1,5] To improve the leaking of plasma new finding colloid could be used. For example: HES, gelofusin, hemacel, etc.

Severe organ impairment in dengue infection usually are complications resulting from a prolonged or recurrent shock. However some dengue patients may manifest a special damage to an organ on system, reason why these occurrances have been named "clinical forms of dengue with visceral predominance" in occasions associated to an extreme severity and death. Dengue patients frequently present some kind of liver involvement, that usually recoverable.[1] Clinical finding of liver involvement in dengue infections includes the presence of hepatomegaly and increased serum liver enzymes. Hepatomegaly is frequent and is commoner in patients with DHF than in those with DF. Transaminase levels are also higher in DHF/DSS than in DF and tend to return to normal 14 to 21 days after infection.

In dengue infections, elevations in serum AST appear to be greater, and return to normal more rapidly than ALT levels. If we found dengue virus infection cases with elevated serum AST & ALT please used crystalloid ringer acetate or physiologic salt. It was to prevent the complication using ringer lactate in patient with liver damage. Usually in a case with healthy liver organ can metabolizes the ringer lactate crystalloid. But if this case have liver damage the ringer lactate crystalloid cannot be metabolized and the result; could promote the severe liver dysfunction and the complication such as DIC and bleeding can occur. In a subgroup of predominantly DHF/DSS patients, severe liver dysfunction occurs and is a marker of poor prognosis.[8]

In a Malaysian study of DF and DHF patients with liver invonvements resulted that ALT and ALP (alkaline phosphatase) levels were significantly higher in DHF patients with spontaneous bleeding than those without bleeding.[9] Dengue viral antigens have been found within hepatocytes, and the virus appears to be able to replicate in both hepatocytes and Kupffer calls, a nd dysregulated host immune responses may play an important causative role in liver damage. Liver damage may also be potentiated by the intake of drugs (such as acetaminophen and anti-emetics) during the early phase of the illness.[8] Hepatic failure is a rare, severe and potentially fatal complication of dengue hemorrhagic fever.[10-12]

In our cases, all of them had liver involvements, as seen on the liver enlargements (more than 2 cm) and the elevation of serum liver enzymes. In case 2 and 3 direct hyperbilirubinemias were found, consistent with the presence of jaundice. In case 2 the liver involvement brought the patient into a fulminant hepatic failure condition, that might be correlated with his severe bleeding manifestation after using ringer lactate.Therefore that crystalloid should be changes by ringer acetate solutions; The result all cases with had liver involvement were improved along their disease's improvements.

In some unusual cases, dengue infections may also present signs and symptoms involving the central nervous system (CNS), such as headache, seizures, neck stiffness, depressed sensorium, behavioural disorders, delirium, paralysis and cranial nerve palsies. Such neurological conditions were attributed to plasma leakage into serous spaces, hemorrhage, shock, and metabolic disturbances in severe dengue infections. Acute liver failure is considered to be another factor causing CNS manifestation. The detection of dengue IgM and the isolation of dengue viruses from the cerebrospinal fluid of patients with neurologic disorders indicate the neurovirulance of dengue viruses and their capability of causing encephalitis.[13]

In all of our cases the patients had encephalopathy that might be correlated to the elevated liver enzymes; it might be due to using ringer lactate in the first resuscitation of dengue shock syndrome cases which had liver damage due to dengue virus infection. Based on these experiences please choose other crystalloid such as ringers acetate and physiology-saltz to change the ringer lactate that usually used in the first resuscitations. In case 1 and 3 the patients had electrolyte imbalance (hyponatremia and hypocalcemia) that could play a role in these neurological disturbance. In case 3 the patient had seizure might be caused by the electrolyte imbalance. All of those CNS manifestations were recovered along with their disease's improvement, and no sequela was observed.

Dengue viral infection may also present some myocardial damage – particularly in adults, with little

electrocardiographic expression. Myocardial dysfunction can be seen patients with DHF, approximately 20% of those who developed DHF have a LV ejection fraction of less than 50%, and are likely to return to normal within a few weeks. The pathogenic mechanisms of cardiac dysfunction are not well established; alternation of autonomic tone and prolonged hypotension may play a role. Electrocardiographic abnormalities have been reported in 44-75% of patients with viral hemorrhagic fever, and prolongation of the PR interval or sinus bradycardia commonly occurs, and some have reported atrioventricular block in variable degrees.[14,15] The underlying mechanisms were postulated to be immune in origin, although myocarditis may be a contributory factor.[16] In an Indian study of children with dengue haemorrhagic fever, there was no correlation between myocardial involvement and clinical severity.[17] Myocardial involvement of dengue infections run a benign course without long-term complication. Dengue myocarditis is exclusively asymptomatic with no long term sequelae.[18]

In case 3 bradyarrhytmia was found on the early recovery phase, that might be caused by myocardial injury. There was no symptom of unstable hemodynamic on the patient, and the ECG was return to normal the day after.

Dengue infection usually has transient renal function abnormalities and urinalysis may help the physicians to look for dengue infection. Proteinuria and abnormal urine sediment are the most common renal manifestation observed in patient with dengue infection[19], although according to a Thailand study, abnormal urinalysis (proteinuria, hematuria and pyuria) are not correlated with the severity of disease.[20] Acute kidney injury with acute tubular necrosis due to shock and multiorgan failure, resulting in rhabdomyolysis, haemolysis with haemoglobinuria, proteinuria, and thrombotic microangiopathy, have been described in patients with dengue infection.[21] Acute renal failure can be happened because of extensive capillary leak, hypotension, and severe disseminated intravascular coagulation, which lead to hypoxia/ischemia and multiple organ dysfunction, although this complications can occur without bleeding manifestations or shock.[19]

In case 3 the patient had abnormal renal function test on the critical phase that returned to normal on the recovery phase. The acute kidney injury was improved along with the disease's improvement.

A primary or secondary antibody response can be observed in patients with dengue virus infection. In primary dengue virus infection, IgM antibodies develop rapidly and are detectable on days 3–5 of illness, reach its peak at about 2 weeks post infection and then decline to undetectable levels over 2–3 months. Anti-dengue virus IgG appears shortly afterwards. Secondary infection with dengue virus result in the earlier appearance of high titers of IgG before or simultaneously with the IgM responses.[22-24] The late presenting IgM can be due to variable rapidity which IgM develops among patients: 80% of patients had detectable IgM antibody by day 5 of illness, 93% by day 6-10, and 99% of patient by day 10–20.[23,24] Secondary infections are more likely to result in DHF/DSS, although not all DHF/DSS cases are secondary infections.[25,26]

In our cases, all of them had positive results for immunoglobulin M and G antidengue. In case 1, the initial dengue serologic examination on the 6th day of illness resulted negative, and the repeated examination on the 11th day of illness had initial positive results on the 5th and 7th day of illness, strongly suggested secondary dengue virus infections.

In recent years, articles have been published that bring into question the accuracy of WHO 1997 dengue classification for regarding it as too stern, much too dependent on laboratory result, and for not including dengue patients with other severe forms of the illness, such as the particular damage to the Central Nervous System (encephalitis), to the heart (myocarditis) or to the liver (severe hepatitis).[27–29] For this reason, the TDR/WHO (Program of Training and Research on Transmissible Diseases of The World Health Organization) has sponsored an international study, named DENCO (Dengue Control), of which one of the components was of clinic, and which main purpose was to obtain information from a high number of patients with confirmed dengue and find out a better way to classify them, as well as to identify those signs of alarm that could be useful to improve the protocol of management of dengue cases. The study had a consistent result in the proposal of a binary classification of the disease: dengue and severe dengue.[1,7]

The criteria of severe dengue include: a) severe plasma leakage, expressed in hypovolemic shock, and / or breathing difficulty due to excess accumulation of fluid in the lungs; b) severe bleeding according to the criteria used by doctors; and/or c) severe organ involvements, include severe hepatitis due to dengue (transaminase >1000 units), encephalitis due to dengue, or serious damage to other organs such as dengue myocarditis (Figure 7).[1,3,7] This severity criterium has 95% sensitivity and 97% specificity.[1,7] DENCO criteria could also identify some signs and symptoms that occurred in patients 1 day before the deterioration of conditions. These warning signs allowed early identification of dengue patients who were heading toward a severe dengue and doctors had a chance to start early treatment by replacing fluid intravenously and improve patient's prognosis. Abdominal pain or painful abdominal palpation was a significant risk factor in adults and children, as well as mucosal bleeding, and thrombocytopenia with a platelet counts less than 10,000/mm^3. In adults, the other danger sign was the presence of lethargy, which sometimes turned to irritability, hypoalbuminemia, and increased hematocrite.[1,7]

In all of our cases, there were organ involvements that made the disease's manifestation more severe. According to the WHO criteria, one of 3 cases didn't fulfill the DHF criterias by WHO (case 3). By applying the revised dengue classification, all of them were classified as severe dengue. In case 2 there was evidence of severe plasma leakage, severe bleeding (gastrointestinal bleeding), and severe

organ involvement (encephalopathy, liver involvement). In case 1 there were severe plasma leakage and severe organ involvement, but there was no severe bleeding manifestation (only petechiae). In case 3 there was severe plasma leakage, no sign of bleeding, and there was severe multiorgan involvement (encephalopathy, acute kidney injury, liver and cardiac involvement). Moreover, from the clinical history all of them had several warning signs before their condition deteriorated. If the patients had come before their critical phase, those identifiable warning signs might be helpful to alarm the clinician to give fluid therapy in sufficient amount to replace the losses caused by the plasma leakage.

Management of dengue virus infection is relatively simple, inexpensive and very effective in saving lives so long as correct and timely interventions are instituted. The key is early recognition and understanding of the clinical problems during the different phases of the disease, leading to a rational approach to case management and a good clinical outcome. In the febrile phase, when the clinical features are indistinguishable between severe and non-severe dengue cases, monitoring for warning signs and other clinical parameters is crucial to recognizing progression to the critical phase. In the critical phase, shock can occur when a critical volume of plasma is lost through leakage. With prolonged shock, the consequent organ hypoperfusion results in progressive organ impairment, metabolic acidosis and disseminated intravascular coagulation, and this in turn leads to severe haemorrhage causing the haematocrit to decrease in severe shock. Those who improve after defervescence are said to have non-severe dengue. Those who deteriorate will manifest with warning signs. Cases of dengue with warning signs will probably recover with early intravenous rehydration, but some cases will deteriorate to severe dengue. If the patient survives the 24–28 hour critical phase, a gradual reabsorption of extravascular compartment fluid takes place in the following 48-72 hours. Respiratory distress from massive pleural effusion and ascites will occur at any time if excessive intravenous fluids have been administered.[3]

In our cases, all of the patients were observed intensively in pediatric intensive care unit and treated according to WHO protocol. In case number 2 and 3 transfusions of fresh frozen plasma were indicated considering the abnormal coagulation profiles. In case 2 packed red cells transfusions were given individually according to the patient's conditions. No complication or sequelae was found. All the involved organs recovered along with the improvement of the disease.

To make sure us for the future helping to dengue virus infection cases in 2011 "update management of dengue complication in pediatric" should be learned carefully and applied it in the community hospital.

Patients require emergency treatment and urgent referral when they are in the critical phase of disease, i.e. when they have:

- Severe plasma leakage leading to dengue shock and/or fluid accumulation with respiratory distress;
- Severe haemorrhages;
- Severe organ impairment (hepatic damage, renal impairment, cardiomyopathy, encephalopathy or encephalitis).

All a patient with severe dengue should be admitted to a hospital with access to intensive care facilities and blood transfusion. Judicious intravenous fluid resuscitation is the essential and usually sole intervention required. The crystalloid solution should be isotonic and the volume just sufficient to maintain an effective circulating during the period of plasma leakage. Plasma looses should be replaced immediately and rapidly with isotonic crystalloid solution or, in the case of hypotensive shock, colloid solutions (Texbox M). If possible, obtain haematocrit levels before and after fluid resuscitation.

There should be continued replacement of further plasma losses to maintain effective circulation for 24–28 hours. For overweight or obese patients, the ideal body weight should be used for circulating fluid infusion rates (texboxes J and K). A group and cross-match should be done for all shock patients. Blood transfusion should be given only in cases with suspected/severe bleeding.

Fluid resuscitation must be clearly separated from simple fluid administration. This is a strategy in which larger volumes of fluids (e.g. 10–20 ml boluses) are administered for a limited period of time under close monitoring to evaluate the patient's response and to avoid the development of pulmonary oedema. The degree of intravascular volume deficit in dengue shock varies. Input is typically much greater than output, and the input/output ratio is of no utility for judging fluid resuscitation needs during this period.

The goals of fluid resuscitation include improving central and peripheral circulation (decreasing tachycardia, improving blood pressure, pulse volume, warm and pink extremities, and capillary refill time < 2 second) and improving end-organ perfusion – i.e. stable conscious level (more alert or less restless), urine output š 0.5 ml/kg/hour, decreasing metabolic acidosis.

Treatment of Shock

The action plan for treating patients with compensated shock is as follow (Textboxes D and N, Figure 2.2):

- Start intravenous fluid resuscitation with isotonic crystalloid solutions at 5-10 ml/kg/hour over one hour. Then reassess the patient's condition (vital signs, capillary refill time, haematocrit, urine output). The next steps depend on the situation.
- If the patient's condition improves, intravenous fluids should be gradually reduced to 5–7 ml/kg/hr for 1–2 hours, then to 3–5 ml/kg/hr for 2–4 hours, then 2–3 ml/kg/hr, and then further depending on haemodynamic status, which can be maintained for up to 24–28 hours. (See textboxes H and J for a more appropriate estimate of the normal maintenance requirement based on ideal body weight).

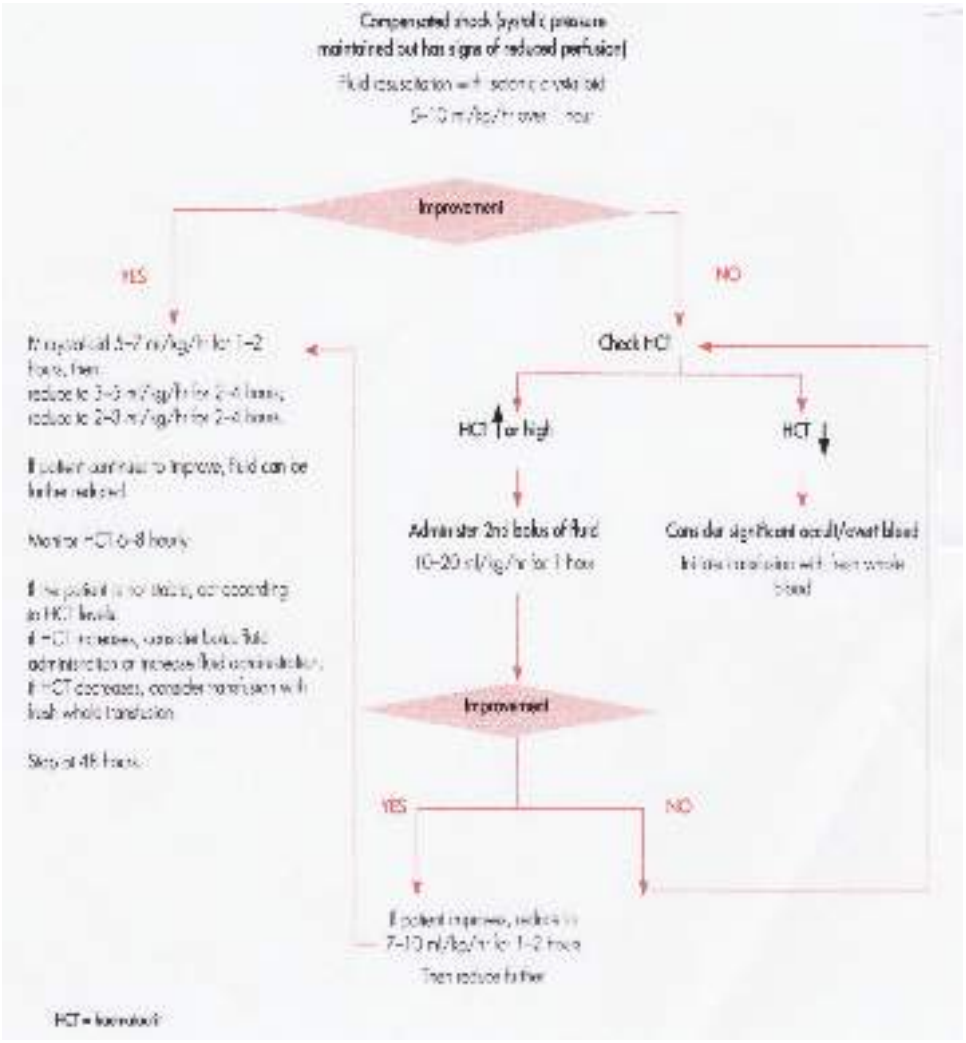

Figure 4. Algorithm for fluid management in compensated shock

- If vital signs are still unstable (i.e. shock persist), check the haematocrit after the first bolus. If the haematocrit increases or still high (> 50%), repeat a second bolus of crystalloid solution at 10-20 ml/kg/hr for one hour. After this second bolus, if there is improvement, reduced the rate to 7–10 ml/kg/hr for 1–2 hours, and then continue to reduce as above. If haematocrit decreases compared to the initial reference haematocrit (< 40% in children and adult females, < 45% in adult males), this indicates bleeding and the need to cross-match and transfuse blood as soon as possible (see treatment for haemorrhagic complications).
- Further boluses of crystalloid or colloidal solutions may need to be given during the next 24–28 hours.

Patients with hypotensive shock should be managed more vigorously. The action plan for treating patients with hypotensive shock is as follows (Textboxes D and N, figure 2.3):

- Initiate intravenous fluid resuscitation with crystalloid or colloid solution (if available) at 20 ml/kg as a bolus given over 15 minutes to bring the patient out of shock as quickly as possible.
- If the patient's condition improves, give a crystalloid/colloid infusion of 10 ml/kg/hr for one hour. Then continue with crystalloid infusion and gradually reduce to 5–7 ml/kg/hr for 1–2 hours, then to 3–5 ml/kg/hr for 2–4 hours, and then to 2–3 ml/kg/hr or less, which can be maintained for up to 24–48 hours (textbox H).
- If vital signs are still unstable (i.e. shock persist), review the haematocrit obtained before the first bolus. If the haematocrit was low (< 40% in children and adult females, < 45% in adult males), this indicates bleeding and the need to cross-match and transfuse blood as soon as possible (see treatment for haemorrhagic complication).
- If the haematocrit was high compared to the baseline value (if not available, use population baseline), change intravenous fluids to colloid solutions at 10-20 ml/kg as a second bolus over 30 minutes to one hour. After the second bolus, reassess the patient. If the condition improves, reduce the rate to 7–10 ml/kg/hr for 1–2

hours, then change back to crystalloid solution and reduce the rate of infusion as mentioned above. If the condition is still unstable, repeat the haematocrit after the second bolus.

- If the haematocrit decreased compared to the previous value (< 40% in children and adult females, < 45% in adult males), this indicates bleeding and the need to cross-match and transfuse blood as soon as possible (see treatment for maemorrhagic complication). If the haematocrit increases compared to the previous value or remains very high (> 50%), continue colloid solutions at 10–20 ml/kg as a third bolus over one hour. After this dose, reduce the rate to 7–10 ml/kg/hr for 1–2 hours, then change back to crystalloid solution and reduce the rate of infusion as mentioned above when the patient's condition improves.
- Further boluses of fluids may need to be given during the next 24 hours. The rate and volume of each bolus infusion should be titrated to the clinical response. Patients with severe dengue should be admitted to the high-dependency or intensive case area.

Patients with dengue shock should be frequently monitored until the danger period is over. A detailed fluid balance of all input and output should be maintained.

Parameters that should be monitored include vital signs and peripheral perfusion (every 15–30 minutes until the patient is out of shock, then 1–2 hourly). In general, the higher the fluid infusion rate, the more frequently the patient should be monitored and reviewed in order to avoid fluid overload while ensuring adequate volume replacement.

If resources are available, a patient with severe dengue should have an arterial line placed as soon as practical. The reason for this is that in shock states, estimation of blood pressure using a cuff is commonly inaccurate. The use of an indwelling arterial catheter allows for continuous and reproducible blood pressure measurements and frequent blood sampling on which decisions regarding therapy can be based. Monitoring of ECG and pulse oximetry should be available in the intensive care unit.

Urine output should be checked regularly (hourly till the patient is out of shock, then 1-2 hourly). A continuous bladder enables close monitoring of urine output. An acceptable urine output would be about 0.5 ml/kg/hr. Haematocrit should be monitored (before and after fluid boluses until stable, then 4–6 hourly). In addition, there should be monitoring of arterial or venous blood gases, lactate, total carbon dioxide/bicarbonate (every 30 minutes to one hour until stable, then as indicated), blood glucose (before fluid resuscitation and repeat as indicated), and other organ functions (such as renal profile, liver profile, coagulation profile, before resuscitation and as indicated).

Changes in the haematocrit are a useful guide to treatment. However, changed must be interpreted in parallel with the haemodynamic status, the clinical response to fluid therapy and the acid-base balance. For instance, a rising or persistently high haematocrit together with unstable

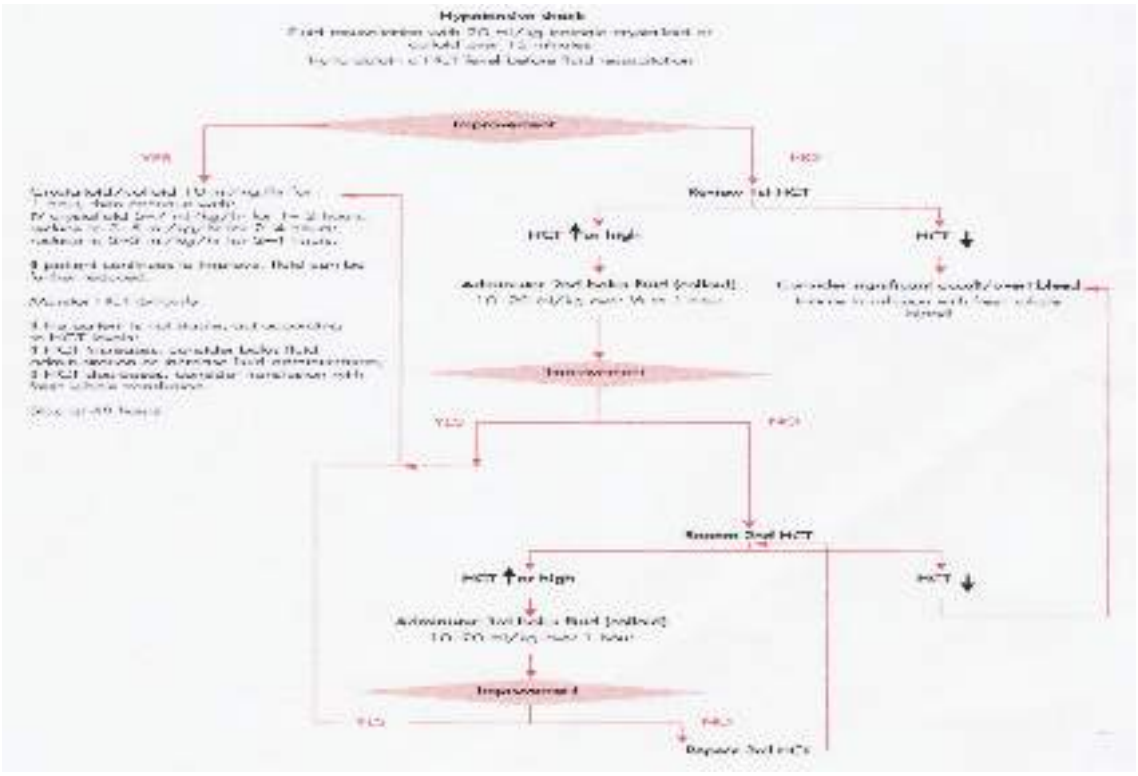

Figure 5. Algorithm for fluid management in hypotensive shock

vital signs (particularly narrowing of the pulse pressure) indicates active plasma leakage and the need for a further bolus of fluid replacement. However a rising or persistently high haematocrit together with stable haemodynamic status and adequate urine output does not require extra intravenous fluid. In the latter case, continue to monitor closely and it is likely that the haematocrit will start to fall within the next 24 hours as the plasma leakage stops.

A decrease in haematocrit together with unstable vital signs (particularly narrowing of the pulse pressure, tachycardia, metabolic acidosis, poor urine output) indicates major haemorrhage and the need for urgent blood transfusion. Yet a decrease in haematocrit together with stable haemodynamic status and adequate urine output indicates haemodilution and/or reabsorption of extravasated fluids, so in this case intravenous fluids must be discontinues immediately avoiding pulmonary oedema.

Treatment of haemorrhagic complications

Mucosal bleeding may occur in any patient with dengue but, if the patient remains stable with fluid resuscitation/ replacement, it should be considered as minor. The bleeding usually improves rapidly during the recovery phase. In patients with profound thrombocytopaenia, ensure strict bed rest and protect from trauma to reduce the risk of bleeding. Do not give intramuscular injections to avoid haematoma. It should be noted that prophylactic platelet transfusion for severe thrombocytopaenia in otherwise haemodynamically stable patients have not been shown to be effective and are not necessary (14).

If major bleeding occurs it is usually from the gastrointestinal tract, and/or vagina in adult females. Internal bleeding may not become apparent for many hours until the first black stool is passed.

Patients at risk of major bleeding are those who:
- have prolonged/refractory shock;
- have hypotensive shock and renal or liver failure and/or severe and persistent metabolic acidosis;
- are given non-steroidal anti-inflammatory agents;
- have pre-existing peptic ulcer disease;
- are on anticoagulant therapy;
- have any form of trauma, including intramuscular injection.

Patient with haemolytic conditions are at risk of acute haemolysis with haemoglobinuria and will require blood transfusion.

Severe bleeding can be recognized by:
- persistent and/or severe overt bleeding in the presence of unstable haemodynamic status, regardless of the haematocrit level;
- a decrease in haematocrit after fluid resuscitation together with unstable haemodynamic status;
- refractory shock that fails to respond to consecutive fluid resuscitation of 40–60 ml/kg;
- hypotensive shock with low/normal haematocrit before fluid resuscitation;
- persistent or worsening metabolic acidosis ± a well-maintained systolic blood pressure, especially in those with severe abdominal tenderness and distention.

Blood transfusion is life-saving and should be given as soon as severe bleeding is suspected or recognized. However, blood transfusion must be given with care because of the risk of fluid overload. Do not wait for the haematocrit to drop too low before deciding on blood transfusion. Note that haematocrit of < 30% as a trigger for blood transfusion, as recommended in the Surviving Sepsis Campaign Guideline (15), is not applicable to severe dengue. The reason for this is that, in dengue, bleeding usually occurs after a period of prolonged shock that is preceded by plasma leakage. During the plasma leakage the haematocrit increases to relatively high values before the onset of severe bleeding. When bleeding occurs, haematocrit will then drop from this high level. As a result, haematocrit levels may not be as low as in absence of plasma leakage.

The action plan for the treatment of haemorrhagic complications is as follows:
- Give 5–10 ml/kg of fresh-packed red cells or 10–20 ml/kg of fresh whole blood at an appropriate rate and observe the clinical response. It is important that fresh whole blood or fresh red cells are given. Oxygen delivery at tissue level is optimal with high levels of 2,3 di-phosphoglycerate (2,3 DPG). Stored blood loses 2,3 DPG, low levels of which impede the oxygen-releasing capacity of haemoglobin, resulting in functional tissue hypoxia. A good clinical response includes improving haemodynamic status and acid-base balance.
- Consider repeating the blood transfusion if there is further blood loos or no appropriate rise in haematocrit after blood transfusion. There is little evidence to support the practice of transfusing platelet concentrates and/or fresh-frozen plasma for severe bleeding. It is being practiced when massive bleeding can not be managed with just fresh whole blood/fresh-packed cells, but it may exacerbate the fluid overload.
- Great care should be taken when inserting a naso-gastric tube which may cause severe haemorrhage and may block the airway. A lubricated oro-gastric tube may minimize the trauma during insertion. Insertion of central venous catheters should be done with ultra-sound guidance or by a very experienced person.

Treatment of complications and other areas of treatment

Fluid overload

Fluid overload with large pleural effusions and acites is a common cause of acute respiratory distress and failure in severe dengue. Other causes of respiratory distress include acute pulmonary oedema, severe metabolic acidosis from severe shock, and Acute Respiratory Distress Syndrome

(ARDS) (refer to standard texbook of clinical care for futhure guidance on management).

Causes of fluid overload are:
- Exessive and/or too rapid intraveneous fluids;
- Incorrect use of hypotonic rether than isotonic crystalloid solutions;
- Inappropriate use of large volumes of intravenous fluids in patiens with unrecognize severe bleeding;
- Inappropriate transfusion of fresh-frozen plasma, platelat concetrates and cryoprecipates;
- Continous of intravenous fluids after plasma leakage has resolved (24–48 hours from defervescence);
- Co-morbid conditions such as contingental or ishaemic heart disease, chronic lug and renal disease.

Early clinical features of fluid overload are:
- Respiratory distress, difficulty in breathing;
- Rapid breathing;
- Chest wall in drawing;
- Wheezing (rather than crepitations);
- Large pleural effisions;
- Tense ascites;
- Increased jugular venous pressure (JVP)

Late clinical features are:
- Pulmonary oedema (caugh with pink or frothy sputum ± crepitations, cyanosis);
- Irreversible shock (heart failure, often in combination with ongoing hypovolaemis)

Additional investigations are:
- The chest x-ray which shows cardionegaly, pleural effusion, upward displacement of the diagphragm by the ascites and varying degress of : bat's wigs" appearance ± Kerley B lines suggestive of fluid overload and pulmonary oedema;
- ECG to exclude ischaemic changes and arrhythmia;
- Arterial blood gases;
- Echocardiogram for assessment of left ventricular function, dimensions and tegional wall dyskenia that may suggest underlying ishaemic heart disease;
- Cardiac enzym.

The actions plan for the treatment of fluid
- Oxygen therapy should be given immediately.
- Stopping intravenous fluid therapy during the recovery phase will allow fluid in the pleural and peritoneal cavities to return to the intravascular compartment.

This results in diuresis and resolution of pleural effusion and ascites. Recognizing when to decrease or stop intravenous fluids is key to preventing fluid overload.

When the following signs are present, intravenous fluids should be discontinued or reduced to the minimum rate necessary to maintain euglycaemia:
- Sign of cessation of plasma leakage;
- Stable blood pressure, pulse and peripheral perfusion;
- Haematocrit decreases in the presence of a good pulse volume;
- Afebrile for more than 24–48 days (without the use of antipyretics);
- Resolving bowel/abdominal symptoms;
- Improving urine output.

- The management of fluid overload varies according to the phase of the disease and the patient's haemodynamic status. If the patient has stable haemodynamic status and is out of the critical phase (more than 24–48 hours of defervescence), stop intravenous fluids but continue close monitoring. If necessary, give oral or intravenous furosemide 0,1–0,5 mg/ kg/ dose once or twice daily, or a continous infusion of furosemide 0,1 mg/kg/ hour. Monitor serum potassium and correct the ensuing hypokalaemia.
- If the patient has stable haemodynamic status but is still within the critical phase, redusce the intraveonous fluid accordingly. Avoid diuretics during the plasma leakage phase because they may lead to intravascular volum depletation.
- Patients who remain in shock with low or normal haemotocrit levels but show signs of fluid overload may have occult haemorrhage. Futher infusion of large volumes of intravenous fluids wil lead omly to a poor outcome. Careful fresh whole blood transfussion should be initiated as soon as posible. If the patient remains in shock and th haemocrit is elevated, repeated small boluses of a colloid solution may help.

Other Complications Of Dengue

Both hyperglycaemia and hypoglycaemia may occur, even in the absence of diabetes melitus and/or hypoglycaemic agents. Electrolyte and acid-base imbalance are also common observations in severe dengue and are probably related to gastrointestinal losses through vomiting and diarrhoea or to the use of hypotonic solutions for resuscitation and correction of dehydration. Hyponatraemia, hypokalaemia, hyperkalaemia, serum calcium imbalances and metabolic acidosis (sodium bicarbonate for metabolic acidosis is not recommended for pHš 7.15) can occur. One should also be alert for co-infections and nosocomial infections.

Supportive care and adjuvant therapy

Supportive care and adjuvant therapy may be necessary in severe dengue. This may include:
- Renal replacement therapy, with a peference to continous veno-venous haemodialyis (CWH), since peritoneal dialysis has arisk of bleeding;
- Vasopressor and therapies as temporary measure to prevent life-thrething hypotension in dengue shock ang during induction for intubation, while correction of intravascular volume is being vigorously carried out;

– Futher treatment of organ impairmant, such as severe hepatic involvement or encephalopathy or enchepalitis;
– Futher treatment of cardiac abnormalities, such as conduction abnormalities, may occur (The latter usually not requiring interventions).

In this context there is little or no evidence in favour of the use of steroids and antravenous immunoglobulins, or of recombinant Activated factor VII.

Refer to standard textbook of clinical care for more detailed information regarding the treatment of compilations and other areas of treatment.

SUMMARY

In 2009, the study Dengue Virus Infection in Patient at Dr. Soetomo hospital found a new serotype variant, it was subtype DEN I and phylo genetic study showed as DEN I genotype IV that correlated with phylo genetic dengue virus came from China which had severe clinical performance of Dengue Virus Infection. Three cases of dengue with unusual manifestations have been reported.

Two classification systems have been applied to address clinical assessment of our patients. Based on the WHO classification, one of our cases did not fulfill the DHF criteria (WHO 1997). By applying the new revised dengue classification all the cases were classified as severe dengue. Several warning signs were present in all patients before their conditions deteriorated. The new revised dengue classification could have helped in detecting severe dengue cases earlier and thus provide the clinicians time to manage severe dengue cases better. All the patients were treated according to WHO protocols and all of the involved organs recovered along with the improvement of the disease.

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PROFILE OF COMMUNITY ACQUIRED PNEUMONIA IN CHILDREN AT SOETOMO HOSPITAL SURABAYA IN 2007–2008

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ABSTRACT

Background: Community Acquired Pneumonia (CAP) is one of the most important health problem affecting children all over the world. Clinical findings, laboratory and radiological examination of CAP may largely vary from mild to severe. Objective: To report profile of CAP in children hospitalized at Soetomo Hospital Surabaya in 2007–2008 Methods: This research was a retrospective study. Data of children with primary diagnosis of CAP in 2007–2008 were obtained from medical records of the Department of Child Health Soetomo Hospital Surabaya. The diagnosis CAP was based on WHO criteria (pneumonia clinical syndrome). The clinical features of illness, laboratory and radiological examination were recorded and presented descriptively. Results: During the study period, 438 patients were diagnosed as CAP. More than half (83.4%) patients aged 3 months–3 year. Beside cough and tachypnea, most common symptom and signs were chest indrawing (76.2%) and fever (23.8%). Leucocytosis (39.6%). Bacteria was found in 8.2%. Accompanying diseases (i.e congenital heart disease, neurological and gastroenterological disorders) were found in 36.4%. One hundred fifty seven patients (35.8%) had malnutrition. Patchy infiltrate was found in 80.8% chest X-ray examination. Mortality was found in 4.3%. Conclusions: Community acquired pneumonia in children still count as a major problem at Soetomo Hospital Surabaya.

Key words: *children, community acquired pneumonia, clinical features of illness*

INTRODUCTION

Community Acquired Pneumonia (CAP) is one of the most important health problem affecting children all over the world. Clinical findings, laboratory and radiological examination of CAP may largely vary from mild to severe. The term "community-acquired pneumonia" (CAP) refers to pneumonia in a previously healthy person who acquired the infection outside a hospital.[a,2] The World Health Organization has defined pneumonia solely on the basis of clinical findings obtained by visual inspection and timing of the respiratory rate. The cause of CAP is often difficult to establish. The most effective methods are often invansive and cannot always be justified and serological diagnosis is too late to be of any therapeutic use. Despite the progress made in the diagnosis of pneumonia, it takes a few days to identify the causative microorganism in the blood or sputum samples and the etiology of half of all patients with CAP remains uncertain. Physicians need reliable data on the relative prevalence of different etiological agents in the patients area of residence, in addition to the clinical, laboratory and radiological findings in order to initiate antibiotic treatment empirically. The relative frequency of etiological agents varies among different geographical areas. The profile of community acquired pneumonia in children at Soetomo hospital Surabaya is not known The present study was undertaken to determine the profile of CAP in children hospitalized at Soetomo Hospital Surabaya in 2006.

MATERIAL AND METHODS

This research was a retrospective study. Data of children with diagnosis of CAP in January 2007–December 2008 were obtained from medical records of the Department of Child Health Soetomo Hospital Surabaya. The diagnosis CAP was based on WHO Criteria (Peumonia Clinical

Syndrome). The clinical features of illness, laboratory and radiological examination were recorded and presented descriptively. No patients had received the pneumococcal conjugate or polysaccharide vaccines. The radiologist assigned standardized and mutually exclusive diagnoses that included normal, patchy infiltrate, lobar consolidation and pleural effusion.

RESULTS

During the study period, 438 patients were diagnosed as CAP. More than half (83.4%) patients aged 3–36 month. Beside cough and tachypnea, most common symptom and signs were chest indrawing (76.2%), and fever (23.8%). Leucocytosis (39.6%). Bacteria was found in 8.2%. Accompanying diseases (i.e congenital heart disease, neurological and gastroenterological disorders) were found in 36.4%. One hundred fifty seven patients (35.8%) had malnutrition. Patchy infiltrate was found in 80.8% chest X-ray examination. Mortality was found in 4.3%.

Table 1. Profile of patients with CAP

Characteristic	No	Percentage
Sex, male	273	62.3
Age at diagnosis		
< 3 month	52	11.8
3–36 month	365	83.4
> 36 months	21	4.8
Clinical characteristic		
Chest indrawing	334	76.3
Fever	104	23.7
Nutritional status		
Well nourished	281	64.2
Moderate nourished	150	34.2
Severe nourished	7	1.6
Presence of accompanying Disease	120	52.9
Chest x-ray.		13.7
Normal	60	4.8
Lobar consolidation	21	0.7
Pleural effusion	3	80.8
Patchy infiltrat	354	

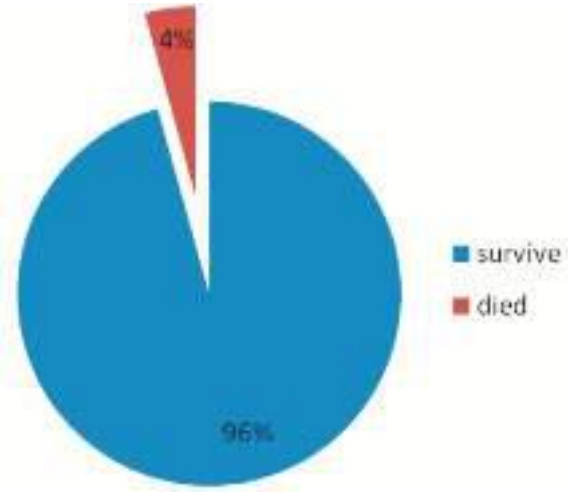

Figure 1. Mortality of CAP

DISCUSSION

During the study period, 438 patients were diagnosed as CAP. We showed that children with aged 3 months–3 year had the greatest degree of CAP, indicating that the infant have at most as many epidoses of pneumonia as older children. The clinical features all patients who diagnosed as CAP in this study were cough and tachypnea, based on the WHO criteria. The other clinical features that were most strongly associated with pneumonia were chest indrawing (76.3%), and fever (23.7%). Pneumonia should be suspected if tachypnea occurs in a patient younger than two years with a temperature higher than 38° C (100,4° F). Measurement of tachypnea requires a full one-minute count while the child is quiet. The World Health Organization's age-spesific criteria for tachypnea are the most widely used: a respiratory rate of more than 50 breaths per minute in infants two to 12 months of age; more than 40 breaths per minute in children one to five years of age; and more than 30 breaths per minute in children older than five years.[2]

Accompanying diseases (i.e congenital heart disease, neurological and gastroenterological disorders) were found in 36.4%. One hundred fifty seven patients (35.8%) had malnutrition. Patchy infiltrate was found in 80.8% chest X-ray examination. Complications of CAP such as respiratory failure occurred in 8.8% cases and sepsis in 10.5%, leading to mortality of 4.3%. The mortality from pneumonia is high particularly in patients with associated co-morbid conditions. Severe CAP requiring intensive care unit (ICU) admission, spread of radiographic infiltrates and

previous treatment with immunosupressive drugs have all been associated a poor outcome.[3,4] The mortality in our study was 4.3%. Analysis with student t test, malnutrition (p = 0,036) and accompanying diseases (p = 0,029) have significant correlation with the mortality of CAP.

CONCLUSIONS

Community acquired pneumonia in children still count as a major problem at Soetomo Hospital Surabaya.

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ASSOCIATION BETWEEN ATYPICAL DEPOLARIZATION IN CELL-DYN 3200 AND THE PRESENCE OF PLASMODIUM SPP IN BLOOD IN Dr. SOETOMO HOSPITAL SURABAYA

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ABSTRACT

Background*: Malaria is a parasitic disease worldwide with a high morbidity and mortality. A rapid and accurate method is needed to detect the presence of malaria parasites in blood. A flagging system atypical depolarization (atypdep) in CBC results from Cell-Dyn 3200 has been related with malaria infection.* ***Materials and Methods****: An observational cross sectional approach with 48 samples obtained from inpatients of the Dr.Soetomo Hospital, Surabaya. Samples were screened by Cell-Dyn 3200 analyzer for atypdep flagging in CBC. Positive samples were later confirmed by microscope to detect malaria parasites.* ***Results****: From 48 samples with atypdep flagging, 7 samples were malaria positive on peripheral blood smear (13.1%). Most frequent atypdep flagging was seen in malignancy (18.7%), and approximately 54.6% of the samples were not accompanied by fever symptoms. Lekositosis and anemia each were found in 20 samples (41.6%) and thrombocytopenia in 33.3%.* ***Conclusion****: The presence of atypdep flagging in Cell-Dyn 3200 does not necessarily indicate the existence of malaria or it could be said that atypdep flagging is not always associated with presence of malaria infection. The usage of an atypdep flagging in non-endemic areas such as Surabaya is just an alert sign to evaluate malaria infection rather than a screening method to detect malaria.*

Key words: *Malaria, atypical depolarization, hematology analyzer*

INTRODUCTION

Until now, Malaria remains the most important parasitic disease worldwide and causes health problems especially for those living in endemic areas. Early diagnosis relies crucially on clinical suspicion. A clinician suspecting the disease has to request explicitly malaria examination by blood smears. Lack of clinical suspicion is a well-known factor for a missed diagnosis, which contributes substantially to patient morbidity and mortality in this disease.[1] Of the 300 – 500 million cases of malaria infection which are estimated to occur annually, approximately 2–3 million of these are fatal.[2] the high frequency of severe clinical complications and mortality in endemic regions is exacerbated by delayed or inefficient treatment, limited access to clinical and laboratory services and the increasing influence of drug resistance.[3,4]

The female anopheline mosquito transmits malaria parasites, and after infecting a new host, the parasites are carried in the blood to the liver where they undergo a hepatic stage of multiplication. After a period of 9 to 16 days, the parasites return to the bloodstream and infect red cells. The typical spiking fever of malaria occurs when the red cells rupture and release free parasites.[3]

Patients with symptoms of fever and malaise in nonendemic areas will usually consult a clinician. However, in many countries malaria ranks as a relatively infrequent cause of pyrexia and thus may not be considered as part of the differential diagnosis. This is especially true if a complete clinical/travel history is not obtained. In such situations, clinicians may only initially request general screening tests such as a full blood count (FBC).[3] while a diagnosis of malaria can be established by microscopic examination of thin and thick blood film[4], although the investigation does not necessarily indicate the existence of parasites.

Microscopic investigation of stained thick and thin blood smears has been the reference standard for malaria detection and species identification for decades. Recently, a number of alternative diagnostic approaches have evolved,

including detection of *Plasmodium* species DNA stained with acridine orange in a quantitative buffy coat analysis, PCR methods, and assays based on detection of circulating *Plasmodium* species specific antigens. Recent studies using automated hematology analyzers have demonstrated unexpected abnormalities in differential white blood cell plots and reticulocyte histograms from patients with malaria.[6]

In normal blood samples, the only depolarizing WBC events are eosinophils. With Cell-Dyn multiangle polarized scatter separation (MAPSS) analysis, normal eosinophils viewed in the polarized-90° versus depolarized-90° (NEU EOS) plot form a distinct cloud of events that are color coded green. The depolarization of these cells is due to a component of eosinophil granules (Figure 1).[3]

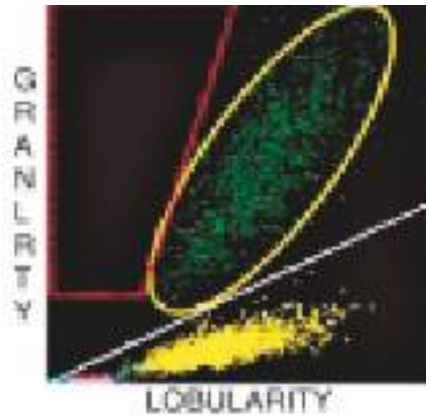

Figure 1. Granularity (90° depolarization axis) versus lobularity (90° polarization axis) plot showing typical Cell-Dyn 3200 eosinophil depolarization pattern. Normal eosinophils are located within the area demarcated by the yellow oval line, and the atypical depolarization region indicated by the red broken line does not normally contain any events.[3]

During the intraerythrocytic stage, a malaria parasite digests and breaks down hemoglobin to its constituent parts heme and globin. The globin is used as a protein source by the parasite and the heme is converted by an enzyme (heme polymerase) to hemozoin or malaria pigment. The parasite initiates this process because heme is toxic to the parasite whereas hemozoin is not. In contrast to nondepolarizing heme, hemozoin has a distinctive ability to depolarize light.

In the malaria parasite cell cycle, the malaria-infected red cells rupture at the schizont stage and the parasites are released together with hemozoin aggregates into the plasma. By an as-yet-unknown mechanism, circulating phagocytic WBCs (monocytes and neutrophils) then ingest the liberated free hemozoin. Consequently, normally nondepolarizing monocytes and neutrophils will depolarize light when they contain aggregates of hemozoin. this will cause appearance of abnormal dots on neu-eosin scatter plot.[3]

Research on detection of hemozoin by hematology analyzer has been done[1,2,3,4,5,6,7]. It was reported that the presence of one or more atypical depolarizing events can be attributed to malaria. Discovery of the abnormal depolarization pattern in patients with unknown fever should be considered to possibility of malaria infection, so microscopic examination by stained thick and thin blood smears as a confirmation needs to be done. A study in Portugal by Hanscheid et al[5] reported that diagnosing of malaria by detection of hemozoin using hematology analyzer obtain 95% sensitivity and a 88% specificity. While a South African study found a 72% sensitivity and 96% specificity.

In the Dr.Soetomo Hospital, Surabaya, atypical depolarizing events are often found in complete blood count results. Surabaya is not a malaria endemic area, but Dr. Soetomo Hospital is a referral hospital for the eastern Indonesian region, so that the patients are estimated to come from various regions. Most patients were examined with a diagnosis of other diseases, without any suspicion of malaria infection. Based on the fact, the researchers wanted to know whether the presence of atypical depolarization was actually due to malaria infection. If this was true, is it possible that existence of atypical depolarization (Atypdep) could be used a screening marker for malaria in non-endemic areas such as Surabaya? Is there any association between the presences of atypdep flagging with the plasmodium in the blood in non-endemic areas such as Surabaya?

MATERIALS AND METHODS

This research was done in the Laboratory of the Department of Clinical Pathology, Dr.Soetomo Hospital Surabaya during February to May 2010. Samples were obtained by selecting CBC results of inpatients in Dr. Soetomo Hospital. Samples of venous blood with EDTA anticoagulant were examined for CBC with a Cell-Dyn 3200 Hematology analyzer. CBC results showing atypdep flagging were included in this study. These samples were examined by thin blood smear examination with Giemsa staining to find and determine the types of parasites. Samples were considered positive when parasites were found in thin blood smears. The numbers of parasites were counted per 1000 erythrocytes, and samples were considered negative if in the 50 fields of emersion parasites were not found. Examination was conducted by 2 persons, a laboratory technician and a medical doctor. This study design was a descriptive observational study through cross-sectional approach, data and results were presented in the form of tables and figures.

RESULTS

During the period of the study 48 samples were obtained that fullfield the criteria (males 64.5%, females 35.5%)

with a variety of diagnosis. Most of the atypdep flagging was found in adult patients (60.4%) and also in 5 samples of neonates. Lekositosis and anemia were each found in 20 samples (41.6%), while thrombocytopenia was found in 16 samples (33.3%). Sample characteristics can be seen in Table 1. Of the 48 samples collected, only 7 samples were malaria positive with a thin smear examination, or about 13.1% only. Of the positive samples, almost all of them showed fever and a history of malaria endemic areas. Of malaria positive samples there were 5 samples showing anemia and thrombocytopenia (71.4%). Of all samples collected, atypdep appears most in the group of malignancy or tumor disease as much as 9 people, or 18.7%. (Table 2)

There are some patterns of atypical depolarizing events, some of which can be seen in figure 2a,2b,2c. Parasites were found among malaria positive patients in various phases (trophozoit, schizont, gametocytes).

Table 1. Samples characteristics

Parameter	number	Persentage (%)
Sex		
Male	31	64.5
Female	17	35.5
Age		
< 1 yr	5	10.4
1–< 18 yr	7	14.5
18–60 yr	29	60.4
> 60 yr	7	14.5
Hb level		
< 6 g/dl	-	
6–8 g/dl	4	8.3
> 8–11 g/dl	15	31.2
> 11–18 g/dl	28	58.3
> 18 g/dl	1	2.1
Temperature		
< 38°C	31	64.5
>= 38°C	17	35.5
Platelet level		
< 150,000	16	33.3
150,000–450,000	24	50
> 450,000	8	16.6
WBC		
< 4,000	4	8.3
4,000–11,000	24	50
> 11,000	20	41.6
Persentage of eosinophil		
<=7%	43	89.5
> 7%	5	10.5

Table 2. Clinical diagnosis of the positive atypdep patients

Diagnosis	No of sample	Persentage (%)
Trauma (Traffic accident)	5	10.5
Urinary bladder diverticle and chronic colitis	1	2
Nephrotic syndrome	1	2
Post partum	1	2
DHF	6	12.5
Malignancy and/tumor	9	18.7
Down syndrome	2	4.1
CKD	2	4.1
Hydrocephalus	1	2
Sepsis	3	6.2
BPH	1	2
Kidney stone	2	4.1
Suspected malaria	4	8.3
Combustio	3	6.2
Febris	2	4.1
DM	1	2
Decubitus ulcer	1	2
Pancytopenia	1	2
Hirschprung's disease	1	2
UTI	1	2
Hemolitic anemia	1	2

Figure 2a. Samples showing occasional atypical depolarizing purple events (within yellow broken boundaries).

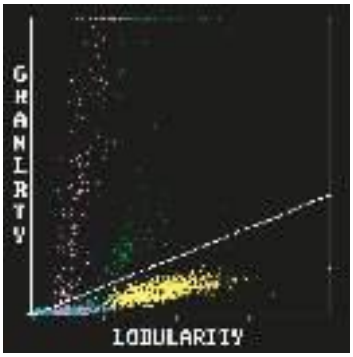

Figure 2b. Samples showing many atypical depolarizing purple events.

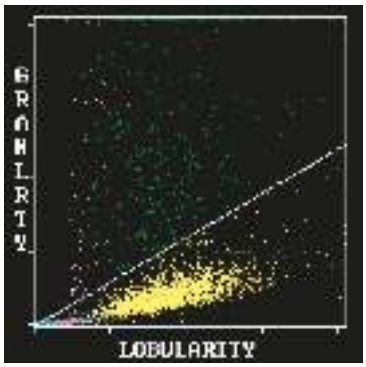

Figure. 2c. Malaria samples with mixture of abnormal depolarizing purple and green events that are not in the position normally associated with typical eosinophils

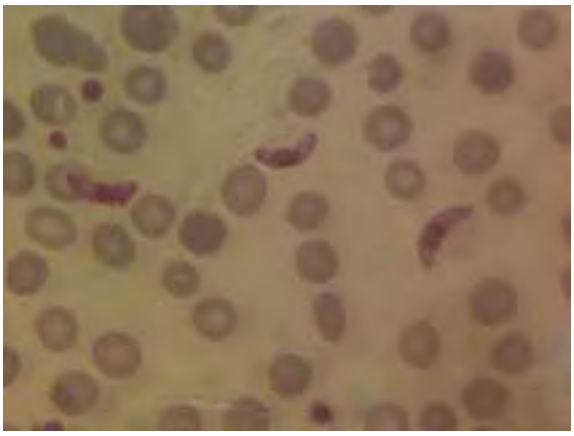

Figure 3. Trophozoit phase of plasmodium falcifarum (banana form)

DISCUSSION

In this study, out of 48 samples with atypep flagging, only 7 samples were malaria positive (13.1%). These results are not in accordance with various previous studies that have been done in several countries reporting that the sensitivity and specificity of atypical depolarization in detecting malaria is very high. This could be due to differences in sampling population In previous studies, samples taken from patients with clinical symptoms of malaria, and also done mostly in endemic malaria areas. While in this study, samples were taken at random, just based on the presence of atypdep flagging on CBC results regardless of clinical symptoms and diagnosis. After confirmation by thin smear examination, only 7 samples were positive for malaria. This shows that for non-endemic areas such as Surabaya (low prevalence), the appearance of atypdep is not yet certain in malaria infection .Therefore, it is important especially for patients with fever whose CBC results show atypdep flagging to confirm this by thick or thin smears in order to prove the existence of malaria infection.

In this study, percentage of atypdep flagging that appeared in diseases without fever and other symptoms of malaria was nearly 65% and many were shown in malignancies this proves that the presence of atypdep is not only caused by the presence of hemozoin or malaria pigment in monocytes or neutrophils, but there may be other causes such as small cell fractions that capable to depolarizing light in addition to eosinophils.

Changes of the parameters such as WBC, red blood cells and platelets in malaria patients are generally not specific. Some studies reported that the occurrence of thrombocytopenia in patients with clinical symptoms of malaria is an important indicator of malaria. Although the frequency of occurrence of thrombocytopenia reported was about 80%,[3,9] but these results varied in different studies that have been conducted. In this study, thrombocytopenia was found in 5 out of samples from the 7 malaria positive samples or approximately 71.4%. Similarly, anemia was found in 5 samples, while the number of WBC showed no characteristic changes .

Of the malaria positive samples, all were imported malaria. All positive samples did not come from endemic areas, however, there was a history of traveling to endemic areas. In this study, one sample showed a negative thin smear with a history of malaria therapy 1 week before. This is consistent with the theory that in patients who are in recovery where parasites can no longer be found in the blood, atypdep flagging can still occur because of atypical depolarizing clearance of malaria pigment is slow. In some individuals, this malaria pigment can remain in circulation until 3 weeks after recovery[3].

There is a reference reporting that pseudoeosinophilia is associated with the emergence of atypical depolarizing.[8] However, in this study, eosinophilia was found just in 5 samples or 10.4%. Also lekositosis as much as 20 samples (41.6%) raises a question, whether lekositosis may be related with the emergence of atypdep ? The emergence of atypdep in neonates as much as 5 samples also need to be considered, whether neonatal blood could influence the occurrence of atypdep flagging. A further study is needed to determine the factors that lead to the emergence of atypdep flagging, so that atypdep flagging is not merely focused on the presence of malaria, but other possible causes as well. However, when atypdep flagging is found, it is important to confirm this by blood smear examination for malaria.

Further studies are needed to determine the factors causing the emergence of atypdep flagging because in this study there are several limitations, among others:

- Detection of plasmodium is influenced by the quality of the staining and the skills and Expertise of examiners
- positive results are influenced by the prevalence
- More samples are needed.

CONCLUSION AND RECOMMENDATION

It was found that the rise of atypdep flagging does not always indicate a malaria infection or it could be said that atypdep flagging is not always associated with the occurrence

of malaria infection because from the 48 samples only 13.1% positive on blood smear. So the emergence of atypdep flagging on Cell-Dyn 3200 instrument can not be used as a screening of malaria in non-endemic areas such as Surabaya.

Additional criteria for non-endemic areas such as Surabaya are needed, when the existence of this atypdep flagging suspicious of malaria infection, for example: 1. Frequency of atypdep appearance in the same patient 2. Existence of thrombocytopenia 3. Presence of clinical symptoms (fever, chills, etc.). This needs to be done by scoring and with a larger number of samples. Moreover, further studies should be conducted to identify other factors leading to the emergence of atypdep flagging.

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THE MONTHLY CHANGING OF THE LOWEST POPULATION DENGUE VIRUS INFECTION IN PATIENT AT SOERYA HOSPITAL SIDOARJO IN 2010

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ABSTRACT

Background: Dengue virus infection is one of the important health problems in Indonesia, although the mortality rate has been decreased but many dengue shock syndrome cases is very difficult to be solving handled. To solve this problem, some factor that influence the prevalence of dengue virus infection should be studied. The Aim of Study: To detect some factor that maintain the higher case of dengue virus infection in patient at the Soerya Hospital Sepanjang, Sidoarjo. Material & Method: Study had been done at Soerya Hospital Sepanjang, Sidoarjo since January 1, 2007 until December 31, 2010. All cases suspected dengue virus infection in patient at soerya hospital were diagnosed based on WHO criteria in 1997 and PCR examination in ITD laboratory. The Result: In 2007, 2008, 2009, the monthly observation showed that decreasing cases of dengue virus infection in patient at Soerya Hospital had been found on September, but in 2010 this event had been found on November. Why this event to be change? It is suggested might be due to global warming in the world and the climate going to influence the environment sanitation. Interaction between agent host and environment becoming increase it might be due to the changing of climate can influence the growing population Aedes Aegyptie and Aedes Albopictus promoting to increase vector for transmit dengue virus infection. It is prominent in sub urban area, with have many peoples don't aware with the bad environment sanitation. And many peoples showed very dynamic for living until the idea good environment do not be thought. By this condition the monthly population dengue virus infection in patient at hospital are going to maintain higher more than six months than usually. On the year 2007, 2008, 2009 the lowest cases found on September. In 2010, the lowest cases had been found on November. The Conclusion: Global warming, increasing sub urban area which have many peoples don't aware with the bad environment sanitation and have highly dynamic peoples for getting some money for their life, could influence the higher cases dengue virus infection in patient at hospital more than 6 months.

Key words: *Monthly changing, dengue virus infection, in patient, in 2010*

INTRODUCTION

Epidemic dengue is a major public health problem in Indonesia, which is tropical mansoon and equatorial zone. Where Aedes Aegypti is wide spread in both urban and rural area where multiple virus serotype are circulating and where dengue is a leading cause of hospitalization and death in children. Cycle epidemic are increasing in frequency and in country geographic expansion is occurring in the dry and wet climate zone with multiple virus serotype circulating.

Dengue inflicts a significant health, economic and social burden on the populations of endemic areas. Globally the estimated number of disability adjusted life years (DALYs) lost to dengue in 2001 was 528. The number of cases reported annually to WHO ranged from 0.4 to 1.3 million in the decade 1996–2005. As an infectious disease, the number of cases varies substantially from year to year. Underreporting and misdiagnoses are major obstacles to understanding the full burden of dengue.

On average, a hospitalized case of dengue cost three times what an ambulatory case costs. Combining the ambulatory and hospitalized patients and factoring in the risk of death, the overall cost of a dengue cases is US$ 828. Children are at a higher risk of severe dengue. Intensive care is required for severely ill patients, including intravenous fluids, blood or plasma transfusion and medicines. Dengue

afflicts all levels of society but the burden may be higher among the poorest who grow up in communities with inadequate water supply and solid waste infrastructure, and where conditions are most favourable for multiplication of the main vector, Aedes Aegypti. Travelers play an essential role in the global epidemiology of dengue infections, as viraemic travelers carry various dengue serotypes and strains into areas with mosquitoes that can transmit infection.

Over the past four years, epidemic dengue activity has spread in Indonesia which more than 35% of country's population lives in urban areas 150.000 cases were reported in 2007 with over 250.000 cases reported from Jakarta, West Java and East Java. The case fatality rate was approximately 1%.

This paper as a part of overview epidemiology dengue in sub urban area of Sidoarjo residence with presented data Dengue cases at Soerya hospital in 2007, 2008, 2009, and 2010.

THE AIM OF STUDY

To detect some factor that maintain the higher case of Dengue virus infection in patient at the Soerya Hospital Sepanjang Sidoarjo.

MATERIAL AND METHOD

Study had been done at Soerya Hospital since January 1, 2007 until December 31, 2010. Diagnoses cases suspected Dengue virus infection in patient at Soerya hospital had been based on WHO criteria 1997 and PCR examination in ITD labolatory.

THE RESULT

In 2007–2009 the monthly observation to Dengue virus infection showed that the decreasing cases of Dengue virus infection in patient at Soerya hospital had been found on September, but in 2010 this even had been found in November (see table 1 & figure 1).

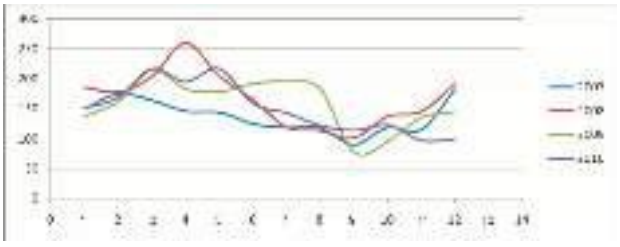

Figure 1. The monthly cases of Dengue virus infection which had been in patien at Soerya hospital in 2007–2010

Table 1. The monthly Dengue virus infection cases in 2007-2010

No.	Month	Year			
		2007	2008	2009	2010
1	Januari	150	186	137	151
2	Februari	175	178	162	169
3	Maret	164	204	215	217
4	April	146	260	183	195
5	Mei	144	205	178	217
6	Juni	125	165	192	159
7	Juli	119	119	195	143
8	Agustus	118	113	183	122
9	September	90	101	77	114
10	Oktober	119	137	95	123
11	Nopember	115	145	135	97
12	Desember	182	191	145	99
Total		1647	2004	1897	1806

The higher prevelancy of dengue virus infection might be due too:

a) The environment sanitation surrounding their house is not enough qualified because the pollution of the factory every day (every early morning and midnight) has been thrown out surrounding the community.
b) The cleaning water is very difficult to get an usually has been contaminated with microbiology.
c) Many people who live in sub urban area with crowded families.

DISCUSSION

The climate changing due to global warming make many cleaner pocked water which are going to spred many parts. Such as at surrounding houses, school, and some found in the field. It support to increase population many mosquito Aedes Aegypti and Aedes Albocpitus if the breeding season of this mosquito occur, there are many larvaes found in pocked water area and in short time it become adult mosquito and do as vector Dengue infections transmit virus from one case Dengue virus infection to other cases who live in surrounding it. Many cases of Dengue virus infection will be increased (see table 1 & figure 1).

This paper focused in Dengue virus infection, Dengue has a wide spectrum of clinical presentations, often with unpredictable clinical evolution and outcome. While most patients recover following self limiting non-severe clinical course, a small proportion progress to severe disease, mostly characterized by plasma leakage with or without haemorrhage. Intravenous rehydration is the therapy of choice; this intervention can reduce the case fatality rate to less than 1% of severe cases. The group progressing from non-severe to severe disease is difficult to define, but this

is an important concern since appropriate treatment may prevent these patients from developing more severe clinical conditions.

Triage, appropriate treatment, and the decision as to where this treatment should be given (in a health care facility or at home) are influenced by the case classification for dengue. This is even more the case during the frequent dengue outbreaks worldwide, where health services need to be adapted to cope with the sudden urgent in demand.

Changes in the epidemiology of dengue, as described in the previous sections, lead to problems with the use of the existing WHO classification. Symptomatic dengue virus infections were grouped into three categories: undifferentiated fever, dengue fever (DF) and dengue haemorrhagic fever (DHF). DHF was further classified into four severity grades, with grades III and IV being defined as dengue shock syndrome (DSS). There have been many reports of difficulties in the use of this classification, which were summarized in a systematic literature review. Difficulties in applying the criteria for DHF in the clinical situation, together with the increase in clinically severe dengue cases which did not fulfill the strict criteria of DHF, led to the request for the classification to be reconsidered. Currently the classification into DF/DHF/DSS continues to be widely used.

A WHO/TDR supported prospective clinical multicentre study across dengue endemic regions was set up to collect evidence about criteria for classifying dengue into levels of severity. The study findings confirmed that, by using a set of clinical and/or laboratory parameters, one sees a clear cut difference between patients with severe dengue and those with non severe dengue. However, for practical reasons it was desirable to split the large group of patients with non severe dengue into two subgroups patients with warning signs and those without them. Criteria for diagnosing dengue (with or without warning signs) and severe dengue should follow guidelines for diagnosis, treatment, prevention and control (new edition WHO 2009). It must be kept in mind that even dengue patients without warning signs may develop severe dengue.

Expert consensus groups in Latin America (Havana, Cuba, 2007), South-East Asia (Kuala Lumpur, Malaysia, 2007), and at WHO headquarter in Geneva, Switzerland in 2008 agreed that: "dengue is one disease entity with different clinical presentations and often with unpredictable clinical evolution and outcome".

The classification into levels of severity has a high potential for being of practical use in clinicians decision as to where and how intensively the patient should be observed and treated (i.e. triage, which is particularly useful in outbreaks), in more consistent reporting in the national and international surveillance system, and as an end-point measure in dengue vaccine and drug trials.

In 2007–2008–2009 we had found many cases with unusually clinical manifestation of dengue hemorrhagic fever. Some of them showed a clinical manifestation that could not predict as usually cases DHF; schedule severity and recovery becoming prolong until ten days. Especially for cases with obesity and severe malnutrition. It may be due to decreasing immunity for doing immune respond reaction. Beside it there were cases showed prolong fever more than seven days. We should aware with a concident infection such as typhoid fever that can be identified with the result positive IgM Salmonella in the blood.

The other cases should be in mind; the underlying disease like as Tuberculosis and urinary tract infection that making the time recovery become prolong.

Dengue virus is a small single stranded RNA virus comprising four distinct serotypes. These related serotypes of the dengue virus belong to the genus flavivirus, family Flaviviridae.

The mature particle of the dengue virus is spherical with a diameter of 50 nm containing multiple copies of the three structural proteins, a host derived membrane bilayer and a single copy of a positive sense, single stranded RNA genome. The genome is cleaved by host and viral proteases in three structural proteins (capsid, C, prM, the precursor of membrane, M, protein and envelope, E) and seven nonstructural proteins.

Distinct genotypes or lineages (viruses highly related in nucleotide sequence) have been identified within each serotype, highlighting the extensive genetic variability of the dengue serotypes. Purifying selection appears to be a dominant theme in dengue viral evolution, however, such that only viruses that are "fit" for both human and vector are maintained. Among them, "Asian" genotypes of DEN2 and DEN3 are frequently associated with severe disease accompanying secondary dengue infections. Intra host viral diversity (quasispecies) has also been described in human hosts.

In 2009 Yamanaka has found dengue virus D1 stereotype IV which showed a severe clinical performance coincidence with primary dengue virus infection.

The various serotypes of the dengue virus are transmitted to humans through the bites of infected Aedes mosquitoes, principally Ae. Aegypti. This mosquito is a tropical and subtropical species widely distributed around the world, mostly between latitudes 35° N and 35° S. these geographical limits correspond approximately to a winter isotherm of 10° C Ae. Aegypti has been found as a far north as 45° N, but such invasions have occurred during warmer months and the mosquitoes have not survived the winters. Also, because of lower temperatures, Ae. Aegypti is relatively uncommon above 1000 metres. The immature stages are found in waterfilled habitats, mostly in artificial containers closely associated with human dwellings and often indoors. Studies suggest that most female Ae. Aegypti may spend their lifetime in or around the houses where they emerge as adults. This means that people, rather than mosquitoes, rapidly move the virus within and between communities. Dengue outbreaks have also been attributed to Aedes Albopictus, Aedes Polynesiensis and several species of the Aedes Scutellaris complex. Each of these species has a particular ecology, behavior and geographical

distribution. In recent decades Aedes Albopictus has spread from Asia to Africa, the Americas and Europe, notably aided by the international trade in used tyres in which eggs are deposited when they contain rainwater. The eggs can remain viable for many months in the absence of water.

After an incubation period of 4–10 days, infection by any the four virus serotypes can produce a wide spectrum of illness, although most infection asymptomatic or subclinical (chapter 2). Primary infection is thought to induce lifelong protective immunity to the infecting serotype. Individuals suffering an infection but with no long term cross protective immunity.

Individuals risk factor determine the severity of disease and include secondary infection, age, ethnicity and possibly chronic disease (bronchial asthma, sickle cell anemia and diabetes mellitus). Young children particular may be less able than adults to compensate for capillary leakage and are consequently at greater risk of dengue shock.

Seroepidemiological studies in studies in Cuba and Thailand consistently support the role of secondary heteritypic infection as a risk factor for severe dengue, although there are a few report of severe cases associated with primary infection. The time interval between infection and the particular viral sequence of infection may also be of importance. For instance, a higher case fatality rate was observed in Cuba when DEN-2 infection followed a DEN-1 infection after an interval 20 years compared to an interval of four years. Severe dengue is also regularly observed during primary infection of infant born to dengue immune mothers. Antibody-dependent enhancement (ADE) of infection has been hypothesized as a mechanism to explain severe dengue in the course of a secondary infection and in infants with primary infections. In this model, neo neutralizing, cross reactive antibodies raised during a primary infection, or acquired passively at birth, bind to epitopes on the surface of a heterologous infecting virus and facilitate virus entry into Fc-receptor-bearing cells. The increased number of infected cells is predicted to result in a higher viral burden and induction of a robust host immune response that includes inflammatory cytokines and mediators, some of which may contribute to capillary leakage. During secondary infection, cross reactive memort T cells are also rapindly activated, proliferate, express cytokines and die by apoptosis in amanner that generally correlates with overall disease severity. Host genetic determinants might influence the clinical outcome of infection, throught most studies have been unable to adequately addres this issue. Studies in America region show the rates of severe dengue to be lower in individuals of Africans ancestry than those in other ethnicgroups.

The dengue virus enters via the skin while an infected mosquito is taking a bloodmeal. During the acute phase of illness the virus is present in the blood and its clearancefrom this compartment generally coincides with defervescence. Himoral and cellularimmune response are considered to contribute to virus clearance via the generation of neutralizing antibodies and the activation of CD 4 and CD 8 T lymphocyte. In addition, innate host defense may limit infection by the virus. After infection, serotype specific and cross reactive antibodies and CD 4 and CD 8 T cells remain measurable for years.

Plasma leakage, haemoconcentration and abnormalities in homeostasis characterize severe dengue. The mechanism leading to severe illness are not well defined but the immune response, the genetic background of the individual and the virus characteristic may all contribute to severe dengue.

Recent data suggest that endothelial cell activation could mediate plasma leakage. Plasma leakage is thought to be associated with fuctional rather than destructive effect on endothelial cell. Activation of infected monocytes and T cells, the complement system and the production of mediators, monokines, cytokines and soluble receptors may also be involved in endhotyal cell dysfunction.

Thrombocytopenia may be associated with alterations in megakaryocytopoises by the infection of human haematopoietic cells and impaired progenitor cell growth, resulting in platelet dysfunction (platelet activation and aggregation), increased destruction or consumption (peripheral sequestration and consumption). Haemorrhage may be a consequence of the thrombocytopenia and associated platelet dysfunction or disseminated intravascular coagulation. In, summary a transient and reversible imbalance of inflammatory mediators, cytokinesiss and chymokinesis occurs during severe dengue, probably driven by a high early viral burden and leading to dysfunction of vascular endothelial cell, derangement of the haemocoagulation system then to plasma leakage, shock, bleeding.

Humans are the main amplifying host of the virus. Dengue virus circulating in the blood of viraemic humans is ingested by female mosquitoes during feeding. The virus then infects the mosquito mid-gun and subsequently spreads systemically over a period of 8–12 days. After this exstrinsic incubation period, the virus can be transmitted to others human during subsequent probing our feeding. The extrinsic incubation period in influence in part by invironmental conditions, especially ambient temperature. Thereafter the mosquito remains infective for the rest of its life. Aedes Aegypti is one of the most efficient vectors for arboviruses because it is highly antrophopilic, frequently bites several times before completing oogenesisi and thrives in close proximity to humans. Vertical transmission of dengue virus has been demonstrated in the laboratory but rarely in the field. The significance of vertical transmission for maintenance of the virus is not well understood. Sylvatic dengue strains in some parts of Africa and asia may also lead to human infection, causing mild illness. Several factors can influence the dynamics of virus transmission including environmental and climate factors, host-pathogen interaction and population immunological factors. Climate directly influences the biology of the vectors and thereby their abundance and distribution it is consequently an important determinant of vector borne disease epidemics.

This overview had also found in our experience in 2007, 2008, 2009, 2010 as follow: The Dengue virus infection cases were one of the top ten infection diseases it had been found at Soerya Hospital in 2007, 2008, 2009 and 2010.

In 2007, 2008, 2009, the monthly observation showed that decreasing cases of dengue virus infection in patient at Soerya Hospital had been found on September, but in 2010 this event had been found on November.

Why this event to be change? It is suggested might be due to global warming in the world and the climate going to influence the environment sanitation. Interaction between agent host and environment becoming increase it might be due to the changing of climate can influence the growing population Aedes Aegyptie and Aedes Albopictus promoting to increase vector for transfering dengue virus infection. It is prominent in sub urban area, with have many peoples don't aware with the bad environment sanitation. And many peoples showed very dynamic for living until the making good environment do not be thought. By this condition the monthly population dengue virus infection in patient at hospital are going to maintain higher more than six months than usually. On the year 2007, 2008, 2009 the lowest cases found on September. In 2010, the lowest cases had been found on November.

CONCLUSION

Dengue inflicts a significant health, economic and social burden on the population of endemic area. Under reporting and mis-diagnosis are major obstacl to understanding the full burden of dengue. Travelers play on an essential role in the global epidemiology of dengue infection as viremia traveler carry various dengue serotypes and strain into areas with mosquitoes that can transmit infection.

Over the past four years, epidemic dengue activity has spread in Indonesia. This paper as a part of overview epidemiology dengue in sub urban area. Presented data dengue that included in the top ten Tropical disease cases at Soerya Hospital in 2007, 2008, 2009, and 2010.

Global warming; increasing sub urban area which have many peoples don't aware with the bad environment sanitation and have highly dynamic peoples for getting some money for their life, could influence the higher cases dengue virus infection in patient at hospital more than 6 months.

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EXPRESSION OF β-XYLOSIDASE ENCODING GENE IN PHIS1525/ *Bacillus megaterium* MS941 SYSTEM

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ABSTRACT

β-Xylosidase encoding gene from G. thermoleovorans IT-08 had been expressed in the pHIS1525/ B. megaterium MS941 system. The β-xylosidase gene (xyl) was inserted into plasmid pHIS1525 and propagated in E. coli DH10β. The recombinant plasmid was transformed into B. megaterium MS941 by protoplast transformation. Transformants were selected by growing the recombinant cells on solid LB medium containing tetracycline (10 µg/ ml). The expression of the β-xylosidase gene was assayed by overlaid the recombinant B. megaterium MS941 cell with agar medium containing 0.2% methylumbelliferyl-β-D-xyloside (MUX). This research showed that the β-xylosidase gene was succesfully sub-cloned in pHIS1525 system and expressed by the recombinant B. megaterium MS941. The addition of 0.5% xylose into the culture medium could increase the activity of recombinant β-xylosidase by 2.74 fold. The recombinant B. megaterium MS941 secreted 75.56% of the expressed β-xylosidase into culture medium. The crude extract β-xylosidase showed the optimum activity at 50° C and pH 6. The recombinant β-xylosidase was purified from culture supernatant by affinity chromatographic method using agarose containing Ni-NTA (Nickel-Nitrilotriacetic acid). The pure β-xylosidase showed a specific activity of 10.06 Unit/ mg protein and relative molecular weight ± 58 kDa.

Key words: *Expression, β-xylosidase,* ***Geobacillus thermoleovorans IT-08, pHIS1525, Bacillus megaterium MS941***

INTRODUCTION

β-D-Xylosidases (1,4-β-D-xylan-xylo-hydrolase, EC 3.2.1.37) are hemicellulases that hydrolyze xylooligosaccharides to xylose and are essential for the complete utilization of xylan. The branching and variability of the xylan structure requires the concerted action of several hemicellulolytic enzymes including endo-1, 4-β-xylanases that hydrolyze the xylan backbone, and β-xylosidases that cleave the resulting xylooligomers into xylose monomers.[1]

Plasmid pTP510 is a recombinant plasmid containing xylanolytic enzyme encoding gene from *G. thermoleovorans* IT-08. The xylanolytic enzymes were expressed intracellularly by the recombinant pTP510/ *E. coli* DH5α. The recombinant plasmid containing three genes: exo-xylanase (*exo-xyl*), α-L-arabinofuranosidase (*abfa*), and β-xylosidase (*xyl*) (GenBank Accession Nos. DQ387046, DQ387047, and DQ345777, respectively).

Escherichia coli plays a prominent role as a heterologous protein production host due to extensive research efforts over the last several decades. Albeit well known and used for protein production, several intrinsic problems hamper the system's unrestrained usage. *E. coli* lacks protein export mechanisms therefore the protein produced accumulates intracellularly, mostly in the form of inactive inclusion bodies due to the high concentrations present. The presence of endotoxins and the inability to attach glycosidic residues constrict its application for pharmaceutical proteins. The Gram-positive bacterium *Bacillus megaterium* offers several advantages over *E. coli* as a protein production system. It does not possess endotoxins and has a high secretion capacity. Compared to *Bacillus subtilis* it is distinguished by higher plasmid stability and a lower intrinsic protease activity which is a significant advantage for a secretory protein production system. Important prerequisites like an efficient transformation system, stable and freely replicating plasmids and the ability to integrate heterologous genes into the genome are met.[2]

The use of a Gram-positive bacterium could facilitate protein production due to the lack of an outer membrane allowing direct secretion of proteins into the growth medium. In contrast to *B. subtilis*, *B. megaterium* does not produce alkaline proteases. Another advantage of this bacterium is the high stability of plasmids during growth, which allows a stable gene expression in long term cultivations and bioreactors. *B. megaterium* has been used for the production of several recombinant proteins, e.g. dextransucrase,[3] and lysozyme specific single chain Fv (scFv) fragment D1.3.[4] Recently, a set of free replication vectors and genetically optimized *B. megaterium* strains for the intra- and extracellular production of affinity tagged recombinant proteins were developed. They were successfully employed for the production and purification of dextransucrase,[5] levansucrase,[3] and penicillin amidase.[6]

Plasmid pHIS1525 is a shuttle vector for *E. coli*/ *B. megaterium,* containing a signal peptide for secretion of heterologous protein of interest into the culture medium. The pHIS1525 system is also equipped with a 6x His-tag sequence for purification and detection of the expressed His-tagged target proteins.[2, 3]

In this work, we reported the expression of β-xylosidase encoding gene from *G. thermoleovorans* IT-08 in the pHIS1525/ *B. megaterium* MS941 system.

MATERIALS AND METHODS

Cultures

Escherichia coli DH10β and *Bacillus megaterium* MS941 were grown in LB (Luria Bertani) medium (composition : 1% NaCl, 1% tripton, 0.5% *yeast extract*). Recombinant *E. coli* DH5α, containing xylanolytic enzyme encoding gene (pTP510) from *G. thermoleovorans* IT-08, was grown in LB medium containing ampicillin (100μg/ ml).

Chemicals

All analytical chemicals and media component used were pure grade and available commercially.

Amplification and sub-cloning

β-Xylosidase encoding gene (*xyl*) was amplified from pTP510 using a pair of primers which designed according to the sequence of β-xylosidase of *G. thermoleovorans* IT-08 (GeneBank no. DQ345777) : F_{xyl} 5'-TTATT*GAGC TC*TCGAATATTCTAACCCAG-3' and R_{xyl} : 5′−CAA GA*GCATGC*AATATTTCAGGAATATATTTAAACC-3'. The *xyl* gene was inserted into plasmid pHIS1525 and propagated in *E. coli* DH10β before transforming into the *B. megaterium*. The recombinant plasmids were analyzed by restriction analysis and sequencing. The recombinant plasmid was transformed into *B. megaterium* MS941 by protoplast transformation method in the isotonic medium containing polietilen glikol.[7] Transformants were selected by growing the recombinant *B. megaterium* MS941 on the solid LB medium containing tetracycline (10 μg/ ml).

Assay of β-xylosidase expression

The expression of the β-xylosidase gene was assayed using 0.5% agar containing 0.2% methylumbelliferyl-β-D-xyloside (MUX). Recombinant *B. megaterium* MS941 cells on LB medium (+Tet 10 μg/ml) were overlaid with suspension of phosphate buffer pH 6.0 containing 0.5% agar and 0.2% methylumbelliferyl-β-D-xyloside (MUX). The assayed Petri plates were incubated at 60^0 C over-night. The β-xylosidase expression was monitored on the UV-trasluminator.

Enzyme assay

The enzyme activity was determined by measuring the release of *p*-nitrophenol from *p*-nitrophenyl-β-D-xylopyranoside (*p*NPX). The mixture of 100 μl enzyme sample and 900 μl of 1 mM *p*NPX, was incubated at 50° C for 30 min. The reaction was stopped by adding 0,1 ml of 0,4 M Na_2CO_3 solution. The release of p-nitrophenol was measured using spectrophotometer UV-vis at λ 405 nm. 1 (one) unit of the β-xylosidase activity was defined as the amount of the enzymes releasing 1 μmol p-nitrophenol equivalent per minute under the assay condition.

Effect of xylose on β-xylosidase activity

The effect of xylose on β-xylosidase activity was studied by producing the enzyme in various concentration of xylose as an inducer and time of addition into the culture medium. The enzyme activities were determined toward *p*NPX as a subtrate. The enzyme production was carried out in 100 ml cotton plugged Erlenmeyers which contained 20 ml fresh LB medium and 1% over-night pre-culture of recombinant *B. megaterium* MS941, and cultivated 37° C 250 rpm. The concentration of xylose was varied: 0, 0.25 and 0.50% (w/v), and the time of addition was varied: 0, 1, 2, 3, and 4 hours after cultivation (OD578 of 0–0.2. The enzyme was harvested after 10 h cultivation. The supernatant was collected by centrifugation at 10,000 rpm, 4° C for 20 min. The β-xylosidase activity was determined toward *p*NPX as a substrate.

Production of recombinant β-xylosidase

The enzyme production was carried out in 500 ml cotton-plugged Erlenmeyers containing 100 ml fresh LB medium, and inoculated by 1% of pre-culture of recombinant *B. megaterium* MS941 and cultivated 37° C, 250 rpm. Expression of Xyl was induced by adding 0.5% sterile xylose at an OD578 of 0.1. The enzyme was harvested after 10 h cultivation. The supernatant I (called secreted enzyme) was collected by centrifugation at 10,000 rpm, 4° C for 10 min. The pellet cell was resuspended in citrate-phosphate buffer pH 6.0 and lysed by two passages through a ultrasonicator at 20 kHz for 60 sec. Debris cells was removed by centrifugation at 10,000 rpm, 4° C for 10 min. The supernatant II called non-secreted enzyme. The β-xylosidase activities of enzymes, both secreted and

non-secreted enzyme were determined toward pNPX as a substrate.

Effect of pH and temperature on β-xylosidase activity

The optimum pH of the crude β-xylosidase activity was determined by measuring the enzymes activity toward *p*NPX as a substrat in pH range of 5–8 at 50° C for 30 min. The optimum temperature of the crude β-xylosidase activity was determined by measuring the enzymes activity toward substrat *p*NPX in defferent temperature range 50–70° C.

Purification of recombinant β-xylosidase

The recombinant β-xylosidase (Xyl) was purified by affinity chromatography method using agarose containing Ni-NTA (*Nickel-Nitrilotriacetic Acid*). Protein was precipitated from supernatant by adding 40% saturated $(NH_4)_2SO_4$. The precipitant was collected by centrifugation at 13,000 rpm, 4° C for 10 min. The desalted protein was resuspended with buffer A (50 mM bufer fosfat pH = 8,0 + 250 mM NaCl + 5 mM imidazole + 5 mM β-merkaptoetanol), then loaded onto a column containing 1 ml Ni–NTA agarose (Qiagen) and pre-equilibrated with buffer A and incubated 2 h at cool room. After discarding the flow through, the column was washed with five column volumes of buffer A, and eluted with five column volumes of buffer B (50 mM phosphate pH 8.0, 250 mM NaCl, 100 mMimidazole and 1 mM β-mercaptoethanol). All of the fractions were analysis by SDS-PAGE method.[8]

RESULTS AND DISCUSSION

Amplification and sub-cloning

Based on the structure analysis, β-xylosidase gene was successfully amplified from pTP510 and sub-cloned in plasmid pHIS1525/ *E. coli* DH10β. The recombinant plasmid named pSMX (Fig.1).

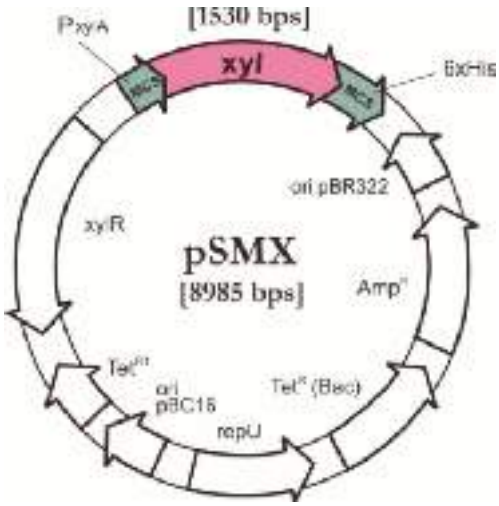

Figure 1. The pSMX map

β-xylosidase expression test

The expression of β-xylosidase in *B. megaterium* MS941 was assayed using methylumbelliferyl-β-D-xyloside (MUX) reagent. This work showed that the recombinant β-xylosidase was successfully expressed by recombinant *B. megaterium* MS941. Among 12 colonies of assayed recombinant *B. megaterium* MS941, there were two colonies (BM1 and BM3) flourescented on the UV transluminator. It was been concluded that the recombinants *B. megaterium* MS941 BM1 and BM3 expressed the β-xylosidase extracellularly. The β-xylosidases catalyse the hydrolysis of glycosidic linkage of MUX, and release xylose and umbelliferon (Fig 2).

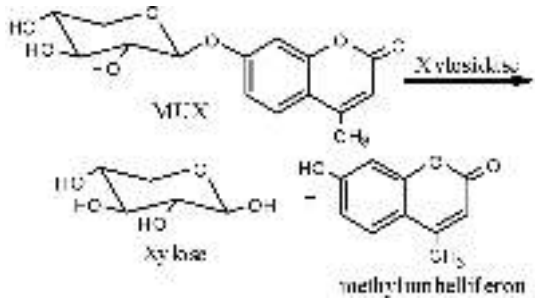

Figure 2. Hydrolysis of MUX by β-xylosidase

The effect of xylose addition

Recombinant plasmid pSMX contains *xylA* promoter (P*xylA*) and *xyl*R repressor. The expression recombinant β-xylosidase in *B. megaterium* MS941 system was xylose-inducible. The addition of 0.5% xylose into ke culture medium at OD_{578} = 0.332 could improve the growing of the recombinant *B. megaterium* MS941.

The influence of xylose concentration and the time of addition into the culture medium toward the activity of β-xylosidase, were studied. The addition of 0.25–0.50% xylose into the culture medium at 2 h cultivation (OD_{578} = ± 0,1) increased the enzyme activity by 2.0–2.74 fold. The enzyme showed an activity of 0.0343 ± 0.0022 Unit/ ml at the absence of xylose. The optimum condition to induce the β-xylosidase activity was the addition of 0.50% xylose at 2 h cultivation (Fig 3.).

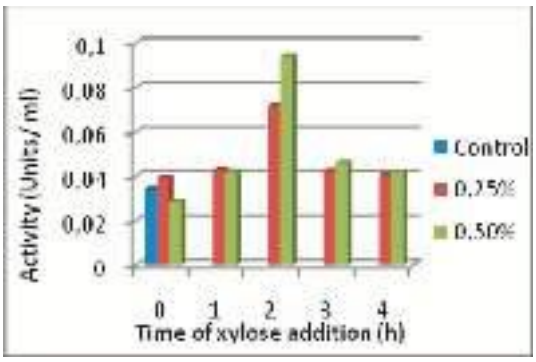

Figure 3. The effect of xylose concentration and time of addition into culture medium toward β-xylosidase activity

Effect of pH and temperature to enzyme activity

The optimum activity of the recombinant enzyme was reached at 50° C and pH 6 (Fig 4.). The crude extracts of β-xylosidase from recombinant *B. megaterium* MS941 the optimum activity at 50° C and pH 6.

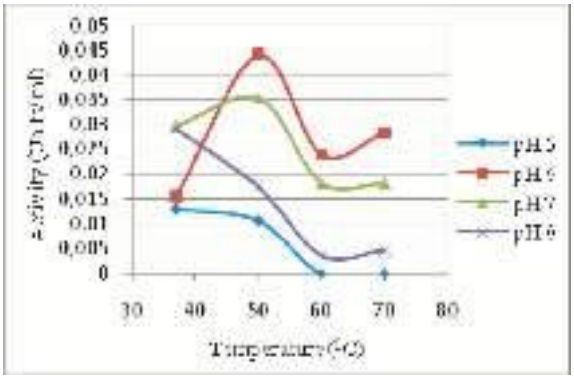

Figure 4. The enzyme activity toward *p*NPX at different pH and temperature

This condition was also reported by Puspaningsih (2005) for recombinant β-xylosidase expressed in pET-xyl/ *E. coli* BL21 DE3 [10]. However, the optimum temperature and pH optimum was different for the recombinant β-xylosidase expressed in pTP510/ *E. coli* DH5α. The enzyme had optimum activity at 70° C dan pH 5. On other hand, the β-xylosidase expressed in the origin strain, *Geobacillus thermoleovorans* IT-08, showed the optimum activity at 70° C and pH 6 This different optimum temperature and pH may be caused by the differential host and surrounding protein. The recombinant pTP510/ *E. coli* DH5α and its origin strain (*Geobacillus thermoleovorans* IT-08) expressed a xylanolytic enzyme, exo-xylanase, α-L-arabinofuranosidase, and β-xylosidase.[9]

Wagschal *et al*. (2008) has synthezed and cloned the *G. thermoleovorans* IT-08 β-xylosidase into pET29b/ *E. coli* BL21 (DE3). The recombinant β-xylosidase (GbtXyl43A) showed the optimum activity at pH 5.0 thermal atability (t1/2) 970 min at 51,2° C.[11]

Enzyme secretion

The analysis of enzyme secretion showed that the recombinant *B. megaterium* MS941 was capable to secrete 75.56% of the expressed β-xylosidase enzyme, into the culture medium. The using of *B. megaterium* MS941 with a detectable extracellular protease deleted showed the improvement the secretion of dextrasucrase [2]. One of the factors in protein secretion is the protein folding. In the production of recombinant penicillin G amidase (PGA) in *B. megaterium*, calcium ion was an important factor in protein folding and maturation. The presence of calcium ion impacted the secretion of PGA protein in *B. megaterium* MS941. The addition of 2.5 mM $CaCl_2$ into the LB medium improved the PGA secretion by 2.6 fold compared to the absence of $CaCl_2$ [6]. The preliminary study, showed that metal ions such as Mg^{++}, Mn^{++}, Zn^{++}, Ca^{++} and Fe^{++} impacted the activity of β-xylosidase from *G. thermoleovorans* IT-08. The addition of 0.5–2.5 mM metal ions improved the β-xylosidase toward substrate *p*NPX.

Purification of recombinant enzyme

The SDS-PAGE analysis showed that the protein purification revealed a pure β-xylosidase protein in fraction B2. The pure enzyme was showed by a single protein band ± 58 kDa on the electrophoregram (Fig 5.)

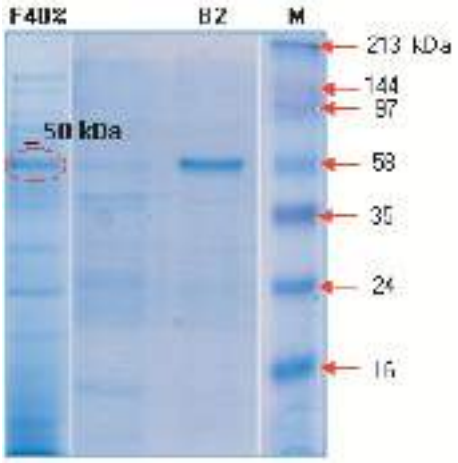

Figure 5. SDS-PAGE analyzed of pure β-xylosidase B2 = fraction 2 of eluted enzyme by buffer B M= protein penanda (16-213 kDa, *Intron Biotechnology*)

The purification of β-xylosidase by affinity chromatography using agarose coloum containing Ni-NTA revealed a pure enzyme with specific activity of 10.06 Units/mg protein, or 24.19 fold compared to the activity of supernatant.

CONCLUSION

β-xylosidase encoding gene from *G. thermoleovorans* IT-08 was succesfully expressed in pHIS1525/ *B. megaterium* MS941.

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THE ROLE OF POLYSACCHARIDE KRESTIN FROM *Coriolus versicolor* MUSHROOM ON IMMUNOGLOBULIN ISOTYPE OF MICE WHICH INFECTED BY *Mycobacterium tuberculosis*

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ABSTRACT

This research was aimed to determine the role of polysaccharide krestin (PSK) with different timing on levels and types of mice immunoglobulin (Ig) isotype which infected by Mycobacterium tuberculosis. This research used 30 adult female mice of Mus musculus strain, polysaccharide krestin was isolated from Coriolus versicolor mushroom, and for infection used Mycobacterium tuberculosis H37Rv (ATCC 27294 T) strain. Provision of polysaccharide krestin was done over 7 consecutive days via gavage. Mycobacterium tuberculosis infection was done 2 times with an interval of 1 week via intraperitoneal. Immunoglobulin isotype serums were analyzed using the ELISA test and the results were analyzed descriptively through the color reaction and OD values. The result showed the highest levels of immunoglobulin was found in the provision of PSK before and after Mycobacterium tuberculosis infection with total 6.280 of OD Ig isotype. Immunoglobulin isotype dominant was IgM with lambda light chain. The conclusion of this research was PSK increased mice Ig isotype levels at the time of provision before, after or before and after infection Mycobacterium tuberculosis. Ig isotype which was formed i.e. IgM, IgA, IgG2b, IgG3, IgG2a, IgG1 with kappa and lambda light chain.

Key words: *Polysaccharide krestin, Mycobacterium tuberculosis, immunoglobulin isotype*

INTRODUCTION

Tuberculosis (TB) is still become a serious problem in the world [12].This bacteria is divided into extracellular and intracellular bacteria[2]. Specific response against extracellular bacteria with produce antibodies by B cells. While in response against intracellular bacteria, the response that happens is the cellular immune response (T cell) [7]. However, intracellular bacteria can induce the development of T cells into Th1 cell phenotype then also can stimulate antibody production by B cells [5].

In the early formation of immunoglobulin molecules (antibodies) by B cells is stimulated by antigen [9]. In mice, the class of immunoglobulin (Ig) based on the H-chain (heavy chain) consists of IgM, IgG, IgA, IgD, and IgE. In mice, IgG consists of four subclasses i.e. IgG1, IgG2a, IgG2b, and IgG3 [23]. In addition, there are 2 types of L-chain (light chain), namely kappa (κ) and lambda (λ) [19].

Some researchers use the immunomodulator as an adjunctive therapy for tuberculosis [18]. *Coriolus versicolor* is a mushroom that commonly used in the treatment of disease. Various active components are isolated from this mushroom, both taking from fruiting bodies or culture mycelium. Active components that are important in the treatment are polysaccharide krestin (PSK) and polysaccharide peptide (PSP). Both PSK and PSP consist of active compounds named β-glucan [16]. Beta (β)-glucan plays a role to activate macrophages and stimulate B cells in the process of antibodies production [3]. Beta glucan increase the production of important *cytokines* there is interleukin-2 (IL-2) which stimulates the differentiation of B cells which are active [29] then the active B cell differentiation into plasma cells (clones plasma) which can produce immunoglobulin [30].

Looking at the capabilities of the PSK on the modulation of immune responses and saw its consumed in a long time in the community without significant side effects, the researcher wanted to investigate how the levels and kinds of immunoglobulin isotype of mice which infected by *Mycobacterium tuberculosis* on providing PSK with

different timing. *Enzyme-linked immunosorbent assay* (ELISA) became selected test for measuring the levels and kinds of immunoglobulin isotype related to the specificity of antigen[7].

METHOD

Stage in PSK isolation from *Coriolus versicolor*

Coarse powder of 200 g *Coriolus versicolor* is added with water as much as 3 l and is heated at a temperature of 80–98° C for 2 - 3 hours. Do extraction twice more with the addition of 2 l of water on the residue, the results obtained in form of supernatant from the three times extractions are ± 2 l [10]. Mushroom extract solution is filtered using Whatman No 41 filter and then its *liofilisasi* supernatant (for 150 ml for ± 24 hours). Precipitation mushroom powder extracts using ammonium sulfate 90% and then dialysis using nitrocellulose membranes for 24 hours [11].

Stage of making PSK solution

3.5 g of Ammonium sulfate is added with 50 ml aquades and 1 g of mushroom powder mixed into one. Stirer solution for 2 h at 4° C and then centrifuged 9000 rpm for 12 min at 4° C. Take the sediment and added 12 ml saline. Polysaccharide concentration is measured using the *phenol-sulfuric acid assay*. Dose of PSK that used is 500 µg[29].

Stage of provisioning PSK and *Mycobacterium tuberculosis* infection

Thirty animals are divided become 6 groups as follows

Table 1. Treatment Group

Group	Provision of PSK on 1st-7th day	*Mycobacterium tuberculosis* infection on 8th and 15th day	Provision of PSK on 23th-30th day
I	–	–	–
II	+	–	+
III	–	+	–
IV	+	+	–
V	–	+	+
VI	+	+	+

Description: (+) indicates treatment
(-) indicates no treatment, were given only aquades
I : As a control, have given only aquades
II : As a positive control, provision of PSK only
III: As a negative control, Mycobacterium tuberculosis infection only
IV : Provision of PSK before infection with Mycobacterium tuberculosis
V : Provision of PSK after infection with Mycobacterium tuberculosis
VI: Provision of PSK before and after infection with Mycobacterium tuberculosis
Mice infected with 0.5 Mc Farland or equivalent to 1.5 ×108 CFU/ml bacteria intraperitoneally.

Stage of analysis Ig isotype

Serum Ig isotype (IgG1, IgG2a, IgG2b, IgG3, IgA, Ig M, kappa and lambda chains) were analyzed with Pierce Rapid ELISA Kit Mouse Mab Isotyping. The reading of OD values by using ELISA reader at a wavelength of 450 nm[4].

RESULT

Table 2. OD Values of Ig Isotype

Kinds of Ig	*Optical Density* (OD) values of Ig					
	K	(K+)	(K-)	P1	P2	P3
IgG1	0,247	0,393	0,304	0,652	0,479	0,755
IgG2a	0,338	0,400	0,426	0,697	0,661	0,872
IgG2b	0,705	0,972	0,908	1,029	0,986	1,041
IgG3	0,414	0,780	0,573	1,000	0,654	1,047
IgA	0,322	0,968	0,953	1,108	1,015	1,155
IgM	0,909	1,286	1,349	1,335	1,420	1,410
Kappa	0,438	0,909	0,806	1,119	0,945	1,077
Lambda	0,584	1,068	1,100	1,112	1,180	1,169

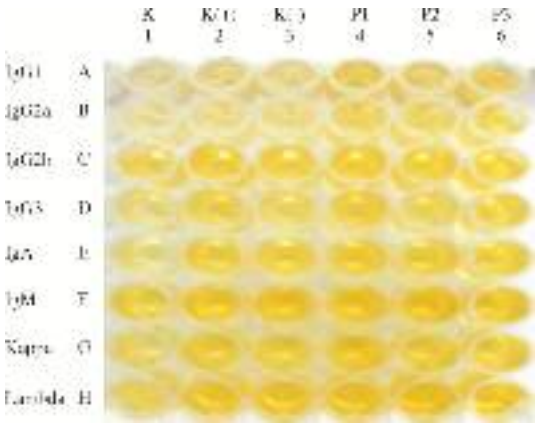

Figure 1. ELISA test for determining the kinds and levels of immunoglobulin Description: K (1A-1H) control, K (+) (2A-2H) positive control is provision of PSK only, K (-) (3A-3H) negative control with *Mycobacterium tuberculosis* infection only, P1 (4A-4H) provision of PSK before infection with *Mycobacterium tuberculosis,* P2 (5A-5H) providing PSK after infection with *Mycobacterium tuberculosis,* P3 (6A-6H) providing PSK before and after infection with *Mycobacterium tuberculosis*.

DISCUSSION

In serum (K) Although there has been color reaction, but not so striking as in serum (K+). This is because the control (K) not be immunized previously with antigens, which meant there was no previous contact with antigens. Color reaction that occurred probably due to the presence of natural antibodies in the body of mice whose concentration is low.

In (K+) provision of PSK only, it has OD values higher than (K). High concentration of immunoglobulin appropriate with the statement of Bellanti (1993), that the potential immunomodulator can increase or make higher levels of certain responses as a whole. According Vetvicka *et al.* (2002), Beta-glucan is known to increase the production of lymphocytes.

In (K-) OD value is higher than (K). This is because *Mycobaterium tuberculosis* can not make invasion of the immune system so it does not decrease the immune response. According Todar (2009), *Mycobaterium tuberculosis* can be multiply after 7–21 days early after infection and Abbas *et al.* (2000) states that the maximum antibody in the primary response can be detected in the third week after immunization. Kresno (2001) states that levels of antibody reduced later and generally only a few can be detected on 4–5 weeks after exposure.

Tuberculosis bacterial population are divided into extracellular and intracellular bacteria[2]. Immunoglobulin which produced by B cells is the major protective immune component for extracellular bacteria that can serves to get rid of microbes and neutralize the toxin [5]. In the fight against intracellular bacteria there are 2 types of reaction are occurred, i.e. The first is killing of intracellular bacteria by macrophages activated through phagocytes in which the activation of macrophages occurs through cytokines, especially IFN-γ, produced by T cells. The second way is with lysis of infected cells by $CD8^+$ T cells. Intracellular bacterial protein can stimulate $CD4^+$ T cells (through MHC class II antigens complex) or $CD8^+$ T cells (through MHC class I antigens complex). Intracellular bacteria induce T cell development into Th1 cell phenotype, because these bacteria stimulates the production of IL-12 by macrophages, and IFN-γ by NK cells, both types of these cytokines promote the development of Th1 cells ($CD4^+$). On the other hand Th1 cells produce IFN-γ which activate macrophages to produce ROI and enzymes that can kill bacteria. IFN-γ also stimulate immunoglobulin isotype production by B lymphocytes [19].

In (K-) has a lower OD value than the P1, P2 and P3. This shows PSK has a role as immunostimulator. This is consistent with the statement of Cui and Chisti (2003), Kidd (2000), and Vetvicka *et al.* (2002), that the PSK is immunostimulator or imunopotensiator.

Polysaccharide krestin contains 34–35% carbohydrate (91–93% glucan) [10]. Beta (β)-glucan is known for stimulate the immune system [24], According to Hong *et al.* (2004), Beta (β)-glucan present in the gut then make contact with macrophages that exist in the intestinal wall which is assisted by M cells (microfold) that are specialized cells and found in the ileum. M cells will take glucan through pinositosis and took it through the intestinal wall where some cells such as macrophages, T cells, B cells and other immune cells have been waiting. Beta(β)-glucan which phagocytosis by macrophages would be degraded into fragments, and then transported to a bone marrow where fragments-glucan degradation results will be released.

According to Chan *et al.,* (2009), these fragments were arrested by the complement receptor (CR3) which located at the cell surface of granulocytes, monocytes, and dendritic cells. These cells with antibodies then activated. Beta (β)-glucan will bind to macrophage on the CR3 receptor, it is combination receptor that has two binding regions. The first area is responsible for binding the type of complement, a soluble blood protein called C3 (or iC3b). C3 will be attached to the specific antibodies then bind to the targeted pathogen and do opsonisasi. The second area in CR3 binding to carbohydrate receptors on cells of yeast or fungus (PSK) that allows macrophages to recognize yeast as "nonself" [14]. From the second signal of PSK, it can help the process of phagocytosis of macrophages in tuberculosis infection.

The highest of OD value was found for the IgM isotype in all treatment groups. Immunization of *Mycobacterium tuberculosis* in live cell form and are conducted twice within an interval of one week makes the immune responses which occured is still primary immune response. According Bellanti (1993), antibodies can be detected after 10 to 14 days after injection of bacterial cells. The first meeting with the bacteria will raise primary immune response. Immune response which raised by imunogen is dominated by IgM.

OD or absorbance values with the second highest concentration in the (K-), P1, P2 and P3 is IgA. High concentration of IgA in serum according to the statement Baratawidjaja (2006) which states that high IgA levels in serum will be found in respiratory and gastrointestinal infections, like tuberculosis. This is supported by Frank (1995) which states that IgA has functions in early antiviral and antibacterial defense by preventing bacterial adhesion to the mucous membranes.

Subclass IgG2b has a higher concentration may be caused by its ability to bind antigens with a form of protein. According to Scott *et al.* (1990), IgG2a, and IgG2b in mice with IgG1, and IgG3 of human have similarities in their ability to embed complement and protein antigens. Polysaccharide krestin (PSK) is a complex polysaccharide binding protein [21] and *Mycobacterium tuberculosis* is a bacterium which contains several proteins that bind to lipids [15]. So, they make subclass of IgG26 has a higher concentration.

According to Scott *et al.* (1990), IgG3 of mice and IgG2 humans have similarity in recognition of carbohydrate epitopes. The existence of higher enough concentration of IgG3 indicated that PSK take a role in increasing the types

of that immunoglobulin. According to Robinson (1995), Beta (β)-glucan is a natural polysaccharide derivatives which having 7-10 monosaccharide units that are classified into the oligosaccharide. Monosaccharide of PSK consists of glucose (74.6%), mannose (15,5%) xylose (4.8% of), galactose (2.7% of), and high fructose (2.4% of) [28]. IgG2a and IgG1 subclass had the lowest concentration in the serum of treatment group, this probably occurred because IgG1 more capable of binding mast cells[25].

Immunoglobulin light chains are divided into two types, namely kappa light (κ) and lambda (λ) chains. According to Tizard (1987) and Bellanti (1993), the ratio between the kappa and lambda light chains are highly variable among species and their combinations are normally present in each individual. Ig isotype highest with total 6.280 is founded on providing PSK before and after infection with *Mycobacterium tuberculosis*. This indicates the role of providing PSK with different times on Ig isotype of mice which infected by *Mycobacterium tuberculosis*.

Provision of PSK before infection with Mycobacterium tuberculosis has function as prevention (preventive) that encourage to increase the number of lymphocytes formation then increases levels of immunoglobulin more optimally, so that levels of immunoglobulin against Mycobacterium tuberculosis infection will further increases and will be further improved with the provision of PSK after Mycobacterium tuberculosis infection as a treatment (curative). Polysaccharide krestin is expected to prepare and boost immunity against disease that will enter the body. Pietro (2003) states that β-glucan is more effective for prevention (preventive) and treatment (curative) of diseases in related with immune system durability.

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RISK FACTOR OF BACTEREMIA IN CHILDREN WITH PNEUMONIA

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ABSTRACT

Background: Pneumonia is known as a frequent cause of morbidity and mortality among children in developing countries. In children,it caused predominantly by bacteria. Bacteremia has been associated with severity and mortalitas of pneumonia. Identify factors caused bacteremia important to prevent severity and mortalitas of pneumonia. Objective: The objective of this study was to identify risk factors of bacteremia in children with pneumonia. Methods: A retrospective study was conducted in children with pneumonia in Dr. Soetomo Surabaya Hospital from January 2007 to December 2008. Blood cultures be performed on all of this patients. Factors associated with bacteremia were identified following review of medical records include clinical features, laboratory , radiology and blood culture results. Results: Frequency of bacteremia was 8,2% (36 patients) of 438 children with pneumonia. Interval from onset of symptoms to hospital admission more than 5 days (22.69 CI 95%), severe malnourished (OR 9.05 CI 95%), anemia (OR 2.44 CI 95%), leucocyt counts less than 5000/mm^3 and more than 20.000/mm^3 (OR 2.41 CI 95%) and paO2 less than 80 mmHg (OR 4.25 CI 95%) were at increased risk of bacteremia in children with pneumonia. Conclusion: Risk factors bacteremia in children with pneumonia included age under 1 year, symptoms more than 5 days, severe malnourished ,anemia, leucosyt counts less than 5000/mm^3 and more than 20.000/mm^3 and paO2 less than 80 mmHg.

Key words: *Risk factors, bacteremia, pneumonia*

INTRODUCTION

Pneumonia is an acute inflammatory disease of the lung parenchyma involving the distal terminal bronchioles, respiratory bronchioles, alveoli ducts, alveoli sacs, and alveoli .. This disease is one of the most common infections in the pediatric age group. In developing countries, researchers estimate that more than 150 million new cases occur annually in children < 5 years. It is well known as a frequent cause of morbidity and a leading cause of mortality among children.[1] One in five of childhood deaths in developing countries have been ascribed to acute respiratory tract infections (ARI) and 90% of these deaths are due to pneumonia.[2]

A variety of microorganisms can cause pneumonia in children-bacteria, viruses or fungi. Pneumonia in developing countries is caused predominantly by bacteria.[1] The frequency of bacteremia in pneumonia patients varies from 4% to 18%.[3] Bacteremic pneumonia are potentially life-threating in children.[4]

Predictor of bacteremia in pneumonia included recent antibiotic treatment, comorbidity disease,increase respiratory rate, temperature, pulse and laboratory abnormalities. [3]

The objective of this study was to identify risk factors of bacteremia in children with pneumonia. A retrospective study was conducted in children with pneumonia in Dr. Soetomo Surabaya Hospital from January 2007 to December 2008. Blood cultures be performed on all of this patients. Factors associated with bacteremia were identified following review of medical records include clinical features, laboratory , radiology and blood culture results. Identify factors caused bacteremia important to prevent severity and mortalitas of pneumonia

SUBJECTS AND METHODS

A retrospective study was conducted in children age 1 months until 5 years with diagnosis community acquired

pneumonia in Dr. Soetomo Surabaya Hospital from January 2007 to December 2008. This formed part of larger study investigating epidemiology study Streptococcus pneumoniae at Surabaya. The diagnosis of pneumonia in this study was made based on clinical symtomps presenting of lower respiratory tract infection. Sex, age, clinical characteristics, laboratory and radiology findings were collected by medical records review.

Patients were defined as bacteremic if a blood culture drawn of presentation to the hospital before antibiotic treatment grew an organism, and not defined as a contaminant.

From the time of admission, we noted duration of symptoms, clinical manifestation, nutritional state, laboratory and radiological findings. We classified temperature into 2 categories, less than 36,5^{o} C, or more than 38,5^{o} C and between 36.5^{o} C and 38,5^{o} C. Nutritional state were classified into 3 categories, well nourished if ideal body weight more than 90%, moderate malnutrition if ideal body weight between 70%–90% and severe malnutrition if ideal body weight less than 70%.

We collected laboratory finding includes hemoglobin level, leucocyte level, C Reactive Protein and blood gas analyse. Anemia if hemoglobin level less than 10 gr/dl. Leucocyt counts classified into less than 5000/cmm, between 5000/cmm and 20.000/cmm and more than 20.000/cmm.

X^2 tests, Fisher's exact test and odds ratio and relative risks with 95% confidence intervals were used to determine whether an association was significant ($p < 0.05$).

Table 1. Characteristic of children with Pneumonia at Dr. Soetomo Hospital, January 2007–December 2008.

Age (months) mean ± SD (range)	12.2 ± 11.2
1–12 month, n (%)	293 (66.9)
13–36 month	126 (28.8)
> 36 month	19 (4.3)
Sex ratio (male: female)	1.6: 1
Interval from onset of symptoms to hospital admission , n (%)	226 (51.6)
< 3 days	199 (45.4)
≥ 3–< 5	13(3.0)
≥ 5	
Nutritional state, n (%)	
Well nourished	281(64.2)
Moderate malnourished	150 (34.2)
Severely malnourished	7 (1.6)
Temperature, n(%)	
< 36.5 C or > 38.5 C	124 (28.3)
36.5 C – 38.5 C	314 (71.7)
Blood culture, n (%)	
Sterile	329 (75.1)
Bacteremia	36 (8.2)
Contaminant	73 (16.7)
Chest X-ray, n(%)	
Normal	60 (13.7)
Lobar consolidation	21 (4.8)
Patchy infiltrate	354 (80.8)
Pleural effusion	3 (0.7)

Table 2. Based line characteristic of children with Pneumonia at dr. Soetomo Hospital January 2007–December 2008

Variable	Bacteremia n (%)	No Bacteremia n (%)	p value
Sex			
Male	25 (5.7)	248 (56.6)	0.169
Female	11 (2.5)	154 (35.2)	
Age group (months)			
1–12 month	27 (6.2)	266 (60.7)	0.307
13–36 month	9 (2.1)	117 (26.7)	0.431
> 36 month	2 (0.5)	17 (38.8)	0.548

RESULTS

We identified 440 patients with pneumonia who met the inclusion criteria for the study. Blood culture was performed on 438 patients, bacteremia was detected in 36 patients (8.2%) and 73 patients (16.7%) had a contaminated bacteria (Table 1)

Of the total cases, ratio male and women was 1.6:1. The mean age was 12.2 months with the majority at the age of 1–12 months as many as 293 (66.9%) children. The majority interval from initial symptoms until it is brought to the hospital was less than 3 days (51.6%) children. Most nutritional status is well nourhised as much as 281 (64.2%) children.

Anemia was found in 251 (57.3%) children, levels of leukocytes less than 5000/mm3 or more than 20.000/mm3 many as 116 (26.5%) children, positive CRP was found in 329 (75.1%) of children and acidosis obtained in 75 (17.1%) children. Preview photos thoracic infiltrates was found in 354 (80.8%) children.

There were no significant difference of based line characteristic children with bacteremia positive and bacteremia negative include sex ang age. (Table 2)

Table 3 shows risk factors several variables on the occurrence of bacteremia in patients with pneumonia of children. From the table, the risk factors of pneumonia were the Interval from onset of symptoms to hospital admission more than 5 days, severely malnutrition, anemia , leukocyte less than 5000/mm^3 or more of 20.000/mm^3 and pO_2 less than 80%.

DISCUSSION

The aetiology of pneumonia in developed countries is predominantly viral, associated with a low case fatality rate, whereas in developing countries bacteraemia is common and associated with a high case fatality rate. Poor sanitation,

Table 3. Risk Factors Bacteremia of children with Pneumonia at dr. Soetomo Hospital January 2007 – December 2008

Variable	Bacteremia positive n (%)	Bcteremia negative n (%)	OR (95% CI)	p value
Interval from onset of symptoms to hospital admission , n (%)				
< 3 days	36 (8.2)	199 (43.4)	1.35(0.69-2.67)	0.386
> 3–< 5	31 (7.1)	168 (38.3)	0.97(0.48-1.97)	0.933
≥ 5	8 (1.8)	5 (1.1)	22.69(6.96-73.9)	0.000*
Nutritional state, n (%)				
Well nourished	26 (5.9)	255 (58.2)	1.52(0.71-3.23)	0.279
Moderate malnourished	8 (1.8)	142 (32.4)	0.52(0.23-1.18)	0.113
Severely malnourished	3 (0.7)	4 (0.9)	9.05(1.94-42.13)	0.001*
Temperature				
< 36.5 C or > 38.5 C	8 (1.8)	116 (26.5)	0.70(0.31-1.59)	0.397
Laboratory findings				
Hb < 10 g/dl	21(4.8)	230(52.5)	2.44(1.04-5.71)	0.034*
Leucocyte < 5,000/cmm or > 20,000/cmm	16(3.7)	100(22.8)	2.41(1.21-4.84)	0.011*
CRP positive	25(5.7)	304(69.4)	0.67(0.26-1.72)	0.404
Blood Gas Analysis				
pH < 7.35	6(1.4)	69(15.8)	0.85(0.29-2.44)	0.766
pO_2 < 80 mmHg	3(0.7)	100(22.8)	4.25(1.18-15.4)	0.018*
pCO_2 > 50 mmHg	5(1.1)	27(6.2)	0.48(0.15-1.60)	0.236
SpO_2 < 95%	7(1.6)	89(20.3)	1.19(0.27-5.31)	0.858
Chest X-ray				
Lobar consolidation	7(1.6)	14(3.2)	1.19(0.27-5.310	0.833

overcrowding, inadequate nutrition, insufficient vaccination coverage, low levels of education, and accumulation of other diseases have been suggested as reasons for the differences in aetiology and mortality. [5]

To reduce mortality from pneumonia in developing countries the problem has to be addressed from a number of aspects, social and environmental as well as medical.[5]

In this comparative study of bacteremic and non bacteremic patients,we found that bacteremia was detected in 8.2% children. The previous study reported frequency of bacteremia in patients pneumonia varies from as low as 4% to as high as 14 to 18%. [3].

We found that bacteremic and non-bacteremic pneumonia patients not differed in baseline characteristics. The study by Spooner et al from Papua New Guinea(PNG) identified firstborn children and female children to have increased bacteremia and mortality risk in children with pneumonia.[5] Meanwhile Jover et al, in research on adult patients with pneumonia, bacteremia get no difference between men and women.[6]

We have identified six independent factors of bacteremia in children with pneumonia.. Five of these significantly associated with bacteremia. Include interval from onset of symptoms to hospital admission has significantly associated with bacteremia, the Interval from onset of symptoms to hospital admission more than 5 days, severely malnutrition, anemia, leukocyte less than 5000/mm^3 or more of 20.000/mm^3 and pO_2 less than 80%. Spooner *et al.* identified that poor feeding, cyanosis, bronchial breathing, and a temperature > 38° C were all associated with bacteraemia.[5]

Interval from onset of symptoms to hospital admission more than 5 days has significantly associated with bacteremia. Previous study reported that history of fever for more than 7 days significantly increased the chance of dying.[5] The onset to hospital admission associated with prior use of antibiotic. Metersky et al,reported that the risk of bacteremia could be predicted by assessing the prior use of antibiotics.[3]

Malnourished children are particularly at risk as demonstrated in this study. A study in PNG reported that malnourished patients had a significantly higher risk of dying.[5]

In our study, bacteremic patients were more likely to be anemic than non-bacteremic patients and to have abnormal leucocyt counts and pO_2 less than 80%. Brandeburg found that bacteremic patients were more likely to have anemia, lower albumin, and elevated blood urea and serum creatinine levels.[7] While Metersky reported that lood urea nitrogen more than 30 mg/dl (11 mmol/L) , sodium less than 130 mmol/L and WBC less than 5,000/mm^3 or more than 20,000/mm^3 were independent predictors of bacteremia in community-acquired patients with pneumonia.[3]

Results of radiological findings in association with bacteraemia have been analysed. They showed that a peripheral homogeneous opacity was the best predictor of bacteraemia.[5] Jover reported that although not statistically significant, pleural effusion was more frequent in bacteremic patients.[6] In this study,the most radiological finding was infiltrate.

There are some limitations in this study. The main weakness of our study is its retrospective design. Collection of some data was therefore incomplete. Another limitation could be the lower number of non-bacteremic cases compared to bacteremic cases.

CONCLUSION

Risk factors bacteremia in children with pneumonia included age under 1 year,symptoms more than 5 days, severe malnourished ,anemia, leucosyt counts less than 5000/mm^3 and more than 20.000/mm^3 and PO_2 less than 80 mmHg.

Bacteremia has been associated with severity and mortalitas of pneumonia. Identify factors caused bacteremia important to prevent severity and mortalitas of pneumonia.

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EFFECT OF CYNAMMYLDEHYDE FROM CINNAMON EXTRACT AS A NATURAL PRESERVATIVE ALTERNATIVE TO THE GROWTH OF *Staphylococcus aureus* BACTERIA

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ABSTRACT

Food is one of the best media for the microorganism to live and grow. Therefore, food is often broken because it has been contaminated by the microorganism. In industry country, approximately 30% of population infected by food borne disease. Food borne disease is caused of phatogen bacteria food borne. Staphylococus aureus is a kind of bacteria that can make food rotten and also it is a phatogen bacteria cause food born disease, no forming spora, positive gram bacteria and the food substance which is contaminated by Staphylococus aureus will cause poisoned becaused of enterotoxin which is heat resisting. Essential oil is antimicrobial and anti bacterial that the most effective, it can inhibit the growing of microba and bacteria. One of the example of essential oil is Cinnamon.sp oil. Cinnamon oil is antimcroba agent for bacteri and fungi because it contain cynammyldehyde and cynammyl alcohol and also eugenol. The aim of this study is to understand the antimcrobacterial potential of cynammyldehyde from cinnamon extract to Staphylococus aureus. This study is laboratory experimantal research. Essential oil from Cinnamon by destilation, then redistilation was done to get cynammyldehyde from cinnamon. Cynammyldehyde was tested to Staphylococus aureus. Test method was done as dilution in the form. From this result, it show that cynammyldehide from cinnamon extract has ability in inhibit the Staphylococus aureus growth. We can conclude that Cynammaldehyde from cinnamon extract has antibacterial effect especially for positive gram bacteria that is Staphylococcus aureus. The optimum inhibiting effort is 0.09%.

Key words: *Cinnamon, Cynammyldehyde, Antibacterial, Staphylococcus aureus*

INTRODUCTION

Food is one of the medium for bacteria growth so it can break due to microorganism contamination. Microorganism can breaks components in the food into simpler compounds. It will changes, decomposition both nutrition and organoleptic.[1]

More than two million people dead because of *food borne disease*. *Food borne disease* caused by pathogenic bacteria of *food borne*. So, it need aan alternative method which eliminate pathogenic bacteria of *food borne disease*.[1] *Staphylococcus aureus* is the kind of decaying food bacteria which pathogenic bacteria of *food borne disease*, not producing spore, Gram positive bacteria and contaminant from it can be toxic because of enterotoksin.[2]

Preservation food is one of the ways to prevent food which contaminated. One of the kind of preservation food is using synthetic materials likes boraks.[3] Boraks is used by people but it has toxicity which danger if consume for along day. Recently, formalin and boraks are agent which have high reactivity so they can reacts with macromolekul on body system. Consuming formalin continuously can effet cancer. Preservation substances which can use is antimicroba and antibacterial substances.[4,5]

Essential oil is the effective antimicroba and antibacterial which can inhibit bacteri and microba growth. One of the kind of essential oil is cinnamon oil. Cinnamon oil is antimicroba to bacteria and fungi,[6] because they have cynammyldehyde, cynammyl alcohol and eugenol,[7] so cinnamon oil can inhibit pathogenic food borne bacteria growth.[8] In industrial country find about 30% population suspect food borne disease. So it need a new method to decrease and eliminate pathogenic bacteria cause of food borne disease.[1]

Laboratory experiment needed to determine the concentration of cynammyldehyde can optimally inhibit *Staphylococcus aureus* bacteria growth. The researches want cynammyldehyde of cinnamon extract can use as antibacterial to keep food quality and it can realize to society. Natural preservation of cynammyldehyde is safe to consume if in appropriate dose.

METHOD AND MATERIALS

Materials

This experiment is laboratory experimental to prove the ability antibacterial cynammyldehyde of cinnamon extract to standard laboratory bacteria such as *Staphylococcus aureus*. Using laboratory tools such as micropipette, petridisc, test tube, test tube rack, spectrophotometer, incubator, brender, and standard oase. The materials are Brain Hearth Infusion, Muller Hinton, aquades steril, Sinamat aldehid, and DMSO.

Bacterial Test

Bacterial test is standard bacteri which sensitive to standard therapy. Bacteria found in microbiologi laboratory Medicine Faculty Airlangga University.

Producing Extract

The material is cynammyldehyde of cinnamon extract. Firstly, determine cinnamon which has thickness about 1,5 mm, long about 1 m and good smell if it broken. After that, wash and dry to produce extract. Producing extract in Research Institute for Industrial Research and Standards Surabaya. Do steam destilation process to get esential oil from cinamon. The cinnamon size is reduced about ± 2 cm by 5 kg, and cinnamon was processed with a tool distiller so it can result essential oil about 5 ml. Next Essential Oil is the next process is Redestilation Oil Bath Process to separate the content of eugenol and cynammyldehyde contained in the essential oil. Bath Oil Redestilation Process is performed to obtain names of cynammyldehyde of 3 ml. The oil lab tested to know the size of the content of cynammyldehyde. In the oil we found water content of 0.03%, cinnamic names of aldehydes 72.86% 18.78% and eugenol. This will be the basis of dilution of cynammyldehyde, which will be tested to *Staphylococcus aureus*.

Preparation of Bacteria Test

Bacteria prepared by creating suspense in accordance with the methods of microbiology laboratory. Bacteria grown in BHI liquid medium, then turbidity adjusted to Mc Farland turbidity standard 0.5 (1×10^8 CFU / ml) and then diluted to concentrations of bacteria 1×10^6 CFU/ml.

Dilution Test Materials

Then performed a serial dilution: 0.18%, 0:14%, 0.10%, 0.06%, 0.02% and then added bacterial suspension with an equal volume of 1 ml so that the concentration is half that of the original, which is 0.09%, 0.07%, 0.05%, 0.03 %, and 0.01%.

Determining the Activity Test Solution

Concentrations that have been given the suspense of bacteria were incubated for 24 hours at 37°C. Furthermore, all media were incubated for 24 hours grown on Muller Hinton for 24 hours at a 37° C to determine the number of colonies that are still growing. And media that have been incubated the absorbance values read using a spectrophotometer at a wavelength of 600nm to determine the percentage of inhibition, respectively - each concentration. Using the formula:[9]

$$\% \text{ inhibition} = [(\text{abs control} - \text{abs sample})/\text{abs control}] \times 100\%$$

Then count of the colony. Each bacterial test was done 5 times. The independent variables in this study were from the names of cynammyldehyde from cinnamon extract that had been serially diluted in several concentrations. Meanwhile, as the dependent variable is the presence of bacterial growth. Data analysis was performed by descriptive statistical One Way ANOVA after the data obtained from 5 times repetition of the *Staphylococcus aureus* bacteria (gram positive).

RESULTS AND DISCUSSION

Results and Characterization of Materials

Experiments with 5 times the repetition in the concentration of 0.01%, 0.03%, 0.05%, 0.07%, 0.09% obtained by the addition of 0.18%, 0.14%. 0.10% 0.06% 0.02% with each of the levels provided and 1 ml of 1×10^6 of Staphylococus aureus bacteria cfu/ml. After incubated for 24 hours and counting the number of bacteria with the spectrophotometer giving the following results.

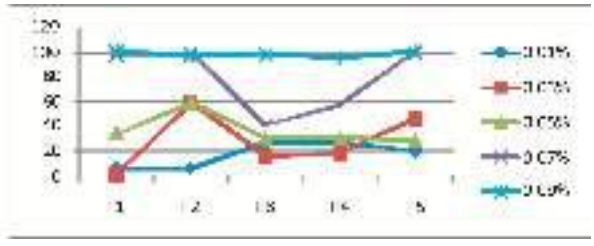

Figure 1. Graph of percentage inhibition

Then, to know the number of bacteria that live at each concentration in every experiment performed with bacterial cultures growing on Muller Hinton solid medium. One plate media in each experiment were divided into 4 sections, and each part drops 50 µl droplets of liquid medium with concentration of 0.01%, 0.03%, 0.05%, 0.07%, and 0.09%. After planting, each plate were incubated in an incubator for

24 hours to determine colony growth on each plate section. These calculations show the following results.

Table 1. Colony count results

Concentrarion of cynammyldehyde	Number of Research				
	1	2	3	4	5
Control +	∞	∞	∞	∞	∞
0.01 %	∞	∞	∞	∞	∞
0.03 %	∞	∞	∞	∞	∞
0.05 %	∞	∞	∞	∞	∞
0.07 %	6	15	8	18	27
0.09 %	4	8	5	10	0

Statistical analysis using one-way annova produces data that has been attached. In descriptive tests 0.01% concentration, the average value is 17.04%, the minimum value is 5.2%, and 27% for the maximum value. For concentration of 0.03%, the average value is 28.12%, 1.7% is the minimum value and the maximum value is 58.8%. For concentration of 0.05%, the average value is 36.56%, 29% is the minimum value, and the maximum value is 59%. For concentration of 0.07%, the average value is 78.8%, 41% is the minimum value, and the maximum value is 99.4%. For concentrations of 0.09% has an average rating of 98.0%, 95% is the minimum value, and the maximum value is 100%. It can be concluded that the highest inhibition at a concentration of 0 : 09% and the lowest at 0.01% concentration.

In the test for homogenity of variances obtained value of significance of the 0.00 ($0.00 < 0.05$ (α)). This results indicate that there are differences of the variance of the inhibition for each concentration. In Annova test showed that the value of F test is 18.945 and P value is $0.00 < 0.05$ (α), it shows that H1 is accepted which means that there is an average difference of inhibition for each concentration. In the post hoc test, it prove that there is a difference between the concentration of 0.01% with concentration of 0.07%, and 0.09%. For the concentration of 0.03%, there is a difference with the concentration of 0.07%, and 0.09%. At a concentration of 0.05%, there is a difference with the concentration of 0.09%.

The activity of Cynammyldehyde against *Staphylococcus aureus*

The results showed that cynammyldehyde from extracts of cinnamon can inhibit the growth of *Staphylococcus aureus*. This would have been due to a chemical compound as cynammyldehyde, eugenol, and alcohol in the extract of cynnamon, especially the compound of cynammyldehyde. That compounds as the active ingredient, which can inhibit growth of *Staphylococcus aureus*. It inhibited the growth of bacteria or bacterial death by an antibacterial agent can be caused by inhibition of the synthesis of cell walls, the inhibition of the cell membrane function, inhibition of protein synthesis, or inhibition of the synthesis of nucleic acids.[10]

Cynammyldehyde from cinnamon extract has the potential to inhibit cell wall synthesis. This is based on the content of cynammyldehyde that is aldehyde compounds.[11] Potential cynammyldehyde from cinnamon extract inhibits *Staphylococcus aureus* by cell wall protein agglomerate, so that the cell wall can not functionate anymore. *Staphylococcus aureus* is a gram-positive bacteria.The cell wall of Gram-positive bacteria consist of a very thick peptidoglycan that provides rigidity to maintain the integrity of the cell. Bacterial cell wall assembly process begins with the formation of peptide chains that will form the cross bridge peptide chains that incorporate glican chains from peptidoglycan to the another chain leading to complete cell wall assembly. If there is damage to the cell walls or any obstacles in its formation can occur in bacterial cell lytic which makes the bacteria lost the ability to form colonies, and it will cause bacterial cell death.

In *Staphylococcus aureus*, the delivery of antimicrobial can inhibit cell wall assembly and cause generate merger glican chain is not connected to cross the cell wall peptidoglycan, being weak structures and cause death of bacteria. Any compound that blocks any step in the synthesis of peptidoglycan will cause bacterial cell wall is weakened and cell lysis.[10] Bacterial cell lysis does not work anymore because the cell wall that maintains shape and protects the bacteria that have a high osmotic pressure. *Staphylococcus aureus* is a gram-positive bacteria that have an osmotic pressure in 3–5 times larger than gram-negative bacteria, making them more susceptible to lysis.[10] Without a cell wall, bacteria can not survive against outside influence and soon die.[12]

Therefore, the lysis of bacteria suspected of interference or inhibition of cell wall Assembly and lysis of the cell wall can explain the bacteriostatic effect of cynammyldehyde of extract of cinnamon. The use of the concentration of cynammyldehyde of different extracts of cinnamon to give different levels of influence in the growth of *Staphylococcus aureus*. At a concentration of 0.07% and 0.09% there are colonies of bacteria which grow, but less in number in comparison with the cultivated in a concentration of 0.01%, 0.03%, 0.05% and the positive control group. Bacterial growth was really inhibited at the concentrations of extract of 0.07% and 0.09%. All indicated that higher concentrations of extract of cinnamon the growth of the bacteria *Staphylococcus aureus* increasingly hampered because the active ingredient in the test solution.

Therefore, this study found that treatment with the potential to inhibit the growth of the bacteria *Staphylococcus aureus* is the initial concentration of 0.07%. In other words, the lowest concentration to inhibit the total growth of *Staphylococcus aureus* is a 0.07%, and the optimal concentrations have the potential to inhibit the growth of the bacteria *Staphylococcus aureus* is 0.09%.

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BIOCOMPATIBILITY OF AZITROMICYN ON CONNECTIVE TISSUE

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ABSTRACT

Background: *periodontal disease is commonly caused by bacteria, especially actinomyces actinomycetemcomitans and porphyromonas gingivalis have an abilty enter epithelial cells* **Objectives:** *to investigate systemic azithromycin as the antibiotic of choice for periodontal disease based on biocomptability test in connective tissue.* **Material and Methods:** *BHK 21 cell lines were exposed to 0.025%, 0.050%, 0.075%, and 0.1% azithromycin solution for seven times. Samples were put in incubator for 24 hours.* **Result:** *Azitrromycin 0.050%-0.1% showed significant difference between life cells percentage and control, however, azithromycin 0.025% revealed insignificant difference with control.* **Conclusion:** *0.025% azithromycin was considered biocompatible with connective tissue and 0.050% was not.*

Key words: *azithromycin biocomptability, connective tissue, periodontal disease*

INTRODUCTION

Until nowadays, infectious diseases still become very prominent diseases in many developing countries, including Indonesia, and a lot of effort had been done to eliminate these problems.

Periodontitis is an infectious disease caused by bacterial accumulation on tooth surface that cause inflammation, bleeding on probing, pocket formation, periodontal attachment loss, tooth mobility, and tooth lost[1].

Since it was known that most periodontal disease was caused by bacteria, the idea of antibiotics treatment was emerged, the periodontal pathogenic bacteria in the oral cavity will recolonize rapidly after scaling and root planning. The ability of Actinobacillus actinomycetemcomitans to penetrate the soft tissue make it protected from scaling and root planing.[1] This is the rationale for the need of antibiotics in the successful treatment of peridontal disease.

Some classes of antibiotics like penicillin, amoxicillin, erythromycin, azithromycin, tetracycline, metronidazole, and clyndamisin are widely used in dental treatment.[2]

Azithromycin is the latest generation of macrolides, erythromycin-derived but slightly differs in chemical compounds.[3] Azithromycin is a broad spectrum antibiotics, works effectively to gram-positive aerobic, gram-negative aerobic, anaerobes obligates such as Bacteroides fragilis, Fusobacterium sp, and Peptostreptococcus sp. Azithromycin was stated to be effective againts Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis.[4] Some researches supported this opinion by declaring that azithromycin was effective as adjuntive therapy to patients with advanced periodontitis and deep pocket.[5] Azithromycin was also effective for treating *Porphyromonas gingivalis*-involved refractory periodontitis.[1] Systemically-administered azithromycin was shown to be 4–8 times more effective. Its local usages are also expected to be more effective than erythromycin, and required a lower concentration than eritromisin.[6] In order to minimize the side effects of systemic-administered azithromycin and to make it more economical and affordable, the authors consider to prepare azythromycin as a local preparations. However its biocompatibility test needs to be carried out to determine the optimum concentration of therapeutical doses which will not harm the gingival and the surroundings tissues. Biocompatibility test can be performed in cell culture, tissue culture, or culture organ.[7] Connective tissue cells (fibroblasts) are one of cell types which is suitable for biocompatibility observation.

Connective tissue cells (fibroblasts), which is often used in cell culture techniques are the L-929 cells, from rat's lung fibroblasts derivation, and BHK-21 cells, hamster's kidney fibroblasts derivation.[8] Fibroblasts is the largest component of the pulp and periodontal ligament. In periodontal tissue, fibroblasts synthesize collagen and extracellular matrix that preserve the health of periodontal ligament.[9]

The question is whether the lower the concentration of azithromycin, the more biocompatible to the connective tissue cells? It is hypothetized that the lower the concentration of azithromycin, the more biocompatible to the connective tissue cells. The purpose of this study is to determine the biocompatibility of azithromycin at various concentrations to connective tissue, as the basis to produce local azithromycin preparations

RESEARCH METHOD

Preparation of azithromycin solution

Azithromycin powder was prepared to four different concentrations: 0.025%, 0.050%, 0.075%, and 0.1%. The powder is digitally weighed, and diluted in aqua bidestillata to reach certain concentration. These solutions were sterilized with ultra violet.

Preparation of cell culture

The monolayer cell lines of BHK 21 with Eagle's MEM medium was grown in culture bottles, then incubated with 37° for 2 × 24 hours. The goal was that cells can live and repopulate. After 2 × 24 hours, 21 BHK cells are harvested and in re-suspensed in Eagles MEM medium with approximate density 2 × 106 cells / ml. Then it was divided in 35 petri dishes, for each concentration of azithromycin and control needed seven petri dishes. Prepared azithromycin solution respectively 0.025%, 0.050%, 0.075%, and 0.1% was introduced to the petri dishes. For each concentration of azithromycin was 7 times replicated. A cell culture with Eagles MEM medium without azithromycin was used as a control. Then the petri dishes was incubated for 24 hours.

After 24 hours, Eagle's MEM was discarded, then the petri dishes were washed with 20 ml PBS (Phosphate Buffer Saline) twice to clean up the waste products of cell metabolism, only the cells would be left on the petri dishes.

In order to observe and count the changes, the cells attached to the Petri dishes ought to be removed with 0.1 ml trypsyn versene 0.25%. Eagle's MEM medium were used again to obtain cell suspension. To count the cells, 0,1 ml were taken from each suspensions, and added with 0.9 ml added tryphan blue, then brough into the hemocytometer.[7]

The results were obtained by calculating the average number of living cells and dead cells of each box. The calculation were performed with the aid of a microscope with 100 times macnification. The living cells were brightly colored, while the dead cells will absorb the blue color. This calculation were performed for all concentration of azithromycin and control groups. The calculations of each concentration obtained were compared to control group, to determine the biocompatibility azithromycin at different concentrations

RESULTS AND DATA ANALYSIS

The precentage of total cells in azithromycin administered with various concentrations after 24 hours can be seen in table 1 below (the results can be seen in appendix I)

Table 1. The average value and standard intersection number of living cells after treatment for 24 hours

Group	N	Average (%)	deviation
Control	7	100	0,00
0,025%	7	95,11	3,69
0,050%	7	94,76	3,25
0,075%	7	94,61	3,02
0,1%	7	86,23	12,18

The test results showed that after 24 hours the group with 0.025% azithromycin, had the highest percentage of living cells while the 0.1% group had the lowest.

Summary of test results can be seen in table 2 (see Annex III).

Table 2. Summary of test results t-test

KGroup	Kontrol	0,025%	0,050%	0,075%	0,1%
KCOntrol					
0,025%	-				
0,050%	X	-			
0,075%	X	-	-		
0,1%	X	-	-	-	

Description: "x" means no significant difference

From Table 2 it might be observed that treatment with Azithromycin concentration of 0.050%–0.1% showed no significant difference in the percentage of living cells to control, whereas treatment with azithromycin 0.025% showed no significant difference against control. This means that azithromycin at concentrations of 0.025% was biocompatible to connective tissue cells, whereas azithromycin concentrations above 0.050% tend not to be biocompatible.

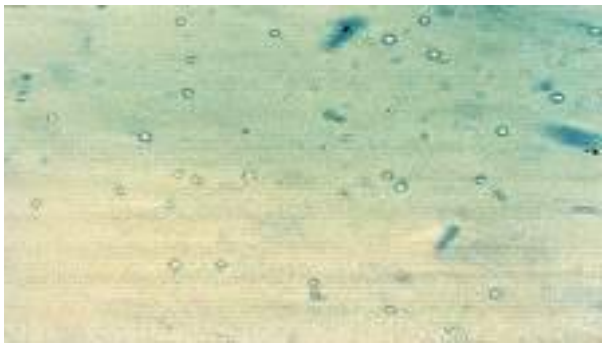

Figure 1 Image of BHK-21 cell lines when a head count by hemositometer
Description: A live cell = B = cell death

DISCUSSION

Recently, research in antibiotics therapy has developed rapidly especially as an adjuvant in aggresive periodontal treatment. Microbiology research data states that mechanical treatment (scaling and root planing) alone was not able to completely eliminate bacterial pathogens, such as Actinobacillus actinomycetemcomitans from local and subgingiva, therefore antibiotic application was considered to be effective as an adjuvant.[6]

Azithromycin is an effective antibiotic against gram-positive aerobic bacteria, gram negative, and strict anaerobes such as Bacteroides fragilis, Fusobacterium sp, Peptostreptococcus sp. In addition, azithromycin also showed good activity against Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis.[4]

Its antimicrobial activity had been proven against oral infections such as periodontitis, periodontal abscess, acute infection of the oral cavity and others. Azithromycin as adjuvant treatment, had shown to be beneficial in reducing deep pockets (> 6mm) in patients with severe periodontitis.[5]

Azithromycin was used in this study, because its broad spectrum activity and effective for the periodontal disease, especially when resistance to tetracycline and erythromycin had been developed,1 while metronidazole would be effective when combined with the other antibiotics.[3]

However, the side effects of systemic adiministered azithromycin should not be nglected. In order to minimize the side effects and increase its effectivity, a new delivery route shall be developed. Local administration of azythhromicin may be suitable for these purposes to support the periodontitis treatment. The local preparations shall not exceed the biocompatibility dose which had been proven not to harm the exposed tissues.

For these reasons we do the biocompatibility test for azithromycin against connective tissue cells (fibroblasts). Fibroblast cell was chosen as the object of exposure because it consists of 65% of gingival fibroblasts as connective tissue cells. In addition, the largest cellular component of the periodontal ligament is fibroblas.[21]

In this study using fibroblast cell cultures was prepared from Baby Hamster Kidney (BHK-21) because according to Ma'at (1999), the best culture material is derived from young tissue cells or embryonic fibroblasts and BHK-21 cells is able to grow and subcultured easily. In addition, BHK-21 fibroblast cells have been frequently used as materials in the dentistry for biocompatibility test.

The biocompatibility of azithromycin at various concentrations on fibroblast cell cultures are presented in Tables 1 and 2. It may be observed that azithromycin at concentrations more 0.050% were not biocompatible to the fibroblast cell culture. Although at a concentration of 0.050% of the average percentage of living cells is still relatively high at 94.76 ± 3.25, but it was statistically different. In azithromicyn-tretaed groups with concentrations 0.025%, 0.050%, 0.075%, and 0.1% was the average number of living cells in a row is 95.1%, 94.76%, 94.61%, and 86.23%. This shows that the higher concentration of azithromycin, the more toxic to the cells. Intensity of cell death depending on the levels of drugs that come into contact with cells, tissue or organ. Cell death increased as a result of azithromycin at a high concentration can be caused by the nature and chemical structure of azithromycin that may interfere with the living cells[15].

Azithromycin inhibits sythesis of bacterial protein at the ribosomal subunits 50 S[16]. Increased doses of chemicals and drugs have altered some vital functions of cells, which manifests as changes in homeostatic mechanisms associated with protein synthesis and cause changes in membrane permeability.[15] If the permeability change, it will cause an increase in intra-cell movement of to the extra cell, so that the it may lose metabolites necessary to preserve life. Azithromycin dissolve in water which cause it to be more easily in penetrating the cell's membrane, and may cause intra-cell disturbances and may cause cell death[16]. In this study, the dead cells absorb the blue color from blue tryphan because of the disruption in cells' membrane permeability. Azithromycin concentrations below 0.050% was shown to be biocompatible. Azithromycin toxicity was increasing as the concentration increase. This means that the drug has the potential capability to cause tissue destruction. This is in accordance with the opinion stating that all substances may be considered toxic, depend on its dosages.[20]

CONCLUSIONS AND SUGGESTIONS

Conclusion

Azithromycin was shown to be biocompatible to tissues at concentrations below 0.050%.

Suggestions

More researches shall be done to demonstrate the effectivity of azithromycin in biocompatible concentration to inhibit bacteria that cause periodontal disease before preparing local azithromycin for clinical usages.

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HEPATITIS VIRUS INFECTION IN REPEATEDLY TRANSFUSED THALASSEMIA PATIENTS

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ABSTRACT

Patients of thalassemia who are conventionally treated by a regular transfusion regimen, are at a risk of developing transfusion transmitted infections, including hepatitis. The present study was conducted to evaluate the prevalence of hepatitis virus infection in repeated transfused thalassemia patients. A total of 83 patients of thalassemia who had received at least 10 transfusions were tested for HBs Ag, anti HBs and anti-HCV using ELISA. Amongst these patients, HBs Ag, anti HBs and anti HBC were detected in 1.2%, 26.5% and 12% patients respectively. the prevalence of HBV and HCV infection were in agreement with the findings in other study.

Key words: *Thalassemia, repeated transfusion, Hepatitis viral infection*

INTRODUCTION

Thalassemias are inherited disorders of hemoglobin (Hb) synthesis. Their clinical severity widely varies, ranging from asymptomatic forms to severe or even fatal entities. Worldwide, 15 million people have clinically apparent thalassemic disorders. Reportedly, disorders worldwide, and people who carry thalassemia in India alone number approximately 30 million. These facts confirm that thalassemias are among the most common genetic disorders in humans; they are encountered among all ethnic groups and in almost every country around the world. Thalassemia major (Cooley anemia) is characterized by transfusion-dependent anemia.[1]

The management of thalassemia major essentially comprises of regular blood transfusion and a life long iron-chelation therapy. Thalassema patients are prone to develop complication such as transfusion transmitted infection particularly hepatitis virus infection.[2,3]

In developed country, prevalence of Hepatitis B infection in blood dependent patients are varies from 0.53% in Shiraz Iran to 22,5% in Palestine.[4,5] Meanwhile Hepatitis C infection prevalence varies from 15.7% to 37.9%.[3–5]

In case of hepatitis B, since an effective vaccine is available, immunization against this virus before transfusion management is started would effectively protect against transfusion transmitted hepatitis B. However, since no such vaccine is so far available against hepatitis C, the only effective protective measure against this virus is provision of HCV negative blood for transfusion. Therefore, screening of transfused blood for HCV in not only mandatory, but also it is essential to use the most sensitive screening methods with least possible false-negative results.

The aim of this study was to look into the prevalence of Hepatitis virus infection in repeated transfused thalassemia major patients in our setup.

PATIENTS AND METHODS

This study was conducted at Hematology Oncology Outpatient Clinic, department of Pediatric, dr Soetomo Hospital Surabaya from June to November 2009. A total of 83 cases of Thalassemia that have been followed up routinely and had been transfused, as a part of their management, irrespective of their age, sex, and history of jaundice were included in this study. A detailed clinical data was noted included age, interval of transfusion, Hemoglobin level and Hepatitis B immunization status.

All the patients who met the inclusion criteria tested for HBs Ag, anti-HBs and anti-HBC using ELISA. Informed consent was taken for each patient involved. About five ml of patient's blood sample was collected by a clean venepuncture. Positive result of HBs Ag was

considered as Hepatitis B infection and anti-HCV positive was considered as Hepatitis C infection. The result was reported descriptively and expressed as mean ± standart deviation (SD).

RESULT

In a total of 83 patients of thalassemia enrolled the study, 49 were males and 34 were females. The age at the time of this study ranged between 2 yrs and 18 yrs with a mean age of 10.6 yrs. The interval between transfusions varied between 2 to 6 weeks in different patients with Hemoglobin level ranged between 4,4 – 12 g/dL with mean 7,62 g/dL.

HBs Ag were detected in 1 (1,2%) patient and Anti HBs antibodies were detected also in 22 (26,5%) patients. Eleven (13%) of patients have no history of Hepatitis B immunization. Anti-HCV antibodies were detected in 10 (12%) of patients. All of these patients have no history of jaundice and clinical evidence of hepatitis viral infection before entered the study. The characteristic of patients were summarized in tabel 1.

Tabel 1. Characteristic of Patients with Non Hepatitis Infection, HBV Infection and HCV Infection

	Non Hepatitis infection (n= 72)	HBV infection (n=1)	HCV Infection (n=10)
Sex			
M	44 (61.1%)	1	6 (60%)
F	28 (38.9%)	-	4 (40%)
Age (years)	10.6 ± 3.6	8	10.8 ± 4.3
Interval of transfusion (weeks)	3–6	4	2–6
History of HBV vaksinasion			
Yes	41 (56.9%)	-	1 (100%)
No	31 (43.1%)	1	-
Hemoglobine level (g/dL)	7.6 ± 2.4	8.9	8.1 ± 2.6

DISCUSSION

Patients with severe thalassemia require medical treatment, and a blood transfusion regimen was the first measure effective in prolonging life. In the process of experimenting with blood transfusion, it was found to provide patients with many benefits, including reversal of the complications of anemia, elimination of ineffective erythropoiesis and its complications, allowance of normal or near-normal growth and development, and extension of patients' life spans. Blood transfusion should be initiated at an early age when the child is symptomatic and after an initial period of observation to assess whether the child can maintain an acceptable level of Hb without transfusion.[1]

The major complications of blood transfusions are those related to transmission of infectious agents, especially HCV, HBV and HIV infections.[1,2] In this study prevalence of HBV infection was 1.2%. Among developed country, study in Iranian patients showed the prevalence varied from 0.53–6% [4,6], but it is lower than in Palestine patients which revealed 22.5% of the blood transfusion dependent patients. [5] Report from England in 1991–1997 showed that the prevalence of Hepatitis B infection associated with transfusion was 0.57%.[7]

HBV infection can be prevented by a immunization. Although 11 (13%) of our patients have no history of HBV infection, only 1 or 83 Thalassemia patients in this study has HBs Ag positive. Means, screening of HBs Ag done by Indonesian Red Croos was effective to prevent Hepatitis infection in the transfusion dependent patients.

HCV infection has gained importance particularly as one of the major complications in multiply transfused patients during the last decade. This is especially true for counties where HCV is more prevalent in general population and therefore also amongst blood donors. The prevalence of HCV seropositivity in multiply transfused β-thalassemia patients has been observed to vary greatly, varies from 15.7% to 37.9%.[3–5] But study by Younus resulted a high prevalence of HCV seropositivity (42%).[2] In our study, Anti-HCV antibodies were detected in 10 (12%) of patients, which was lower than previous study in developed country.

Although Indonesian Red Croos' screening of HBV and HCV infection was effective and the prevalence of HBV and HCV infection were in agreement with the findings in other study, serious attempts have to be made to ensure a safe blood transfusion, so as to cut down the prevalence of HCV hepatitis in multiply transfused thalassemic patients. Education regarding transfusion transmitted infections, including HCV, HBV & HIV infections, is of prime importance.

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BASIC MECHANISM OF HYPERBARIC OXYGEN IN INFECTIOUS DISEASE

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ABSTRACT

Hyperbaric oxygen therapy (HBOT) is the inhalation of 100 percent oxygen inside a hyperbaric chamber that is pressurized to greater than 1 atmosphere (atm). HBOT causes both mechanical and physiologic effects by inducing a state of increased pressure and hyperoxia. HBOT is typically administered at 1 to 3 atm. While the duration of an HBOT session is typically 90 to 120 minutes, the duration, frequency, and cumulative number of sessions have not been standardized. HBO has been use widely in treating gangrene diabetic, stroke, osteomyelitis and accelerating wound healing. The use of HBO in infectious disease is wide, so the mechanism of hyperbaric oxygen in infectious disease should be well-understand. This understanding could bring the proper and wise management of infectious disease and to prevent the side effect of each therapy.

Key words: *HBO, infectious disease, mechanism, proper and wise mechanism*

INTRODUCTION

This review would discuss the basic mechanism of action of hyperbaric oxygen in infectious disease. It will present the evidence for the bacteriostatic and bactericidal effect of hyperoxia and hyperbaric oxygen on microbial organisms in vitro and in in vivo model of infections. It will also examine the effect of oxygen on the activity of antimicrobial agent and on the function of immune defense mechanisms.

Regulation of Oxygen Delivery to Tissues

Tissue oxygen tensions are effected mainly by the concentration on inspired oxygen , cardiac output, local blood flow, cellular metabolism and substrate availability. (Kehrer JP *et al*, 1990; Sheffield PJ, 1988; Silver IA, 1984). Different partial pressures of oxygen (pO_2) are normally found in various body compartment. The pO_2s may the oven lower. In bacterial osteomyelitis, the pO_2 range from approximately 100 mm Hg within pulmonary alveoli to 15 mm Hg in the liver parenchymal cell. In traumatized or septic tissues, pO_2s may be even lower. In bacterial osteomyelitis, the pO_2s of bone is lowered by 50%; in experimental abscesses pO_2s may measure as low as 0 mm Hg (Hays RC, Mandell GL, 1974) within individual cells, pO_2s are heterogeneous and are much lower than extracellular pO_2s. For example, pO_2s in mitochondria are less than 1 mm Hg (Wilson DF, Erecinska M, 1984).

Normoxia (15%–21%) is defined in this review as the fractional inspired oxygen (FIO_2) concentration necessary to maintain aerobic metabolism and homeostasis in the body. Oxygen tensions outside this normal range will be devined as follows: Anaerob (less than 0.01% O_2), hypoxia (12% O_2 or less), hyperoxia (45%–100% O_2), and hyperbaric oxygen (any O_2 tension greater than I atmosphere absolute pressure or 760 mm Hg).

General Mechanism of Action of Oxygen in Infections

Hyperoxia and hyperbaric oxygen (HBO) increase oxygen tensions in tissue to levels which inhibit microbial growth by inhibiting various microbial metabolic reactions. Hyperoxia and HBO by themselves also exert direct bacteriostatic and bactericidal effects on selected microorganisms because of increased generation of reactive oxygen species or free radical (Jamieson D et al, 1986; Raffin TA et al, 1977). Free radicals are lethal for microorganisms that ether lack or possess limited antioxidant defenses. HBO is a unique antibacterial agent.

At doses used clinically, HBO is usually bacteriostatic. Not all doses of HBO have an antibacterial effect. The use of HBO at pressures of 1.5 ATA or less promotes the growth of aerobic bacteria in vitro (Olodart RM, 1966).

Hyperbaric oxygen also raises oxygen tensions in hypoxic tissue to levels necessary for the killing of bacteria by neutrophils (Mader JT et. Al, 1980). While phagocytosis remains unaffected by low oxygen tensions (Karnovsky ML, 1968) killing of microorganisms by the oxidative burst is dependent on oxygen tensions. (Babior BM, 1978; Beaman L *et al*, 1984; Hasset DJ, Cohen MS, 1989) polymorphonuclear leukocytes (PMNs) from patients with chronic granulomatous disease lack the enzyme NADPH-oxidase necessary for oxygen –dependent killing of such pathogenic bacteria as *Pseudomonas aeruginosa and Staphylococces aereus* (Mandell GL, Hook EW, 1969).

Hyperoxia and HBO also influence the activity of selected antimicrobial agens belonging to the following categories antimetabolites, protein synthesis inhibitors and reduction – oxidation cycling agent. Oxygen tensions also influence the pharmacokinetics of antimicrobial agent. For example, hypoxemia (PaO_2-32mm Hg) prolongs (2-fold) the serum half –life of aminoglycosides hypoxemia effects both the absorption from muscle as well as the climination of these antimicrobials (Miphij MJ *et al*, 1978).

Hyperbaric oxygen can also effect the outcome of infections indirectly by influencing tissue repair and regeneration responses in infected necrotic tissue.for example, hypoxia (12% O_2, 1 ATA) retards healing of skins wounds and thus probably favors bacterial growth (Knighton DR *et al*, 1986) hyperbaric oxygen (100% O_2, 2 ATA, 2t, twice daily)does not effect the healing of vascularized, full-thickness skin wound , but enhances wound closure in ischemic wound (Kivisaari J, Niinikoski J, 1975).

Significantly decreased in uninfected bone of rabbits after exposure to HBO (100% O_2, 2ATA) (Stelner B *et al*, 1984) the hemodynamic changes induced by hyperbaric oxygen may be the results of increased oxygen delivery of tissue. It is also possible that negative inotropic effect on myocardium play a role in these changes. As for as can be judged from work with a model of antibiotic – controled pepsis , the presence of sepsis per se does not cause may hemodynamic changes during exposure to HBO (Muhvich KH, 1986).

Susceptibility of anaerobic and aerobic bacteria to HBO

Pathogenic bacteria are classified in terms of the partial pressure of oxygen in which they grow. By definition , anaerobic bacteria can not survive in normal oxygen tensions because they lack antioxidant defenses. As such they are very susceptible HBO . for example, hyperbaric oxygen (3 ATA for 18 hours) is completely bactericidal for *Clostridium perfringens* in vitro (Hill GB, Osterhaut S, 1972) however, there are differences in susceptibility to oxygen among Clostridium spesies. Hyperbaric oxygen (100% O_2, 2 ATA) block the germination of *C. perfringens* spores in vitro, but is not bactericidal for the spores (Demello FJ *et al*, 1970) facultative anaerobic bacteria are able to grow in normoxia hyperoxia by increasing the synthesis of antioxidant enzymes (Gregory EM, Fridovich I, 1973).

The growth of some aerobic bacteria is enhanced by hyperoxia, but is inhibited by HBO. For example, oxygen tentions upo to 1 ATA enhance the growth of *Escherichia coli*, whereas oxygen tensions greater than 2 ATA inhibit growth in vitro. (Olodart RM, 1966) hyperoxia (100% O_2, 1 ATA) enhances the the growth of *P. aerogenesa* in vitro (Park MK et. Al, 1991); hyperoxia (0.2 ATA to 0.87 ATA) enhances the growth of *Corynebacterium diphtheriae* in vitro (Gottlieb SF *et al,* 1974).

Prolonged in vitro exposure to oxygen tensions greather than 1.5 ATA inhibith the growth of several aerobic and facultative anaerobic bacteria. Hyperbaric oxygen (greather than 1.5 ATA) is bacteriostatic for *E. coli* (Bochme DE *et al*, 1976; Brown OR, 1972) *P. aerugenesa*, (Bornside GH *et al*, 1975, *C. diphtheriae, Lactobacillus casei*, (Gottlieb SF, 1979) and Vibrio anguillarum (Keck PE *et al*, 1980) however, a 1 hour intermittent exposure to HBO (100% O_2, 2 ATA every 8 hours) has no effect on the growth of *Pherugenosa* or *S. aureus* (Brown GL *et al*, 1979) prolonged in vitro hyperbaric oxygen exposure (2.9 ATA O_2, 24 hours) is also bacteriostatic for the following enteric bacteria *Salmonella thyposa*, *S. schottmuelleri, S. paratyphi, shigella dysenteriae, S.flexneri,* and *Proteus vulgaris* (Bornside *et al*, 1975) the growth of *Strepstococcus (enterococcus) faeculis* is partially in habited by 2.9 ATA O_2 however, an alpha hemolytic strain of streptococcus is not inhibited by HBO (Gottlieb SF, 1979) possibly because of the presebse of a hyaluronic acid-containing capsule (Cleary PP, Larkin A, 1979).

Hyperbaric oxygen is bactericidal for aerobic and facultative anaerobic bacteria usually only at pressures and/ or durations which are greather than can be used clinically. For example, HBO is bactericidal for *P.aeroginosa , Proteus vulgaris , and S. typhosa.* at 3 ATA for 24 hours and for E. coli at 20 ATA when treated for 6 hours (Bornside *et al*, 1975).

Mechanisms of Bacteriostatic Effect of HBO

HBO inhibits the growth of aerobic facultative anaerobic bacteria by inducing a variety of metabolic effect involved with the syntesis of proteins. Nucleic acids and essential cofactors metabolic reactions: membrane transport function are also effected. These effects where achieved with the use of hyperbaric oxygen in viyo.

Inhibition of Amino Acid and Protein Biosyntesis

Exposure of E. coli to hyperbaric oxygen (100% O_2 at greater than 3 ATA) causes a rapid inhibition of growth and respiration (Brown OR, 1972) the inhibitory effect HBO are most likely caused by free radicals and other reactive oxygen based molecules, because hyperoxia (100% O_2, 1 ATA) inhibits growth of a superoxide dismutase-deficient double mutant of E.coli (sod A sod B) (Carlioz A, Touati D, 1986) free radicals probably

inactivate a bacterial enzyme (dihydroxyacid dehyoratase) involved in amino acid biosynthesis (Brown OR, 1975). Dihydroxyacid dehydratase catalizes the formation of alpha-ketoisovalerate, an intermediate in the formation of valine and leucine. Hyperbaric oxygen (100% O_2 4.2 ATA) deceases the specific activity of dihydroxyacid dehydratase by 78 % (Brown OR, 1975). The inhibition of amino acid biosynthesis by HBO eventually leads to increase level of tRNA, which is responsible for inducing stringency response.The stringency response is characterized by increased level of tetra- and penta- phosphorylated guanosine which inhibit bacterial carbohydrate, lipid and nucleotide synthesis and enhance proteolysis (Cashel M, 1975). The end result is cessation of bacterial growth.

The inhibition by HBO of protein synthesis in bacteria may also be caused by free radical- induced block in the transport of substrates use in RNA transcription. Hyperoxia or the superoxide anion free radical inhibit the transport of lactose, guanosine and methylglycopyranoside in to E.coli (Forman HJ *et al*, 1982). Hyperoxia also inhibit the transport of protons and the synthesis of ATP in bacterial membranes (Wilson DM *et al*, 1976). However it appears that the growth inhibition caused by HBO begins long before a drop in ATP level occurs (Mathis RR, 1976). The mechanism of decreased transport cused by HBO is thought to be the oxidation of sulfhydryl-containing protein involved in transport of metabolic substrates. Free radicals are able to inactivate other bacterial proteins with key enzymetic function by oxidizing sulfhydryl-containing animo acids such as metionine play a key role in defending against this type of oxidative damage to proteins (Brot N *et al*, 1981).

Decreased Levels of Key Cofactors of Metabolic Reactions

Hyperbaric oxygen also inhibits bacterial growth by decreasing the levels of thiamine and of both the reduced and oxidiced forms of nicotinamide adenine dinnucleootide (NAD, NADH) (Brown OR, 1983) thiamine pyrophosphate is an essentral coenzyme in carbohydrate metabolism and NADPH production; NADPH is a critical cofactor in a wide range of metabolic reactions. The mechanism of the decrease in NAD is in inhibition of the de nooa NAD syntesis pathway and possibly also and increase in catabolism of NAD (Gardner PR, 1990).

Decreased Synthesis in Increased Degradation of DNA and RNA

Hyperbaric oxygen can also inhibit bacterial growth by directly blocking RNA transcription and DNA syntesis, for example, HBO (4,2 ATA) inhibits RNA transcription and DNA systhensis in both stringent and relaxed strains of E. coli after a 30 minute exposure (Brown OR, 1983).

Electron microscopic studies show ultrastructural evidence of degradation of nucleic acids and ribosomal proteins in *P.aeruginosa*, after bacteriostasis induced by prolonged exposure to HBO (100% O_2 ,2.9 ATA) for 24 hours (Clark JM, 1971). *P. aerugioesa* undergo market changes in morphologic appearance when exposed to oxygen at pressures that do not induce bacteriostasis (100% O_2 ,2 ATA). These abnormal shape changes are reversible (Kenward MA *et al*, 1980).

Another important mechanism of oxygen-induced toxicity to bacteria is via injury to DNA. Production of to superoxide anion in vitro and in vivo has been linked to mutations in bacteria, HBO is mutagenic induced the reversion of a tryptophan auxotroph (E.coli WP 2 hcr) to prototrophy. Paraquat toxicity for E. coli is in large part due to superoxide radical production (Hassan and Fridovich, 1978). Paraquat is highly mutagenic for two strains of S. typhimurium (Moody and Hassan, 1982). Both base-pair substitution and frameshift mutations were noted in DNA from the *Salmonella* strains. cell containing high levels of SOD are more resistant to toxicity and mutagenecity than cell containing normal levels of this enzyme.

From a quantitative standpoint, an important cellular source of superoxide is the nonenzymatic oxidation of cytochrome intermediates of the electron transport chain in mitochondria.

Superoxide is also generated by the cytochrome-P-450 substrate-oxygen complexes in the endoplasmic reticulum. Another cellular organelle producing tonic oxygen spesies is the peroxisome. Here H_2O_2 production occurs by oxidation of substrates such as long chain fatty acids. In all these cellular organelles, the generation of tonic oxygen spesies is dependent on tissue oxygen tensions (Turrens JF *et al*, 1982) Xantine oxidase is a major source of O_2 in ischemic and hypoxic tissue that undergo re- oxygenetation by blood reflow (McCord JM,1985). In summary, the presence of an adequate amound of molecular oxygen is necessary for oxygen- dependent killing by PMNs and macrophages to occur. A variety of enzymatic and nonenzymatic celluler reactions also normally result in the production of O_2 and H_2O_2. The production of these molecules is enhanced by increasing tissue oxygen tensions. Free radicals are highly reactive and if not removed by scavengers, may cause extensive cellular injury.

Bacterial Defense Mechanism Against Free Radicals

For protection against the free radicals generated during normal aerobic metabolism, cells have developed antioxidant defence mechanism. Three main antioxidant enzymes are known. Superoxide dismutase (SOD) is an extremely efficient O_2 (GSH peroxidase) catalyzes the reduction of hydrogen peroxide to water and dioxygen, and is capable of converting tonic lipid peroxides into nontonic products.

Superoxide onion may undergo spontaneous dismutation to form hydrogen peroxygen. The rate of reaction is enhanced markedly by the presence of superoxide dismutase (SOD). Dismutation of two O_2 radicals results in the formation of one hydrogen peroxide molecule. Catalase subsequently converts hydrogen peroxide to water and oxygen. The role of catalase is probably more important during hyperoxic conditions than in normoxic conditions. In the presence of trace amount of transition metals ,

particularly iron, hydrogen peroxide may participate in the Fenton reaction. This reaction serves to produce the highly reactgive OH. Radical, removal of H_2O_2 by catalase is important in order to prevent lipid peroxidation of membranes by OH.

Free radicals may also be inactivated by reacting with low molecular weight substances located in the cellular membranes or in the cytosol. Tocopherol (Vitamin E) is in antioxidant located in membranes. Ascorbate, beta-carotene and sulfhydryl-containing compound such as cysteine, cysteamine and gluthatione are water soluble antioxidant compound. Inder normal metabolic conditions, these free radical cellular injury. However, if host devense mechanism atre overwhelmed, damage to eukaryotic cells as well as procarotic cells will occur. (Freeman BA, 1982).

It is clear that primary mechanism of toxicity of HBO for eukaryotic cells and for microorganism is through the generation of free radicals, and other tonic oxygen spesies. Mammalian cells have various antioxidant defense and utilize free radical reactions for bacterial killing. Augmentation of endogenous host antioxidant defenses may permit use of higher doses of HBO than are currently possible in the treatment of infectious disease states. One of the rationales for using hyperbaric in infections is the potential to exploit the enhanced of selected microorganism to tonic oxygen molecules.

Role of Superoxide and Hydrogen peroxide in Bacterial Killing by Hyperoxia and hyperbaric oxygen

The superoxide anion radical appears to be particularly important in bacterial killing (Gregory EM, 1974) several in vitro studies have shown that the absence of the enzyme responsible for the detoxification of O_2, namely superoxide dismutase (SOD), increases the susceptibility of many anaerobic and facultative anaerobic bacteria to oxygen (McCord JM, 1971) on the other hand , by raising bacterial levels of SOD , the susceptibility of the bacteria to oxygen can be diminished in vitro. For example, SOD levels in *B. fragilis* can be raised 5-fold by exposure to 2% O_2 (Privale CT, 1979). The increased SOD activity markedly reduces killing of these bacteria by HBO (Gregory EM, 1973) killing of *S. sanguis* can also be prevented by increasing SOD activity : dimenthylsulfoxide (apermeable OH. Scavenger) does not protect againt free radical toxicity (DiGuiseppi J, Fridrovich I, 1982) studies with SOD and catalase devicient mutants of E. coli confirm that SOD is more important than catalase in protecting against the growth inhibition caused by hyperoxia (Schellhorn HE, Hassan HM, 1988).

In some strains of bacteria such as L. plantarum high levels of Mn^{2+} appear to be an effective substitute for SOD in protecting against the toxic effect of O_2. other bacteria such as N. gonorrhoeace are particulary susceptible to a different toxic oxygen spesies, namely H_2O_2. in these bacteria resistance to oxygen induced killing is associated with high levels of catalase, the enzyme responsible for detoxification of H_2O_2 . Aditional antioxidant defence such as peroxidase and high levels glutathione also contribute to survival of these bacterial in aerobic conditions (Archibald FS, Duong MN, 1986).

Work done by Beaman et al,(1985) has shown that surface associated SOD and high levels of catalase in *Nocardia asteroides* act together to resist oxygen dependent microbicidal activity of human PMNs. Microoganism with adequate antioxidant defenses are resistant to toxic actions of O2 and may use the production of toxic oxygen spesies to injure host cells for example, virulent strains of listeria monocylogenis exhibit maximal production of H_2O_2 and O_2. Virulence is correlate with survival of *Listeria monocytogenesis* in macrophage monolayers. The exogeneous H_2O_2 damage macrophages. An avirulent strain of *L. monocytogenes* does not release H_2O_2 or O_2 in significant amounts (Godfrey RW, Wilder MS, 1985).

It is not clear if damage to bacterial cytoplasmic membrane caused by HBO is significant enough to be considered an important mechanism of HBO induced killing. In the case of E. coli, very few broken cells and no evidence of membrane lipid peroxidation are seen after the bacterial have been killed by HBO in vivo (Harley JB *et al*, 1981). However the presence of a capsule appears to protect bacteria against oxygen-induced damage, in the case of *Streptococcus pyogenes* the presence of a hyaluronic acid capsule increases resistance to the bacteriostatic effect of oxygen. Removal of the capsule from an encapsulated St*reptococcus* strain using hyaluronidase digestion increases susceptibility of this bacterium to the toxic effect of oxygen (Cleary PP, Larkin A, 1979).

Genetic Mechanism of Bacterial Resistance to Oxygen

Two regulatory genes responsible for the increased resistance of bacteria to hyperoxia have been indentified and are known as the soxR and oxyR regulons. Hyperoxia and superoxide induce the synthesis of 30 proteins; approximately 20 of these proteins are regulated by the soxR or the osyR regulons. (Christman MF *et al*, 1985; Greenberg JT *et al*, 1990; Storz G *et al*, 1990; Walkup LKB, Kogama T, 1989). Many of these bacterial proteins are enzymes involved in detoxification of free radicals and repair of free radicals damage; example are SOD , endonuclease IV, and glucose 6-phosphate dehydrogenase (Greenberg JT *et al*, 1990; Tsavena JR, Weiss B, 1990). Example of these proteins include the antioxidant enzymes hydroperoxidase 1 catalase. NAD(P)H- dependent alkyl hydroperoside reductase, and glutathione reductase, exposure to toxic oxygen species induces the synthesis of several other protective proteins whose specific identify remains to be characterized (Christman MF *et al*, 1985; Demple B, halbrook j, 1983; Storz G *et al*, 1990).

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