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Research Report

COMPARATIVE STUDY ON THE INTENSITY OF *Mycobacterium leprae* EXPOSURE BETWEEN HOUSEHOLD AND NON-HOUSEHOLD CONTACT OF LEPROSY

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ABSTRACT

Leprosy stills a public health problem in West Sulawesi which has a Case Detection Rate (CDR) around 43.69/100.000 population. Household contacts of leprosy are a high risk group to be infected, due to droplet infection mode of transmission of the disease. A nose swab examination and serological study was conducted to detect exposure of M. leprae of people who live in leprosy endemic area. Detection of M. leprae in the nasal cavity will represent the exposure rate from outside and the measurement of specific antibody is represented the result of exposure to the immune system. Two group of inhabitants (30 household contacts of leprosy and 30 non-household contacts) were involved in the study. They live in Banggae district, a leprosy endemic area of Majene Regency, West Sulawesi. Sixty nose swab samples and sixty capillary blood samples from the same invidividuals of the two groups were collected and sent to Leprosy laboratory of the Institute of Tropical Disease, Airlangga University Surabaya. A Polymerase Chain Reaction (PCR) was performed to the nose swab samples for detection of M. leprae. The blood samples were examined serologically to measure the level of anti PGL-1 antibody. PCR examination of nose swab samples showed 1/30 positive result in the household contact group and also 1/30 positive result in non-household contact of leprosy (statistically no significant difference, $p > 0.05$). Serological study showed higher sero-positive result in the household contact group (15/30 or 50%) compared to non-household contact (11/30 or 36%), but statistical calculation revealed no significant difference between the two groups ($p > 0.05$) on sero-positive results of leprosy. It is concluded that household and non-household contact in leprosy have the same risk to be affected by the disease. The term of household and non-household contact need to be redefined. The possible role of exposure from the environment was also discussed, especially from non-human resource of M. leprae.

***Key words*:** *leprosy – sero epidemiology – PGL-1 antibody*

INTRODUCTION

Leprosy is a chronic infectious disease caused by *M. leprae* and primarily affect the peripheral nerves, secondary to skin and other organs. The complication of the disease can cause some disabilities and social problem in the community. Close contact is one condition that increased the risk of transmission. From several contact surveys, it is reported that more leprosy patients found and live in the same house, indicates that household member of leprosy patient is a high risk group for affected the disease.[1] Droplet infection mode of transmission seems the main route of transmission.[2] After the lepra bacilli enter the body, the immune response will be induced to eradicate the microorganism. Specific antibody to *M. leprae,* the anti Phenolic Glycolipid-1 (PGL-1) antibody is also developed. The level of antibody is correlated with the antigen load of the bacilli, which means that level of antibody is represented the amount of *M. leprae* in the body.[3] From this point of view, the intensity of *M. leprae* exposures to individual could be measured by examining the presence of *M. leprae* in the nasal cavity and study the level of specific antibody on the bacilli.

AIM OF STUDY

The aim of this study is to compare the intensity of *M. leprae* exposure between the healthy household contacts group and the non-household contacts of leprosy, by detection of *M. leprae* in the nasal cavity and measurement the specific antibody to *M. leprae* of the same individuals as an immunologic response to the infection.

MATERIAL AND METHOD

Sixty adult healthy individuals from Banggae subdistrict, Majene, West Sulawesi, (figure 1) consisted of 30 household contacts of leprosy patients (live in the same house with the leprosy patients) and 30 non-household contacts were involved in the study.

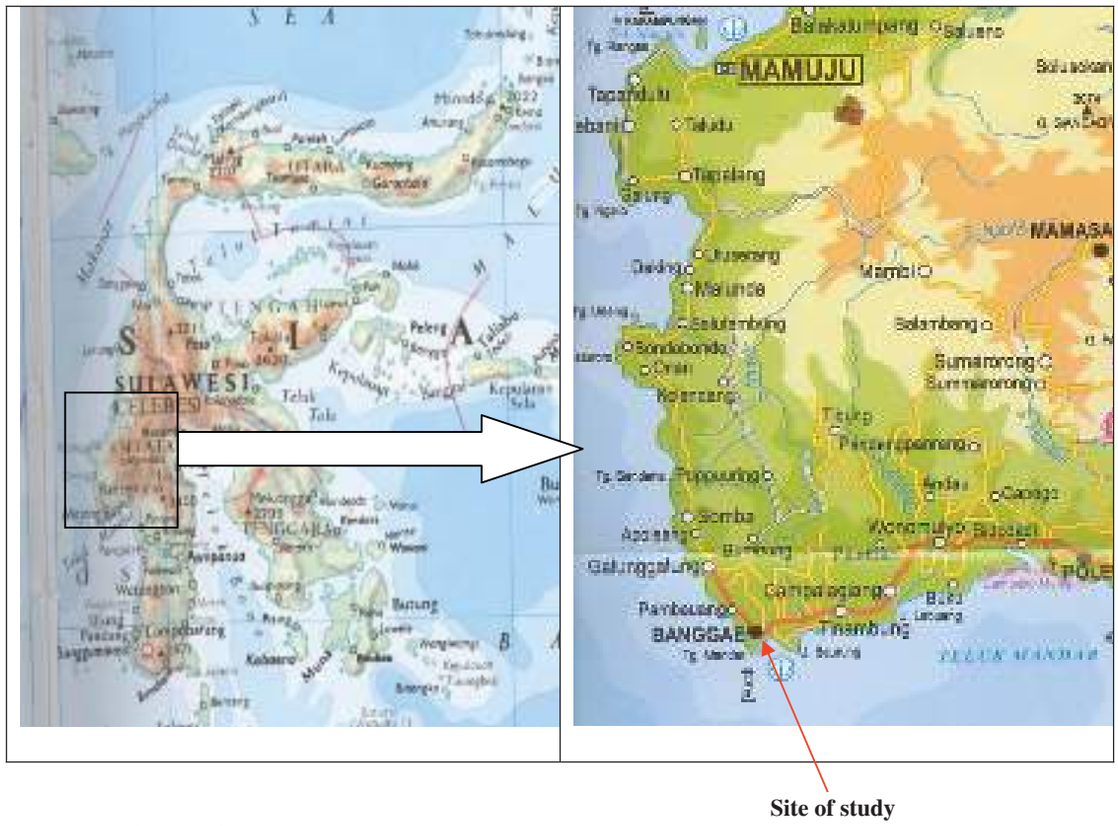
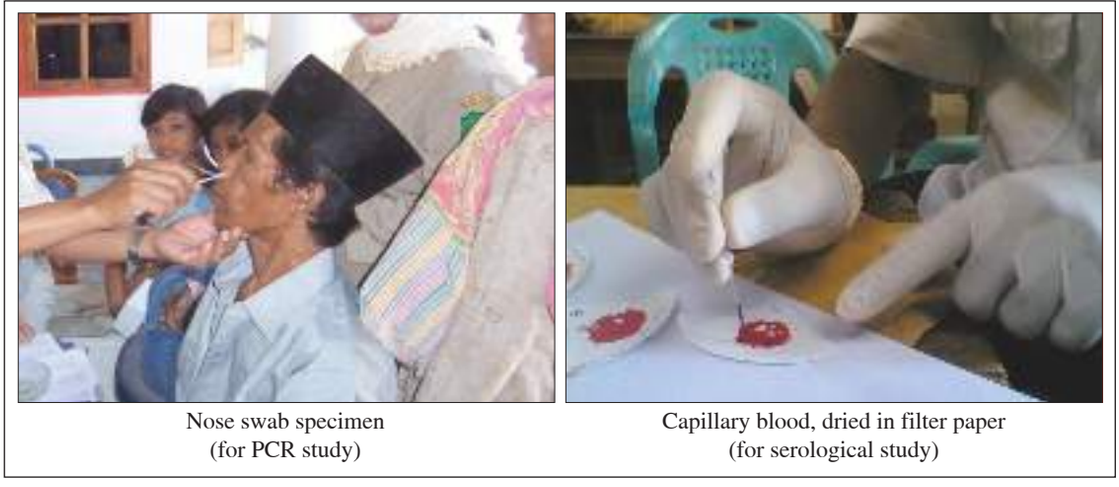


Figure 1. Geographic area of the study



Nose swab specimen
(for PCR study)

Capillary blood, dried in filter paper
(for serological study)

Figure 2. Collection of specimens

From each patient a nose swab specimen was collected and 100 ul capillary blood was collected by finger tip punctured and dried in the filter paper (figure 2). These 60 pairs of specimens were brought to Leprosy lab in the Institute of Tropical Disease, Airlangga University, Surabaya. A Polymerase Chain Reaction (PCR) test were performed to detect *M. leprae* in the nose swab specimens, while the dried capillary blood was examined serologically to measure the level of anti PGL-1 antibody using the ELISA technique.[4] The results will be analyzed to compare the positive PCR results of the nose swab specimens and also to compare the immunologic response to *M. leprae* between the two groups. The dried blood in filter paper are diluted in distilled water for two hours and shaked. This diluted blood was used as a specimens for ELISA study to measure the level of IgM anti PGL-1 antibody and using the conversion value, the results were converted to serum equivalence value.[5] By Biolise program in computer, the Optical Density (OD) value was converted to unit.ml. Using cut off value 605 u/ml, sero-positive result were established.[6]

RESULTS

Using the Lp1-Lp4 nested primer that amplify the *Rlep* region of *M. leprae* DNA (99 bp), the household contact group showed 1/30 positive PCR result, compared to 1/30 positive result in the non-household contact group. Statistically there is no significant difference between the two goups in the positive PCR results ($p > 0.05$). In serological examination, after a conversion to achieve the serum equivalency and using the cut off 605 u/ml for IgM anti PGL-1 (ELISA), 15/30 samples from the household contact group showed sero-positive results, compared to 11/30 sero-positive in the non-household contact group. Although the number of sero-positive is higher in household contact group, statistically no significant difference between the two groups in the sero-positive results. Also when the two datas (PCR and serology of leprosy) are combined, still no significant difference between the two groups ($p > 0.05$).

DISCUSSION

The route of transmission in leprosy mainly by droplet infection, since multibacillary leprosy case will harbour many lepra bacilli in his nasal cavity.[7] Prolonged contact, intimate and continuously with leprosy patients are the condition for affected the disease.[8]

The existence of *M. leprae* in the nasal cavity could be either from outside, aspirated during respiration, or secretion from the nasal mucous as a secretion from leprosy lesion in the nasal cavity.[9,10] Household contacts of leprosy fulfill these criteria and become the high risk group. When the bacilli enter the body, the immune response will develop. Although the anti PGL-1 antibody is not effective to eradicate the *M. leprae* infection, it is a useful parameter for monitoring the infection.[11] The level of this specific antibody is correlated with the amount of *M. leprae* in the body.[12] Based on previous serological surveys in endemic and non-endemic areas, the cut off IgM and IgG anti PGL-1 (ELISA) can be calculated. The level 605 u/ml for IgM anti PGL-1 and 615 for IgG anti PGL-1 was used as the cut value. Those who have the IgM anti PGL-1 level >605 are considered as a sero-positive case. Most of serological studies use serum samples, which originally from venous blood samples. The use of capillary blood which is dried on the filter paper has been introduced since 2007 and very useful for collecting blood samples from field that located long distance from the laboratory.[13,14] Using a conversion coefficient, the equivalence value of anti PGL-1 antibody in serum can be obtained.[15]

Sub-clinical leprosy is a term for healthy individual who live in leprosy endemic area, with high level of IgM anti PGL-1 in serological examination. These sub-clinical leprosy cases still show no sign of clinical leprosy, but they are potential to progress toward manifest leprosy.[16] In this study the serological examination result showed around 43% of the inhabitants showed sero-positive, which means that they are already exposed to *M. leprae* and induced the humoral response. Since the level of antibody correlates with the antigen load, once can assume the load of bacilli in the body is also more than normal people in other areas. Although it is hypothesized that household contacts will get more *M. leprae* exposure than those non-household contact of leprosy, this study showed that by cross sectional study both groups of study only showed 1/30 PCR (+) for *M. leprae* in the nose swab cavity. This means that airborne infection of *M. leprae* in the household and non-household contacts is similar, or in other word the *M. leprae* infection source not only from leprosy patient in the house, but maybe from other patients or environment. From the serological study, the results showed the same phenomena, but the level of antibody in sero-positive cases showed a different pattern.

Household contacts with sero-positive anti PGL-1 antibody showed a higher incidence and higher (figure 1).

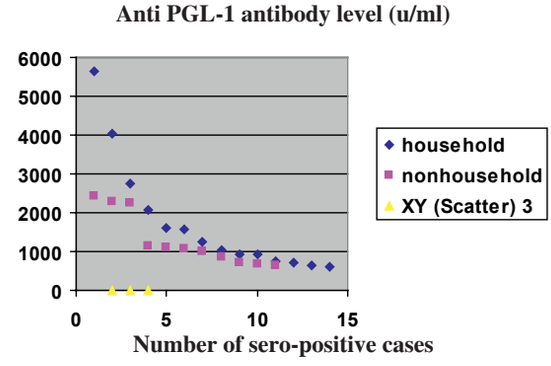


Figure 3. Distribution of serological level of sero-positive cases among household and non-household contacts of leprosy

Since the immune response need a certain duration before it is developed, once can assume that household contacts have more antigen load (*M. leprae*) in their body. Prolonged contact with leprosy patient in the same house might cause the accumulation of antigen and induce high level of specific antibody production. Those sero-positive contacts with high level of antibody (sub-clinical leprosy) need special attention to avoid progression towards manifest leprosy in the future.[17]

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Research Report

THE CHANGING CLINICAL PERFORMANCE OF DENGUE VIRUS INFECTION IN THE YEAR 2009

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ABSTRACT

Background: *Dengue (DEN) virus, the most important arthropod-borne human pathogen, represents a serious public health threat. DEN virus is transmitted to humans by the bite of the domestic mosquito, Aedes aegypti, and circulates in nature as four distinct serological types DEN-1 to 4).* ***The aim of Study:*** *To identify Dengue Virus Serotype I which showed mild clinical performance in five years before and afterward showed severe clinical performance.* ***Material and Method:*** *Prospective and analytic observational study had been done in Dr. Soetomo Hospital and the ethical clearance was conduct on January 01, 2009. The population of this research is all cases of dengue virus infection. Diagnosis were done based on WHO 1997. All of these cases were examined for IgM & IgG anti Dengue Virus and then were followed by PCR examination to identify Dengue Virus serotype.* ***Result and Discussion:*** *DEN 2 was predominant virus serotype with produced a spectrum clinical illness from asymptomatic, mild illness to classic dengue fever (DF) to the most severe form of illness (DHF). But DEN 1 usually showed mild illness. Helen at al (2009–2010) epidemiologic study of Dengue Virus Infection in Health Centre Surabaya and Mother and Child Health Soerya Sidoarjo found many cases of Dengue Hemorrhagic Fever were caused by DEN 1 Genotype IV. Amor (2009) study in Dr. Soetomo Hospital found DEN 1 showed severe clinical performance of primary Dengue Virus Infection as Dengue Shock Syndrome two cases and one unusual case.* ***Conclusion:*** *The epidemiologic study of Dengue Virus Infection in Surabaya and Sidoarjo; in the year 2009 found changing predominant Dengue Virus Serotype from Dengue Virus II to Dengue Virus 1 Genotype IV which showed a severe clinical performance coincident with primary infection.*

Key words: *Changing Clinical Performance, Dengue Infection.*

INTRODUCTION

Dengue (DEN) virus, the most important arthropod-borne human pathogen, represents a serious public health threat. DEN virus is transmitted to humans by the bite of the domestic mosquito, Aedes aegypti, and circulates in nature as four distinct serological types DEN-1 to 4. DEN virus has been recognized in over 100 countries, and 2.5 billion people live in areas where DEN virus is endemic.[16]

Dengue, an emerging arboviral and arthropod borne disease, is a major cause of morbidity throughout the tropical and sub-tropical regions of the world.[1] Dengue virus (DV) infection with any 1 of 4 serotypes produces a spectrum of clinical illness, ranging from an asymptomatic or mild febrile illness to classic dengue fever (DF) to the most severe form of illness, dengue hemorrhagic fever (DHF). DHF is characterized by plasma leakage and a hemorrhagic diathesis near the time of differences, typically after 5 days of fever.[2] In severe DHF, morbidity and mortality are the result of hypotension and shock, at times accompanied by severe coagulation abnormalities and bleeding. Since early hospitalization and careful supportive care can reduce the case-fatality rate of DHF, the rapid identification of patients at risk for developing DHF is desirable in regions where DV is endemic.

Dengue hemorrhagic fever is one of the important health problem in Indonesia, although the mortality rate has been decreased but many dengue shock syndrome cases is very difficult to be solving handled. Natural course of dengue virus infection is very difficult to predict of the earlier time

of severity occur; It is may be due to the new variant of dengue virus that infect a child could be severe and can not be identified earlier.

Previous study show that some of DEN 2 and DEN 3 virus cases could show a clinical performance of severe dengue virus infection such as dengue shock syndrome.

Based on Halstead hypothesis, the severe dengue virus infection could be correlated with secondary infection. The infant cases show a severe clinical manifestation.

In Thailand and Cuba, many cases of dengue virus infection were identified as secondary infection and some of them showed dengue shock syndrome, but this case did not found in other countries. Moren (1980) found that the differences of growing dengue virus in monocyte could be a predictor of severity or mild cases for dengue virus infection.

The first outbreak of DHF in Indonesia was reported in Java Island in 1968, all types (Den VI-4) were isolated from patient in Jakarta in 1973–1974. Indonesia has approximately 100.000 annual dengue cases. Since then some outbreak in other cities and island were reported and the type of circulating DEN virus varies in each province and island. Based on Setiati TE et al (2006), recently predominant type as follow: Jakarta DEN V3; Palembang DEN V3; Bandung DEN V2; Manado DEN V1; Merauke DEN V3; Yogyakarta DEN V3.

In the year 2009, Dengue Virus Team of Institute Tropical Disease had done epidemiologic study in Surabaya.

MATERIAL & METHOD

Prospective and analytic observational study had been done in Dr. Soetomo Hospital and the ethical clearance was conduct on January 01, 2009. The population of this research is all cases of dengue virus infection that in Tropical ward of children, diagnosis were done based on WHO 1997. Cases of dengue virus infection were collected & involving in research based on inform concern. All of these cases were examined for IgM & IgG anti dengue virus and then followed by PCR examination to identify dengue virus serotype.

Blood examination should be done everyday. X-Ray examination were also done base on clinical performance of Pleural Effusion & Ascites. Data of all cases dengue virus infection should be analyze using method of Kruskal Walles & Mann Whitney and Regression Logistic multivariet.

RESULT & ANALYSIS

150 cases of primary and secondary of dengue virus infection were studied. Dengue virus was isolated from vero cell and 120 samples have positive CPE. 70 samples were found as serotype by doing RT-PCR examination.

Serotype DEN 1: there ware only 3 cases (see table 3) consisted of 2 cases had age 1-4 years and 1 had age 5–14 years. They showed a severe clinical performance as DSS 2 cases and 1 case as unusual case (see table 1).

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

	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)*

Table 2. Distribution of Clinical Performance of Dengue Virus Infection

	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)*

Serotype DEN 1 was usually mild case but in this study 1 case showed a severe clinical performance as DSS and identified as primary infection (see table2).

Table 3. Distribution of Primary and Secondary infection and Serotype that were correlated with clinical Performance of Dengue Virus Infection

	Clinical Performance & Diagnostic				
Type of Infection	**DF**	**DHF**	**DSS**	**UNUSUAL**	**Total**
Primary					
DEN 1	0	0	1*	0	1
DEN 2	16	7	0	2	25
DEN 3	0	0	0	0	0
DEN 4	0	0	0	0	0
Total	16	7	1	2	26
Secondary					
DEN 1	0	0	1	1	2
DEN 2	14	19	7	0	40
DEN 3	1	0	1	0	2
DEN 4	0	0	0	0	0
Total	15	13	9	1	44

The second case of DEN 1 was identified as secondary dengue virus infection and the third case was an unusual case which showed secondary of dengue virus infection (see table 3). Based on Yamanaka this serotype DEN 1 might be have genotype IV or mention as DEN 1 genotype IV.

DISCUSSION

Aryati (2005), Fedik (2007), had done an epidemiologic study of dengue hemorrhagic fever cases this in Surabaya, found that DEN virus 2 was a predominant types.

The study in Health Center of Surabaya DEN V2 was predominant in Surabaya (see table 4).

All of them showed clinical manifestation of dengue virus infection with produces a spectrum of clinical illness, ranging from an asymptomatic or mild febrile illness to classic dengue fever (DF) to the most severe form of illness as dengue hemorrhagic fever (DHF). DHF is characterized by plasma leakage and a hemorrhagic diathesis near the time of differences, typically after 5 days of fever (2). Most of them showed severe dengue hemorrhagic fever as the result of hypotension and shock, at the times accompanied by severe coagulation abnormalities and bleeding. Since early hospitalization and careful supportive care can reduce the case-fatality rate of DHF, the rapid identification of patients at risk for developing DHF is desirable in regions where DV is endemic. On the year 2007 13% (7 cases) showed very severe clinical performance of dengue virus infection due to combining virus of DEN 2 and DEN 3 infected in one host of dengue hemorrhagic fever case that could induce viremia.

But based on epidemiologic study in Surabaya & Sidoarjo on 2009 and 2010[27] found many cases of dengue hemorrhagic fever were caused by virus DEN V1 (see table 5).

The clinical performance of cases Dengue Virus Infection who came in health center of Surabaya in year 2008 with 2169 cases showed clinical performance of Dengue Fever 87% and 10% Dengue Hemorrhagic Fever and Dengue Shock Syndrome and 3% unusual manifestation. In the year 2009 with 2268 cases Dengue Virus Infection showed clinical performance of Dengue Fever 71.5% and Dengue Hemorrhagic Fever and Dengue Shock Syndrome 28% and unusual cases of Dengue Virus Infection 0.5% (see table 6).

This finding supported study of mosquito bites to some peoples live surrounding Dengue Hemorrhagic cases who had been admitted in hospital (see table 7).

Table 4. Prevelance Dengue Virus Infection based on serotype virus that was found in Surabaya on the year 2003–2005, 2007, 2008.

Year	DEN V1	DEN V2	DEN V3	DEN V4	D2+D3	Total
2003–2005	0	20 (80%)	4 (16%)	1 (4%)		25
2007	0	46 (87%)	0	0	13%	53
2008	0	20 (100%)	0	0		20

Table 5. Prevalence Dengue Virus Infection in Surabaya & Sidoarjo in 2009–2010.

Year	DEN V1	DEN V2	DEN V3	DEN V4	Total
2009	79 (87%)	6 (6.5%)	0	6 (6.5%)	91
2010 (Jan–Feb)	27 (100%)	0	0	0	27

Table 6. Clinical performance of dengue virus infection in Health Centre of Surabaya

Year	Total Patients	Dengue Fever	DHF + DSS	Unusual
2008	2169	1890 (87%)	216 (10%)	63 (3%)
2009	2268	1601 (71,5%)	656 (28%)	11 (0,5%)

Table 7. Virus Isolation from Mosquito

Mosquito	2008					
	Total	Pool	CPE	Immune staining	PCR	Sequencing
Ae.aegypti	271	12	2	Dengue	D2	D2
Cx.quinquefasciatus	336	10	4	Dengue	D2	D2
Cx.tritaeniorhynchus	131	3	–	–	–	–
Cx.vishnui	71	1	–	–	–	–
Cx.pseudovishnui	42	1	1	Dengue	D2	D2

Table 8. Virus Isolation from Mosquito

Mosquito	2009–2010					
	Total	Pool	CPE	Immune staining	PCR	Sequencing
Ae.aegypti	1784	45	13	Dengue	D1	D1
Cx.quinquefasciatus	74	4	1	Dengue	D1	

Table 7 supported previous epidemiologic study that found DEN V2 as predominant types in the year 2008 but table 8 supported epidemiologic study in the year 2009 found DEN V1 as predominant types. The study in Dr. Soetomo hospital since January 1, 2009 as followed DEN 1 showed clinical performance of Dengue Shock Syndrome 2 cases and unusual case with total 3 cases, DEN 2 were found clinical performance of 30 cases Dengue Fever, 26 Dengue Hemorrhagic Fever 7 Dengue Shock Syndrome and 2 unusual cases, with total 65 cases. DEN 3 were found clinical performance of Dengue Fever 1 case Dengue Shock Syndrome 1 case, with total 2 cases. Den 4 virus was not found. The differences of result were found due to the differences of population of study. But DEN V1 were always found in this study.[27]

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 Beijing which had severe clinical performance of Dengue Virus Infection.

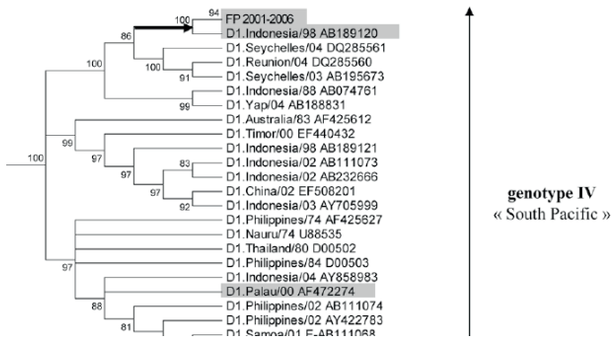

Figure 1. Phylogenetic Dengue Virus in The World

In the year 2009 we have many experience to care severe performance of Dengue Virus Infection with unusual 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 for managing the unusual dengue virus infection we should followed new WHO criteria diagnosis and classification of cases as followed.

During three decades, the World Health Organization (WHO) has recognized and recommended the classification of dengue in: dengue fever (DF) and dengue hemorrhagic

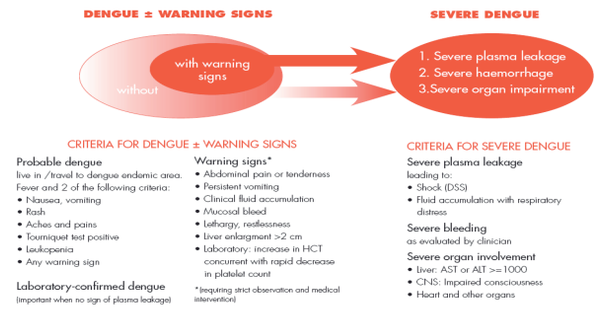

Figure 2. Suggested dengue case classification and levels of severity. Dengue guidelines for diagnosis, treatment, prevention, and control. World Health Organization, UNICEF, UNDP. New Edition 2009

fever (DHF) with or without dengue shock syndrome (DSS).[6] However in some severe cases the clinical manifestations sometimes doesn't fit to these definition and classification. In this WHO recommendation clinical manifestation in DF are mild form than DHF/DSS, but in this case DF with severe hemorrhagic manifestation and that may be life threatening. Dengue can also express itself by means of the so-called "atypical" forms or unusual manifestation.[1,5] These unusual clinical manifestations may delay recognition of potentially severe disease.

Lately, several publications that appeared worldwide emphasize the need to revise the classification of severe dengue.[1] One of the revised dengue classification proposed by DENCO (Dengue Control) has been applied and studied in several countries in Asia and Latin America with good result.[1,7] The DENCO study concluded that 18 to 40% of the cases could not be classified by means of the current WHO Classification, and over 15% of unusual cases with shock could not be classified as severe cases of dengue either, since they did comply with some of the criteria to be regarded as a case of DHF/DSS.[1,7]

The pathogenesis of bleeding in DF is poorly understood. Thrombocytopenia may enhance the risk, but the primary cause of bleeding is unknown. Limited data suggest that activation of coagulation and fibrinolysis play role in the pathogenesis (srichaikul). An imbalance in the regulation of coagulation and fibrinolysis, as in disseminated intravascular coagulation syndrome (DIC), in conjunction with the characteristic thrombocytopenia may contribute to the bleeding tendency in DF.

In the year 2009, the study found that DEN V1 genotype IV showed a severe clinical performance. Of a primary dengue virus infection. This study supported to Gubler

hypothesis which gave information that a new virulent variant DEN V1 can cause a severe clinical performance of dengue virus infection.

SUMMARY

The epidemiologic study of Dengue Virus infection in Surabaya. In the year 2009 found a changing predominant Dengue Virus from Dengue Virus 2 to Dengue Virus 1 genotype 4 which showed a severe clinical performance coincident with primary infection.

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Research Report

CATHETER DURATION AND THE RISK OF SEPSIS IN PREMATURE BABIES WITH UMBILICAL VEIN CATHETERS

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ABSTRACT

Umbilical catheters are frequently required in the management of severely ill premature babies. The risk of complications may increase with duration of UVC use. ***Objective:*** *To determine whether the risk of central line-associated bloodstream infections (CLA-BSIs) and sepsis remained constant over the duration of umbilical vein catheters (UVCs) in high-risk premature neonates.* ***Methods:*** *retrospective analysis. The data were collected from the medical record of high risk premature neonates who had a UVC placed in neonatal care unit of Husada Utama Hospital between April 1st 2008 to April 30th 2011 with purposive sampling. Catheter duration was observed before and after 14 days on placement. Blood and UVC culture was performed to establish the risk of CLA-BSIs and sepsis. Chi-square and logistic regression analysis were performed in the laboratorium data.* ***Result:*** *A total 44 high risk premature babies with UVCs were enrolled (sepsis group: n = 23 and non sepsis group: n = 21). Baseline demographics were similar between the groups. 15 babies in sepsis group have UVCs duration > 14 days, and 8 babies have UVCs < 14 days (p = 0.533). Days of UVC < 14 days show UVCs culture performance in 11 babies with positive evidence, blood culture performance shows negative in 21 babies (p = 0.516). Days of UVC >14 days show blood culture performance in 11 babies with positive evidence, UVCs culture performance is negative in 18 babies (p = 0.456). Burkholderia cepacia and Klebsiella pneumonia mostly appeared in blood culture performance. 25% of UVC culture performance shows Pseudomonas aeroginosa.* ***Conclusions:*** *The catheter duration have no significant difference in risk of sepsis in premature babies with Umbilical Vein Catheters.*

Key words: *Premature babies - Sepsis – days of UVC – CLA-BSIs*

INTRODUCTION

Umbilical catheters are frequently required in the management of severely ill neonates.[1.2] Umbilical-vein catheters (UVCs) can be used for intravenous administration of parenteral nutrition, hypertonic solutions, blood products, and medication. Umbilical-artery catheters (UACs) can be used for blood sampling and continuous monitoring of blood pressure. However, the advantages of umbilical catheters must be carefully balanced against the potential risks. Several life threatening complications have been associated with the use of umbilical catheters including catheter-related infections, intestinal necrosis, thrombosis, cardiac arrhythmias, myocardial perforation, as well as pleural and pericardial effusion.[1.2.3] According to the literature, mechanical adverse events occur in 5 to 19% of patients with a UVC, infectious adverse events in 5 to 26% and thrombosis in 2 to 26%.[4.5]

The incidence of neonatal sepsis is approximately 1 to 10 cases per 1000 live births and 1 per 250 live premature births. The incidence rates of neonatal infection in several referral hospitals in Indonesia is approximately 8.76%–30.29% with the mortality rate is 11.56%–49.9%. The incidence rates of neonatal sepsis in several referrals hospital in Indonesia is 1.5%–3.72% with the mortality rate is 37.09%–80%.[6]

Because the risk of complications may increase with duration of use, UVCs are often removed after relatively short periods and replaced with percutaneous central venous catheters (PCVCs) for maintenance of long-term fluid and nutritional status.[2.7] On the basis of these limited data, the Centers for Disease Control and Prevention

currently recommend use of UVCs be limited to 14 days. In a retrospective review of 230 infants with birth weights 1251 g who were admitted to our NICU and required a UVC and/or PCVC, the apparent proportion of catheters remaining infection free at 20 days (the time at which the last UVC was removed) was 89% for UVCs and 73% for PCVCs.[8]

UVCs comprise a large proportion of central lines inserted in the NICU. Central line–associated bloodstream infections (CLA-BSIs) can complicate PICCs. An estimated 80 000 CLA-BSIs occur in the United States every year. The mortality rate for these CLA-BSIs remains unclear, but recent studies demonstrated a range of 4% to 20%. CLA-BSI extends patient length of stay by an average of 7 days, and the attributable cost is $3700 to $29 000 per infection.[3.5.8]

In this study, we prospectively examined catheter-related bacteremia and associated sepsis complications in long-term use of UVCs.

METHODS

Subjects

The study was retrospectively done at NICU of Husada Utama hospital, conducted for 3 years (April 1st 2008 until April 30th 2011). The premature infants with birth weights less than 2000 g who had a UVC placed on NICU admission were eligible for the study. Infants who required a UVC for exchange transfusion, infants with gastrointestinal abnormalities including gastroschisis and omphalocele, or infants with congenital heart disease with intra cardiac shunting were excluded. The parents or legal guardians of the patients gave informed consent before enrollment.

Umbilical Catheterization

Placement of a UVC was attempted in infants < 2000 g on admission to the NICU. Either a single or double lumen catheter (3.5F diameter, Polyurethane 1270 Catheter; Vygon Healthcare, Gloucestershire, UK) was inserted under sterile conditions. A double-lumen UVC was used if it was technically possible to place one. Care of the catheters was standardized. Catheters were attached to transducers, was changed every 24 hours if the infused concentration of dextrose was >12.5 g/L and every 72 hours for concentrations of dextrose <12.5 g/L. All UVCs had continuous infusion of solutions in the main port. Both infusion and flush solutions contained heparin (1.0 IU/mL for infants >1000 g and 0.5 IU/mL for infants <1000 g or on total parenteral nutrition). All catheter connections were checked hourly to guard against any disconnection.

Catheter placement was confirmed with a chest and abdominal radiograph. The catheter placement was adjusted to place the catheter tip at the inferior vena cava/right atrial junction. We had confirmed the depth of catheter tips using antero-posterior chest X-rays. Ideal position of the UVC was defined as the catheter tip being visible between the 9th and 10th thoracic vertebrae on a chest X-rays. Catheters were sutured in place into the umbilical cord, and tape was then used to secure the catheter to the infant's abdomen.

Blood and tips UVC tips Cultures

Blood culture test was performed in premature babies with suspected sepsis based on clinical symptoms, complete blood test and CRP using VITEX method. Whole blood (0.3–1.0 mL) was placed in sterile Isolator tubes and transported to the microbiology laboratory. Blood was streaked onto blood and chocolate agar plates and then incubated for 5 days under aerobic conditions. UVC tips Cultures were placed in an automated reader (Bact-alert; Biomerieux). Any positive or potentially positive cultures were Gram-stained, streaked on to blood and/or chocolate agar plates (depending on the likely pathogen), and incubated under aerobic conditions. Organisms were isolated by either culture system identified with standard microbiologic techniques.

Definitions

Clinical Sepsis

The definition of infection included symptomatology (eg, temperature instability, increased ventilator settings, increased apnea, bradycardia or desaturations, feeding intolerance, lethargy, or blood pressure instability) and either a single positive blood culture for prospectively defined definite pathogens or multiple positive cultures (≥ 2 within 48 hours) for other organisms from usually sterile site(s) (blood, catheter tip, urine, or cerebrospinal fluid, with at least 1 positive culture from the blood).[1.9.10]

CRBSI

Bacteremia/fungemia in a patient with an intravascular catheter with at least 1 positive blood culture obtained from a peripheral vein, clinical manifestations of infections (fever, chills, and/or hypotension), and no apparent source for the BSI except the catheter. One of the following should be present: a positive semi quantitative (>15 CFU/catheter segment) or quantitative (>103 CFU/catheter segment catheter) culture whereby the same organism (species and antibiogram) is isolated from the catheter segment and peripheral blood; simultaneous quantitative blood cultures with a >5:1 ratio CVC versus peripheral; differential period of CVC culture versus peripheral blood culture positivity of >2 hours.[8.11.12]

Statistical Analysis

Data are presented in distribution tabulation and data analysis was performed with a computer assisted statistical package (SPSS ver. 12.0). Descriptive analysis of catheter duration and risk of sepsis, UVCs and blood culture of the patient were calculated. Chi-square analysis and logistic regression were performed in the laboratorium data.

RESULTS

Data from April 1st 2008 until April 30th 2011 revealed the premature babies with UVCs were 44 samples. All of them were eligible for analysis, 23 in sepsis group and 21 in no sepsis group. The characteristics of the sample are listed in table 1.

Table 1. Characteristic of High Risk Premature Neonates Who had a UVC Placed in NICU

Parameters	Sepsis	No Sepsis	P
	n = 23 (%)	n = 21 (%)	
Gender			.121
Female	7 (38.9)	11 (61.1)	
Male	16 (61.5)	10 (38.5)	
Birth weight (g)	1428.3 (SD 324.33)	1450.0 (SD 321.71)	.52
Gestational age			.467
< 30 week's	9 (50.3)	7 (43.8)	
> 30 week's	14 (50)	14 (50)	
Apgar score			.322
≤ 6	8 (61.5)	5 (38.5)	
> 6	15(48.4)	16 (51.6)	
Mechanical ventilator			.068
Yes	18 (62.1)	11 (37.9)	
No (n-CPAP)	5 (33.3)	10 (66.7)	

Table 1 shows that the results have no significant difference based on the gender, birth weight, gestational age, apgar score in premature babies with sepsis risk treated in NICU Husada Utama hospital. However baby with mechanical ventilator shows to have higher risk compared with n-CPAP. In this case: 18 premature babies with mechanical ventilator and 5 with n-CPAP affected by sepsis. Babies with apgar score less than 6 during the labor have higher risk affected by sepsis, on the other hand apgar score more than 6 shows lower risk.

Table 2. Catheter Duration and The Risk of Sepsis in Premature Babies

Days of UVC	Sepsis	
	Positive n(%)	Negative n(%)
< 14 days	15 (53.6)	13 (46.4)
> 14 days	8 (50)	8 (50)

Chi Square X^2test p = 0.533

Table 2 shows that days of UVC have no significant difference in the risk of sepsis in premature babies treated in NICU Husada Utama hospital. In this study 15 babies with days of UVC less than 14 days were in the risk of sepsis. 8 babies with days of UVC more than 14 days were in the risk of sepsis.

Table 3. Catheter Duration and The Risk of CLA-BSIs

Days of UVC	UVC Culture		Blood Culture	
	+	–	+	–
< 14 days	11 (68.8)	5 (31.2)	7 (25)	21 (75)
> 14 days	10 (35.7)	18 (64.3)	11 (68.8)	5 (31.2)

Chi Square X^2 test p = 0.516; p = 0.456

Table 3 shows that days of UVC have no significant difference in UVC and blood culture result in premature babies treated in NICU Husada Utama hospital. Days of UVC less than 14 days show UVC culture performance in 11 babies suspected sepsis is positive, in fact blood culture performance shows negative in 21 babies. Days of UVC more than 14 days show blood culture performance in 11 babies with blood culture positive evidence, even though UVC culture performance is negative in 18 babies.

Table 4. Pathogens that Caused CLA-BSI in Neonates with UVCs in Premature Babies

Microorganisms	Blood Culture		UVC Culture	
	n	%	N	%
Acinetobacter baumanii	1	2.3	5	11.4
Burkholderia cepacia	4	9.1	4	9.1
Candida albicans	1	2.3	0	0
Enterobacter asburie	1	2.3	0	0
Klebsiella pneumoniae	4	9.1	3	6.8
Escherichia coli	0	0	4	9.1
Pseudomonas aeroginosa	0	0	11	25
Enterobacter cloacae	0	0	2	14.5
Stenotrophomonas maltophila	1	2.3	0	0
No organism growth	12	27.3	15	34.1

Table 4 shows the types of microorganism appeared in blood and UVC culture in 44 premature babies treated in NICU Husada Utama hospital. *Burkholderia cepacia* and *Klebsiella pneumonia* mostly appeared in blood culture performance. 25% of UVC culture performance shows *Pseudomonas aeroginosa*. Of 23 babies suspected sepsis 12 babies show no organism growth on blood culture performance while 15 babies show no organism growth on UVC culture performance.

DISCUSSION

CLA-BSIs are a common cause of morbidity and mortality among neonates.[1,3,6,10] Several factors have been

shown to contribute to the pathogenesis of nosocomial CLA-BSI. Host-related risk factors include age, immunologic immaturity, and severity of underlying disease.[12.13] In this study shows that gestational age < 30 weeks and mechanical ventilator have contributed the risk of CLA-BSI.

The risk profiles of a long term UVC to a long-term PCVC have seldom been compared. On the basis of these limited data, the Centers for Disease Control and Prevention currently recommend use of UVCs be limited to 14 days. However, a survey of nursery directors revealed that some NICUs leave UVCs in place for a longer period of time.[7.14.15] The limited data available after 14 days in this study suggest the possibility of increased infection. Duration of catheter > 14 days show 11 babies with blood culture positive evidence, although not statistically significant, would have potential clinical significance if it were to be substantiated.

Migration of skin organisms at the insertion site into the umbilical catheter tract with colonization of the catheter tip is the most common route of infection for centrally inserted, shortterm catheters.[4.8.9] Contamination of the catheter hub contributes substantially to intraluminal colonization of long-term catheters. Occasionally, catheters might become hematogenously seeded from another focus of infection. Rarely, infusate contamination leads to CRBSI.[1.16]

Important pathogenic determinants of catheter-related infection are 1) the material of which the device is made and 2) the intrinsic virulence factors of the infecting organism.[8.9.11] In vitro studies demonstrate that catheters made of polyvinyl chloride or polyethylene are likely less resistant to the adherence of microorganisms than are catheters made of Teflon, silicone elastomer, or polyurethane.[9.13.15] Some catheter materials also have surface irregularities that enhance the microbial adherence of certain species (eg, coagulase-negative staphylococci, *Acinetobacter calcoaceticus*, and *Pseudomonas aeruginosa*); catheters made of these materials are especially vulnerable to microbial colonization and subsequent infection.[4.7.8] Additionally, certain catheter materials are more thrombogenic than others, a characteristic that also might predispose to catheter colonization and catheter-related infection. This association has led to emphasis on preventing catheter-related thrombus as an additional mechanism for reducing CRBSI.[17]

One study reports that the incident rate of PICC related sepsis is between 2 and 21%. This study suggests that the lower incidence of infection in PICCs, when compared to other UVCs, might be related to the low concentration of bacteria in peripheral areas (50 to 100 colonies of bacteria per cm^2 of skin) when compared to the thorax (1,000 to 10,000 colonies of bacteria per cm2 of skin).[18]

The literature shows that there are microorganisms more prevalent in catheter-related primary sepsis. The gram-positive cocci are responsible for 65% of infections, while the most prevalent are the *Staphylococcus epidermidis* (31%) and the *Staphylococcus aureus* (14%). The gram-negative bacilli account for 30% of infections and the most prevalent are the *Pseudomonas sp* (7%) and the *Escherichia coli* (6%). Infection by *Candida SP* is responsible for the remaining 5% of catheter-related infections.[1.2.19] Coagulase negative staphylococcus was the dominant infection (55.6%) within the first 2 weeks, whereas Gram negative bacteria were dominant pathogens (58.3%) after the first 2 weeks.[20.21] However, the most frequent microorganism isolated in cultures in this study was the *Pseudomonas aeroginosa.*

To avoid contaminating central venous catheters, several measures should be implemented in their insertion and maintenance.[10.11] Central catheter insertion, whether it is a PICC or a UVC, should be aseptic and include measures of barrier precaution such as wearing a cap, mask, sterile gown, sterile gloves and drapes. It is recommended to wash hands with chlorhexidine detergent or alcohol gel before and after contacting with the catheter during UVC maintenance. The dressing has to be changed every seven days or when it is wet or for other reasons taken off, change taps, equipment and extensions every 72 hours and the equipment for parenteral nutrition should be changed every 24 hours, always swabbing the connections and taps of the catheter with 10% concentration of alcohol before handling them.[2.6.11.15]

Adverse events in central catheters were frequent in neonatal populations, both for PICCs and in UVCs. The most prevalent adverse event in PICCs was catheter occlusion, while clinical sepsis prevailed in UVCs.[8.21] PICCs presented a higher frequency of mechanical adverse events, especially catheter occlusion and rupture. However, its use presented very low rates of catheter-related infections; these rates are similar or less than those reported in the literature.[20] Therefore, we assert that PICC is a safe means for parenteral administration in the neonatal population due to the low risk of infection found in this study and in the literature. The use of UVCs resulted in a lower rate of mechanical adverse events: occlusions or ruptures were not found in this catheter in this study. However, the rates of infectious adverse events related to this catheter are the most prevalent.[20.21]

Several limitations should be considered when interpreting our data. We conducted the study on a large cohort of patients over a 3-year period, because our unit has low incidence of CLA-BSI. Several confounding factors still persist in this study. Underlying disease, duration of mechanical ventilator, nosocomial infection were the risk of sepsis in premature babies with UVCs.

CONCLUSIONS

The catheter duration have no significant difference in risk of sepsis in premature babies with Umbilical Vein Catheters. *Burkholderia cepacia* and *Klebsiella pneumonia* mostly appeared in blood culture performance. 25% of UVC culture performance shows *Pseudomonas aeroginosa*.

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Research Report

MYCOBACTERIA AND OTHER ACID FAST ORGANISMS ASSOCIATED WITH PULMONARY DISEASE IN JOS, NIGERIA PULMONARY DISEASE AND ACID FAST ORGANISMS

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ABSTRACT

Objective: *Acid fast bacilli (AFB) for sputum smear microscopy is the affordable method used for prompt diagnosis of tuberculosis in Nigeria despite its lack of specificity and limited sensitivity. The study aims to identify Mycobacterium tuberculosis and other acid fast organisms isolated from sputum of of HIV positive adult patients with pulmonary disease in Jos, Nigeria.* ***Methods:*** *Acid fast organisms isolated from 80 AFB positive sputa of HIV positive adult patients suspected for tuberculosis in Jos, Nigeria were identified for members of M. tuberculosis Complex (M tuberculosis, M bovis, M africanum, M canetti M. microti and M. caprae) by use of spoligootyping, Multiplex Gen Probe, Hain genotype assay and gene sequencing for spoligotype negative isolates.* ***Results:*** *Seven different spoligotypes of M. tuberculosis complex were identified from 70/80 (87.5%) total number of isolates. M. kansasii (1), M. dulvalii (1) Nocardia species (1) and Tsukamurella species (2) were detected from 5/10 spoligotype negative isolates.* ***Conclusion and Recommendation:*** *Although M. tuberculosis is the dominant AFB associated with chronic pulmonary disease in Jos, Nigeria, other clinically relevant mycobacteria were observed in the study. This suggests that other AFB positive microorganisms associated with tuberculosis -like symptoms could be misdiagnosed and incorrectly treated as M. tuberculosis. It is therefore necessary for laboratories in TB high burden countries to step up diagnostic procedures beyond routine smear microscopy.*

Key words: *Acid fast bacilli (AFB) Mycobacteria tuberculosis, Other Mycobacteria species*

INTRODUCTION

Mycobacterium tuberculosis is a pathogenic species of the genus Mycobacteriaceae and the agent of human classical tuberculosis. The less virulent Non Tuberculous Mycobacteria (NTM) found in environments such as dust and running surface waters[1–3] are morphologically indistinguishable from *M. tuberculosis*. Although not transmissible from human to human, NTMs cause opportunistic infection capable of multifocal organ involvement in humans, and more frequently chronic lung diseases.[4-5,2] Infection of the lungs may be similar to classical tuberculosis but more difficult to treat and if necessary, prolonged treatment periods may be required.[6–7] HIV positive and severely immunocompromised persons are at high risk due to very low CD4 counts.[8–10] The lack of sensitive identification methods in most clinical laboratories may predispose to misdiagnosis of NTM disease for tuberculosis especially in resource limited settings that rely only on AFB smear microscopy for TB diagnosis. Although NTMs have been associated with primary disease in severe immunodeficiency conditions, it could also constitute a secondary infection in active TB or after TB therapy.[11] It is therefore necessary to carryout comprehensive clinical and radiological investigations in infected persons, to understand the pathological role of NTM when isolated. Establishment of referral centers including expert physicians in NTM treatment and management has been recommended.

Published studies on Mycobacterium infections are scarce in Nigeria in spite of high burden of HIV and TB and the prevalence of atypical mycobacteria associated

with pulmonary disease is not known. Reports from other countries have demonstrated that atypical mycobacterial infections are associated with HIV positive persons, other immunocompromised patients and transplant receivers.[12–13]

Conventional methods[14-16] for identification of mycobacterium species are time consuming and often not specifically conclusive in species identification, while the newer biochemical (high performance liquid chromatography) and some of the highly specific molecular methods[17-19] are not cost effective for use in routine clinical laboratories. Spoligotyping,[20] a simple PCR based method distinguishes members of *M.tuberculosis* complex in clinical specimens or culture. The procedure, though not cost effective for routine use, has been widely applied in molecular epidemiology and identification of *M tuberculosis* complex.

We identified acid fast bacilli isolated from sputa in Jos Nigeria, where smear microscopy has been the most widely used laboratory method for TB diagnosis. The study examined 80 consecutive isolates from cases of pulmonary tuberculosis.

MATERIALS AND METHODS

Ethical Consideration

The study which was respectively approved by the ethical committee of the Jos University Teaching Hospital and the Plateau State Hospital Jos, Nigeria, was descriptive of a bacterial collection and contained no material of human origin. Personal data were removed from all bacterial cultures to protect the anonymity of the patients. Ethical clearance was granted with no requirement for patient informed consent.

Eighty AFB positive isolates from 94 AFB positive sputa were identified by spoligotyping, GenProbe, Hain genotype and 16s ribosomal DNA gene sequencing. The strains were isolated during January 2008 to December 2009 from 790 total number of HIV patients suspected for tuberculosis in Jos, Nigeria.

Sputum specimens were collected in 1ml solution of 1% cetyl pyridinum chloride (CPC) with 2% sodium chloride and processed for culture on Lowenstein Jensen (LJ) medium.[8] AFB smear microscopy was used for preliminary identification of suspect isolates. AFB positive cultures on LJ slants were subcultured and preserved at -20° C and subsequently shipped to SEEFO NIH TB/imunology Laboratory Mali for spoligotyping and Multiplex GeneProbe. Spoligotyping was performed as described by Kermerbeek et al.[20] Unidentified species were sent to the Norwegian Institute of Public Health Oslo for sequencing.

RESULTS

Seventy of the 80 (88%) total number of isolates were *M. tuberculosis* complex spoligotypes; Latin America Mediterranean Family (LAM) 75.6%, T (10%), Haarlem (4.3%), *M. africanum* (2.9%) EAI (5.7%), F (1.4%). Only one (M. *kansasii*) of the 10 spoligotype negative isolates were identified by geneprobe, 4 others; *M. duvalii* (1), *Norcardia asteroids* (1) and Tsukamurella species (2) were detected by 16s rRNA by gene sequencing while 5/10 isolates were lost to contamination.

These results illustrate the importance of further investigation of AFB cases to exclude other Mycobacteria/ non mycobacterial microorganisms, especially in immunosuppressed patients suspected of having tuberculosis.

Table 1. Genus Actinomycetes isolated from sputa of pulmonary disease cases in Jos, Nigeria N = 80

	No of isolates	%
M. tuberculosis	70	87.5
NTM	2	2.5
Nocardia spp	1	1.2
Tsukamurella spp	2	2.5
Total	75*	93.7*

*Five isolates were lost to contamination

Table 2. Spoligotypes of *M tuberculosis* complex isolated from Jos, Nigeria

MTB Family	Number	%
LAM 10	47	67
LAM 8	6	8.6
HAARLEM	3	4.3
EAI	4	5.7
F	1	1.4
M	2	2.9
T	7	10
Total	70	99.9

DISCUSSION

The detection of 88.5% *M tuberculosis* complex by spoligotyping confirms that *M. tuberculosis* is the major cause of chronic pulmonary disease in Jos Nigeria and that the use of smear microscopy for prompt and presumptive diagnosis of *M tuberculosis* remains an effective and relevant tool especially in a resource limited setting lacking the more sensitive technological implements for more

accurate and rapid diagnosis. The findings in this study agrees with others in some countries where a declining incidences of tuberculosis have been reported following the practice of the directly observed treatment short course (DOTS).[21-22] However, the emergence of drug resistance TB or the non eradication of acid fast bacilli after successful completion of therapy with first line anti tuberculosis drugs remains a concern.

The prevalence of 10/80 (12%) AFB positive and spoligotype negative isolates in this study calls to question the position of some of the cases that failed eradication with consistent acid fast positive smears after completion of treatment with first line anti tuberculosis drugs. The detection of *M. kansasii* (1), *M. duvalii* (1), Nocardia spp (1) and Tsukamurella spp (2) from the 5 available isolates may not be unrelated to such cases. The pathogenic relevance of the isolates could not be explained from the available data in this study even though all five isolates were from sputa of new cases which apparently qualified the patients for recruitment under the DOTS TB treatment program. *M. kansasii* could be clinically relevant as it has been known to cause tuberculosis -like pulmonary disease in humans.[2,23-24] Nocardi spp and Tsukamurella spp have also been associated with pulmonary disease in humans.[8,25-26] There are scare reports associating *M. duvalli* with human infection although it has been reported to have some antigenic relatedness with *M. leprae*[27] and also was reported in HIV patient in India.[28] All three genera (Mycobacteria, Nocardia, Tsukamurella) belong to the same Family Actinomycetales with mycolic acid cell walls.[29-30] Further studies are intended to ascertain the followup treatment outcome of NTM isolates in cases treated with conventional anti TB regimen in Jos Nigeria.

Only 94 of 790 (12%) total number of patients suspected for tuberculosis had AFB positive smear sputa. This is less than 25% estimated prevalence of TB in HIV positive cases in Nigeria. It is possible that some of the patients were unable to expectorate detectable levels of bacilli in sputa due to HIV immunosuppression. HIV and TB endemic countries need to step up laboratory diagnostic facilities to include more sensitive detection methods such as the nucleic acid amplification test (NAAT) to enable effective detection and treatment of NTM as well as other non mycobacteria pulmonary diseases. This would prevent unnecessary rise in drug resistant mycobacteria species.

The concept which suggests that non specific cross immunity develops due to latent TB against the atypical mycobacteria especially in *M. tuberculosis* endemic countries[10] may not significantly apply in HIV/TB endemic communities like Nigeria.

The dominance of LAM 10 Family of *M tuberculosis* in this and a previous study[31] needs to be investigated further to establish the transmission pattern of tuberculosis in Jos. Although LAM is generally reported in other West African countries,[32-34] the unique homogeneity of LAM 10 seen in Nigeria has not been reported elsewhere. We have previously suggested that the dominance of LAM family in Nigeria and West Africa may be a result of the historic interactions between West Africa and South America of which the Nigerian sea coasts served as major export route.[31]

The limitations of the study included the Inability to define the clinical relevance of other acid fast bacilli isolated. However, the results illustrate the importance of investigating for NTMs and other non Mycobacatrial AFB in clinical specimens (sputa) especially in immunosuppressed patients. Such organisms may colonize the airways and cause life threatening diseases. Precise identification of some genera and species requires advanced methodologies which are not readily available in several high TB burden countries.

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Conflict of Interest: None

Author Contributions

AEA and URD conceived and designed the study, CL and YF did the pre analytical processing of specimens and data arrangement, URD did the gene sequencing while AEA, BD, URD, and SM performed the other assays and analyzed the data. AEA, URD and JI wrote the report which was reviewed and approved by all authors.

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Case Report

RECCURENT LARYNGEAL PAPILLOMA

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ABSTRACT

A case of respiratory papillomatosis was reported. The patient suffered from the disease since eight months old with chief complaint progressive hoarseness and dyspnea. It was diagnosed with respiratory papillomatosis and scheduled for performing tracheotomy and continued with the first microlaryngeal surgery (MLS). Decanulation was taken after 2nd surgery of removing papillomas. Finally was reported she got serial of surgery for 22 times during 18 years of age. It was costly and deteriorating quality of life. The problem remains persisted because of frequent recurrences and need for repetitive surgeries. Specimen biopsy for histologic examination was shown the signs of HPV infection, papilomatic coated squamous epithel with mild dysplasia and coilocytosis. The threatening of upper airway obstruction is the main important reason for patient's coming. The patency of airway assessed by Direct Laryngoscopy then the next treatment was decided with schedule of Micro Laryngeal Surgery (MLS). Finally the MLS treatment is just only for temporarily recovery. A further research to define the proper treatment in the future is required, especially for prevention of the diseases related to the viral causes of infection.

Key words: *recurrent, respiratory papilloma, HPV, microlaryngeal surgery*

INTRODUCTION

Respiratory papilloma has been known since the 17th century ago. This disease was first discovered by Marcellus donalus as "*warts in the throat*" that grows on the throat area. Papillomas may grow on the mucosa throughout the respiratory tract. Vocal cords are a common predilection obtained. The growth of tumors usually occurs in multiple and tends to grow recurrent.[1,2]

Papilloma of the nose is rarely obtained. Moreover, it can also be found type of sinonasal papilloma (inverted papilloma) and carcinoma can mimic the form of papilloma in the nose area.[3]

Patients were commonly found in difficulty to breath, dyspnea state, because of airway obstruction and indicated for performing tracheotomy. The disease leads to the expansion of papilloma growth and tends to increase morbidity. Considering from this point, early diagnosis is very important and need some efforts to avoid tracheotomy in patients.[4]

Since now Papilloma is remain a problem because of its frequent relapses and potential to threat airway obstruction that endangers the lives of patients. This problem more over complicated, cause there is no right treatment to overcome this problem so far. Even though various theories have been published but the results are not satisfactory yet.[5]

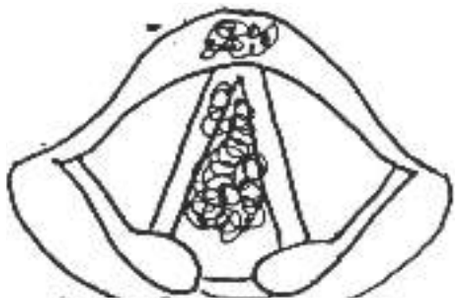
The purpose of this paper is to report a case of laryngeal papilloma in our Departmen, Departement of Otorhinolaryngology Head and Neck, Airlangga University, Dr. Soetomo General hospital.

CASE REPORT

March 17, 1994, in ENT outpatient, a young woman (RAF) 8 months old, was referred from a doctor, ORL-HNS specialist at the Dr. Soedono Hospital, Madiun city. She complaint with hoarsness since 3 months before. She looked cahectic.

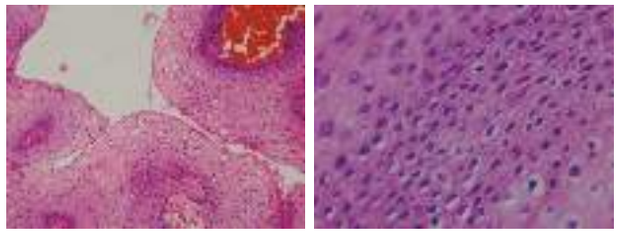
Examination on the ear and nose and thorat, showed no abnormalities. On direct laryngoscopy examination with the following results; Anamnesis hoarseness, sometimes dyspnea, coughing was not found and ate and drank well. Physical examination found mild stridor and intercostals

retractions. Direct laryoscope showed bump of mass which colour white pellucid, uneven, lookslike papilloma pharynx and larynx, in the glottis and supraglottis. It planned for tracheotomy and extirpation with Micro Laryngeal Surgery (MLS).



Picture 1. A scheme of direct laryngoscopy showed mass in the larynx, glottis and supraglottis

One day later the patient was performed tracheotomy and followed with MLS one month later with the following result, was seen mass bumps, which color is translucent white alike papilloma, located in the pharynx, posterior middle aritenoid, cricopharynx at 3, 6 and 9 hours. Then the tumor is extracted until it was clean and performed histopathologic examination.



Picture 2. Specimen from papilloma in the vocal cord biopsy when performing MLS, showing tissue sections shaped papilomatic coated with squamous epithel with mild dysplasia and coilocytosis, epithel in the surface and shown stroma with fibrous.

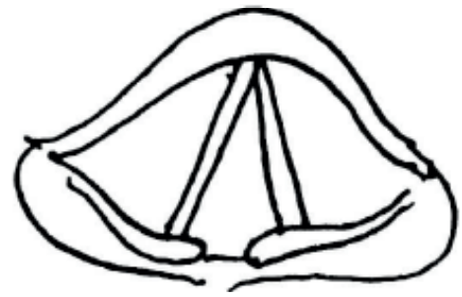
Patient came at May 11, 1994 (13 days later), without any complaints. On examination found trakeocanul installed and functioning properly. The pathologic anatomy result (No. L. 1598/94) with the conclusion: papilloma with coilocytosis (signs of HPV infection).

On September, 1994, four months later papillomas were still in the pharynx and larynx. Second MLS was planned next one month.

Tha patient returned 1 month after the MLS, there was no complaint and the examination didn't find growth of papilloma again. Decanulation was planned.

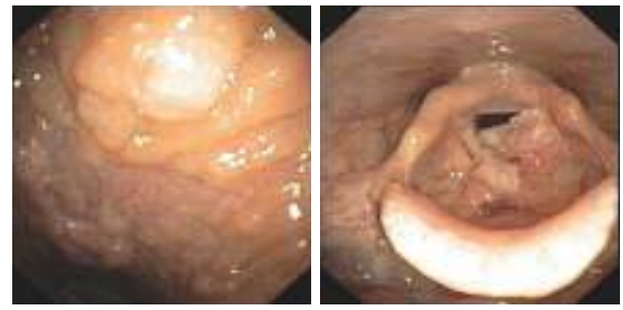
On December, 1994 (one month later), still found little papillomas in the oropharynx and one month later the situation remain similar. Decanulation was performed.

On February 15, 1995, papilloma became the less prominent. Likewise the following months, the situation remains the same until the month of November 1995 (20 months since the patient first came).

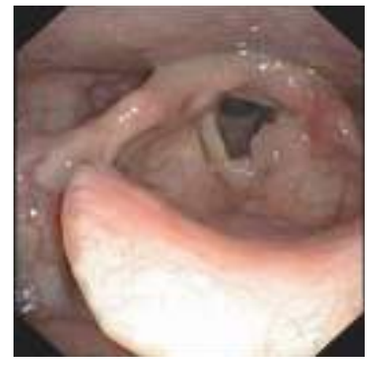


Picture 3. Endoscopic examination (illustration) on larynx, minimal growth of papilloma, and airway is wide enough

Recently status coming, the patient was 18 years old. She had been performed MLS for 22 times surgeries. We recorded the endoscopic examination at August, 2011. The picture wass shown below and after that examination, she performed the 22nd MLS for removed the papillomas.



Picture 4. Endoscopic examination on larynx. Left picture: papillomas growth on the pharynx, right: papillomas in the glottis.



Picture 5. Endoscopic examination after performed the 22nd, showed minimal papillomas in the larynx.

DISCUSSION

It was found a patient with papillomas which age were 8 months old when in the first arrived. Most patients with papilloma have age under 5 years.[6]

In adults, men are tends common occured, but the incidence in children is almost the same.[8] In this case, the patient is a women.

Tumors can grow along the respiratory tract and mouth (aero-digestive tract) and predilection the most common is in the larynx (97.9%–100%).[3,4] The growth of papilloma of the nose, are often in the histopathologic form of inverted papilloma (47%) and fungiformis papilloma (50%) than the cylindrical papilloma (3%).[15]

One of the factors causing papillomas is due to a viral infection. Any signs of HPV infection are found in both patients in the form of coilositosis cells, so that convince suspicion the virus as the etiological factor of disease.[1,9] This can cause by transmission from mother during delivery (60%).[4] But, the gynecological examination form the mother of the patient didn't found signs of condyloma. This possibility can occur because the patient's mother may have recovered from her illness at the time when examination performed (some time later after giving birth).

At first, papilloma is often confused with suspicion of allergic disease, asthma or croup.[5] Similar with the 2nd case, the complaint of runny nose and frequent epistaxis has suffered since 2 years before. Papilloma was diagnosed after one year later after the appearance growing mass in the left nose. Three months later, there were complaints of sound breathing and short of breath. Patients referred with the airway inflammation. But, the thoracic X-ray showed no abnormalities. Finally, the direct laryngoscope showed multiple masses in the pharynx and larynx, suggest papillomas.

Papilloma can show remission with increasing age.[6] In this case, a minimal tumor growth after 20 months later and the MLS has done frequently. Following the Papilloma growth getting fewer and steady, therefore, the tracheo-canule could be pulled out. Based on studies about papilloma that grows outside the larynx, it gives a better response to treatment (MLS).[5]

Serial of Microlaryngeal surgery (MLS) were performed repeatedly to they that need to excise the tumor, because of that, airway is free and sounds normal again. Decreasing of papilloma is expected to facilitate the body's defense system to eradicate the residual lesion, and then would accelerate healing. As is well known, larger size of the tumor, there is a lot of virus and more difficult to control.[17]

All patients performed emergency tracheotomy at the first time came at the emergency room (second case) and tracheotomy preparation for MLS a day after the examination of direct laryngoscope (first case). Actually, tracheotomy could be avoided if the patient came and diagnosed earlier. This procedure will cause a wound that may facilitate the implantation of new lesions in lower respiratory tract. Expansion to the tracheobronchial founded approximately 83% after tracheotomy.[2] This is a concern, especially in the second case where there is growth of laryngeal papilloma, with using tracheocanule can cause new lesions caused by friction of the canule.

Therefore, it's needed to evaluate the subglottic and trachea due to the expansion of laryngeal papillomas. The first case, where the larynx is clear from papilloma, there is no papillomas growth in tracheobronchial region although it has been performed tracheotomy. This is corresponding with the state that the papillomas growth in the trachea is always preceded by a laryngeal papilloma after tracheotomy.[8] We only have two papilloma patients without tracheotomy in our hospital. Examination on 11 and 13 months after first MLS didn't found any papillomas extension to the tracheobronchial. In 14 patients who performed tracheotomy, several of them were found down expansion to the tracheobronchial after 2nd or 3rd MLS (approximately 6–12 months). After that, interval time between MLS more short (1–2 months), even in one case the papillomas expansion has reached the left bronchus after the 23rd MLS (34 months later).

Tracheotomy is necessary when there is upper airway obstruction with grade III Jackson or show signs of respiratory failure. Meanwhile, when in grade I-II, could performed MLS with insufflations anesthesia techniques. However, this technique has never been applied so far, so tracheotomy performed for procedures such as in the case of the first MLS.

Decanulation done as early as possible when conditions are stable and papilloma growth stopped for at least 6 months. Likewise in the first case, growing of the papilloma was slight then pulled out the canule performed 10 months later and next 10 months showed minimal lesion.[5]

In addition, there are also two patients who have been decanulation after 6th and 10th MLS (2 yr and 3yr 5mo). Until tracheal, papilloma growth has stopped.

The existence of a large papilloma growth (diffuse, multiple) possibly because patient with low immune state (since the age of 8 months has reccurence of cough) thereby increasing aggressiveness of the disease. One factor in accelerating the remission of disease is to increase the immunity of patients, namely how to immunotherapy such as vaccination and administration of interferon.[10,16] This treatment is not yet a standard treatment at our institution.

Inverted papilloma of the nose, which its epithelial growth folding in to the stroma. HPV virus is a one of suspected etiology factor, these tumors are potentially associated with multiple papillomas along the respiratory tract and mouth. This is consistent with the results of studies using PCR techniques (Polymerase Chain Reaction) which have found HPV virus types 6, 11, 16 and 18 in the genital tract and respiratory tract. In the genital tract HPV types 6 and 11 found in the exophytic condyloma, but types 16 and 18 are found on flat condyloma with a high degree of dysplasia and invasive carcinoma. Similarly in the respiratory tract, HPV types 6 and 11 associated squamous papilloma and inverted papilloma, while HPV types 16 and 18 are found in squamous carcinomas.[18]

An important thing to differentiated from squamous papilloma is the nature of the invasive and the tendency to malignancy in inverted papilloma. Therefore, patients with inverted papilloma need to be having long-term follow-up of recurrence and risk factors of transformation towards malignancy. Interval changes of malignancy ranging from

5 to 20 years, with the incidence of 1.5 to 2%.[19] There was a report the occurrence of malignancies at the age of 20 years from one patient papillomas since childhood and has performed tracheotomy, ie bronchogenik carcinoma. Eventually the patient died after occured metastasis. Some experts associate inadequate incision and exposure to carcinogens such as radiation with materials, cigarette smoke with risk factor of reccurence and malignancy. Histologic examination found a representation of atypical epithelium and dysplasia.[17]

Aggressiveness of papilloma growth may be explained by histopathology examination, among others associated with the type of papilloma, the degree of cell atipia, mitotic index, the ratio of neoplastic epithelium with the stroma, and the presence of inflammatory cells.[13,15] It required a clear description of histopathology analysis results by including the factors mentioned above. Likewise, signs of viral infection should be included, for example coilocytosis, nuclear inclusion bodies or multinucleated epithelial cells.[15] Where possible to do on a regularly, such information will be able to add the epidemiological data that may be useful in overcoming this disease.

CONCLUSION

It has been reported a case of recurrent Laryngeal papilloma, threatened the airway and lead to obstruction in the larynx.

Tracheotomy should be avoided if patients can come earlier and early diagnosis is established.

The problem was still persisted with the high recurrence in children and treated temporary by Micro Laryngeal Surgery.

Inverted papillomas might have a greater risk for the occurrence of malignant transformation, then long-term follow-up is required.

Following study is necessary to explore further about pathogenesis of suspected viral infection in pregnant patients as resources to find a strategy in the epidemiological approach to disease prevention.

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Case Report

PAIN RELIEVED USING EXTRA ANATOMY PATHWAY IN ACUTE INFECTION

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ABSTRACT

Acute infection is characterized especially by pain as major complaint of patients. In this following case report, it will be shown that pain cause of acute infection can be relieved using acupuncture technique. Acupuncture use meridian as extra anatomy pathway.

Key words: *Pain, Meridian, extra anatomy pathway*

INTRODUCTION

During the long history of traditional Chinese acupuncture, the results of needling acupoints have been described both clinically and theoretically. The concepts of chi, blood, meridians, and acupoints are integral to the understanding and application of Traditional Chinese Medicine (TCM). Since its introduction into Western culture, there were many experiments and writings to attempt to explain these concepts in Western scientific terms. Many early explanations were shallow and simplistic, for example, that acupuncture is simply a primitive way of describing stimulation of the nervous system, or that it is only placebo treatment (Starwynn, 2001).

Recently a growing number of insightful researchers have penetrated further into the common truth between TCM and Western science, and their paths have led into the realms of electromagnetics and quantum physics (Starwynn, 2001).

Acupuncture involves the insertion and manipulation of needles into specific points on the body to relieve pain or for therapeutic purposes. In acupuncture medicine technique, a communication path exists aside from nervous, blood vessels and lymph vessels communication path. Meridian is not a nerve path, not a lymph vessels path, is also not a path of blood vessels. This particular communication path is known as energy communication path (chi) or is specially named as meridian. In acupuncture theory, it is mentioned that chi flows through the body's meridians. If this chi flows is disrupted, complains or symptoms according to the degree of disruption and the meridian where disrupted will appear (Yanfu,[1] 2002).

As early as in the ancient age, people began to use stone needle for medical treatments. Acupuncture therapy uses acupuncture points as the stimulating points and the relationship of meridian as basis of the treatment. Meridian consists of major channel and branches of channels, which refer to the network that runs *chi*, contact the viscera, communicate the internal and external and run through up and down inside the body (Yanfu,[1] 2002)

According to Gellman (2002), the body's bio energy flows through specific channels called meridian and regulates the whole body function of the body's organ. Meridian is channels which connect all the body's components. Aside from connecting all of the body's energy internally, meridian also connects the body's internal energy with external energy (natural energy) through "doors" called acupuncture points or acupuncture points.

Stimulation on acupuncture points will be transmitted meridian communication path. Then stimulation will affect circulation of the existing energy system, creating a healing effect, especially to meridian connected directly to the stimulated acupuncture point (Gellman, 2002). Diameters of the acupuncture points are approximately between on to three millimeters.

The depths from the surface of the skin are according to the place and different in each individual (Wensel, 1980). It has long been known that acupuncture points have some specific characteristics, at superficial acupuncture points, there are high electric potential (can reach as high as 300mV), high electric capacitance (0.1-lmF), low electric resistance, increased skin respiration, high local temperature, radiating light which spontaneously visible from Jing and Yuan points, and sound signals (frequency

2–15 Hz, amplitude: 0.5–l mV). At profound acupuncture points, there are low perception threshold to electric stimulation, high capacitance, electric resonance with the other acupuncture points, high conductivity to isotopic tracers (Starwynn, 2001).

Darras (1992) investigated the pathways of acupuncture meridians in the human body through the injection of radioactive tracers at acupuncture points. Technetium 99 m (99 mTc) as sodium pertechnetate, the most common radioactive tracer in nuclear medicine, has been used. The migration patterns were recorded with a scintillation camera associated with computer imaging capabilities. His findings show that the preferential pathways taken by the radiotracer coincide with acupuncture meridians as described in Chinese traditional medicine. More, it has been established that these pathways are distinguishable from either lymphatic or vascular routes.

CASE REPORT

A 68-year-old mother came up with right foot pain. Right foot pain caused by an iron rod pierced. Iron was piercing of the plantar toward back foot about 3 cm long (see Fig. 1). Diameter of the iron in about 6 mm. Puncture occurred 20 hours ago.

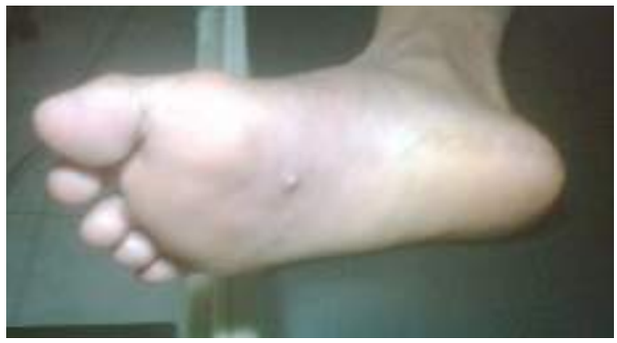

Figure 1. Wound location (private document, Camera in Black Berry Bold 9500)

Injury caused pain. Pain was felt increasing. Injuries also generate signs of inflammation do to infection. The foot swelled especially in the area around the wound, the plantar foot and the dorsum of the foot (Fig. 1). The color of the area around the wound flushed skin, increased body temperature. The temperature increase is felt throughout the body, the patient feels cold. Related to the pain increases, the patient could not stand upright, because the inversion of the foot should be positioned.

After examination, anamnesis and physical examination, the physician who examined propose to do therapy using acupuncture techniques.

Before performing the acupuncture therapy, the physician did conventional therapy in the form:
First, the doctor did conventional therapy in the form:

1. Clean the wound (debridement)
2. Closing the wound using gauze soaked in liquid antiseptic solution (betadine) and then closes the wound using hypafix (Fig. 2A).

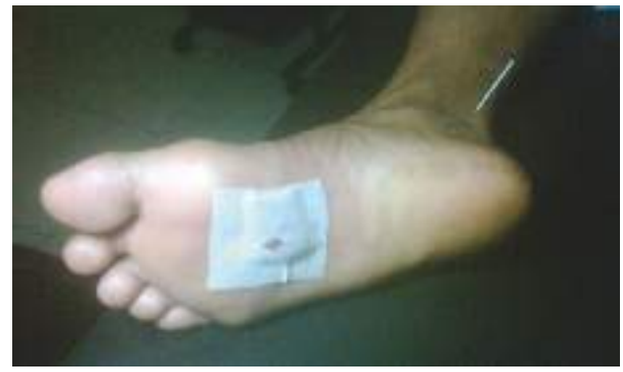

Figure 2. A. Closes the wound using hypafix (private document)
B. Punctured in Ki-3 acupoint (private document)

Furthermore, inspectors perform acupuncture therapy by acupuncture needle (stainless steel) sterile size 0.25 x 40 mm. Puncture perpendicularly 0.3–0.5 inch at the Kidney point-3 (Ki-3, Taixi), on the medial border of the foot. posterior to the medial malleolus, in the depression between the tip of the medial malleolus and Achilles tendon (yanfu,[2] 2002), (Fig. 2B).

Needle direction and rotated counter-clockwise. In this case report, point was chosen as an effective point for the body's energy flow in *kidney meridian* energy lines, especially for unblocked of body energy flow in plantar of foot (Yanfu,[2] 2002). Pain was relieved in about 45 seconds. Patients feel the pain decreased to about 80%. After that, acupuncture needle revoked, the person can stand in the direction normal standing foot (not invertion), a little pain.

CONCLUSION

1. Pain in acute infection can be relieved by puncturing the point of acupuncture.
2. The way of communication used in acupuncture is extra anatomy pathway.
3. This case report impressed the existence of meridian pathway. Another pathway common use in anatomy terminology (Abdurachman, 2005, 2009).
4. This case report impressed the existence of acupuncture points.

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Literature Review

USING LEARNING VECTOR QUANTIZATION METHOD FOR AUTOMATED IDENTIFICATION OF MYCOBACTERIUM TUBERCULOSIS

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ABSTRACT

In this paper, we are developing an automated method for the detection of tubercle bacilli in clinical specimens, principally the sputum. This investigation is the first attempt to automatically identify TB bacilli in sputum using image processing and learning vector quantization (LVQ) techniques. The evaluation of the learning vector quantization (LVQ) was carried out on Tuberculosis dataset show that average of accuracy is 91,33%.

Key words: *Mycobacterium Tuberculosis, Image Processing, Learning Vector Quantization.*

INTRODUCTION

In global terms, there are one billion people infected with tuberculosis at any one time. Eight million new cases arise annually making a prevalence of 16 million cases. Three million persons die annually from TB. However, despite these grim figures, even without the influence of treatment and immunisation, the incidence is not as high as it was in the last century. The annual risk of TB infection in South East Asia is 1 to 2.5 per cent. This represents an upward trend. In Indonesia. there are at least half a million new cases of TB per year and 175,000 deaths. Tuberculosis is the second killer of adults after cardiovascular disease and the most important killer out of all the communicable diseases.

At the local level, diagnosis is best achieved through microscopic examination of the bacillus in a sputum. Culturing the bacillus is expensive and impractical as it takes 6 weeks and X-rays can be misleading. Skin testing is recommended by WHO, however, it is not specific for human TB bacillus. Additionally, the size of the reaction is not always helpful as strong reactions may occur in healthy people with repeated occupational exposure to infectious tuberculosis patients and in those with old healed disease.

The purpose of this study is to automatically identify TB bacilli in sputum smears using image processing methods and learning vector quantization (LVQ) techniques. LVQ systems are flexible, easy to implement, and can be applied in multi-class problems in a straightforward fashion. Because LVQ prototypes are determined in the feature space of observed data, the resulting classifiers can be interpreted intuitively. Consequently, LVQ classifiers are widely used in a variety of areas including image processing tasks, medical applications, control of technical processes, or bioinformatics.

THEORY

Tuberculosis

Tuberculosis (TB) is a contagious disease caused by a germ called "Mycobacterium tuberculosis" or "M. tuberculosis". Germs or "bacteria" are tiny living organisms that reproduce by dividing, and can be shaped like a sphere, rod or spiral. They are present virtually everywhere. Some of them are harmless – others are very dangerous.

TB is manly spread by airbone transmission. The source of infection is a patient with pulmonary (or laryngeal) TB who expectorates bacilli. During coughing, speaking, or

sneezing, the patient produces tiny infectious droplets; these droplets dry out and remain in the air for several hours. Contamination occurs when these infectious droplets are inhaled. Sunlight and ventilation are effective in decontaminating the environment.

The infectiousness of a patient is linked to quantity of bacilli contained in his/her sputa. In adults and older children: sputum obtained spontaneously. Sputum emitted in early morning often shows a higher concentration of *M. tuberculosis*. Sputum smear microscopy allow a simple, rapid and reliable identification of patients, but has a low sensitivity. The reliability of this examination depends also on the proper preparation and interpretation of slides. Quality control checks must be regularly carried out in the laboratory.

Image Processing

The image processing techniques used in this investigation help in enhancing the images and highlighting features needed for the shape description of each bacillus in the image. Separate entities in the images, such as TB bacilli, will be referred to as objects (each object being considered as a separate region).

1. *Image Enhancement.* The main goal of image enhancement is to process an image in some way so as to render it more visually acceptable or pleasing. Some techniques of image enhancement are removal of noise, sharpening of image edges and 'soft focus' (blurring) effect.
2. *Edge Detection.* Edges are simply regions of intensity transition between one object and another.One of edge detection method is Canny edge detector. Canny aimed to develop an edge detector that satisfied three key criteria: A low error rate. In other words, it is important that edges occuring in images should not be missed and that there should be no response where edges do not exist. The detected edge points should be well localized. In other words, the distance between the edge pixels as found by the detector and the actual edge should be a minimum. There should be only one response to a single edge.
3. *Morfhological processing*. The aim is to identify and extract meaningful image descriptors based on properties of form or shape within the image. Morphological operations can be applied to images of all types, but the primary use for morphology (or, at least, the context in which most people will first use it) is for processing binary images and the key morphological operators are the relatively simple ones called dilation and erosion.
4. *Feature Extraction*. The aim is to process the image in such a way that the image, or properties of it, can be adequately represented and extracted in a compact form amenable to subsequent recognition and classification.

Learning Vector Quantization

The LVQ classifier is based on the principle of nearest neighbor, which is demonstrated in the Figure. 1, where Euclidean distance is basically used for calculating distance.

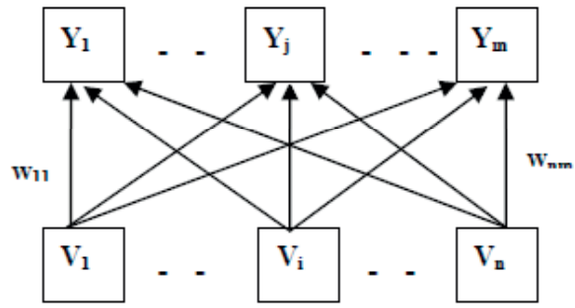


Figure 1. LVQ Architecture

Consider a sequences of labeled input samples {Xi: Xi Є R^n, i = 1, 2,…,N}; each sample input xi is tagged with its "correc class. Let t denote the number of training iterations. Assume that a set of cluster vectors {Ci: Ci Є R^n, i = 1, 2,…,m; m<<N}; have been obtained. The computing similarity and selecting criteria are unmodified but the adaptation is given by firs finding two closest cluster Ci and Cj vector to X and assume their distances to X are S1 and S2 respectively. Correction are made, two cases:

Case 1. Ci and Cj belong to different Classes, but one of them is correct.

If (S1/S2) > [(1-W)/(1+W)]
Ci = Ci-ά(t)[X-Ci]
Cj = Cj+ ά(t)[X-Cj]

Where X and Cj belong to the same class, while X and Ci belong to different classes.

Case II. Ci and Cj belong to same classes

Ck = Ck + e ά(t)[X-Ck]
For k Є {i,j} and X, Ci, Cj belong to the same class.

METHODOLOGY

In the proposed method, we first pre-processed the image of sputum using median filter. The median filter is used to preserving sharp high-frequency detail (i.e. edges) whilst also eliminating noise with minimal degradation or loss of detail in the image. Second, we partitioned the image into objects of defective areas, by performing Watershed Segmentation method. In watershed segmentation, we envisage the 2-D, grey-scale image as a topological surface or 'landscape' in which the location is given by the x,y image coordinates and the height at that location corresponds to the image intensity or grey-scale

value. Third, we extracted the image properties by using single parameter shape descriptors. The aim is simply to characterize a shape as succinctly as possible in order that it can be differentiated from other shapes and classified accordingly. These features extracted are fed to the LVQ, which is a supervised learning method of kohenon network, which uses competitive learning technique. The objective of LVQ based training and classification method performed, is to develop software to improve the process of automated identification of mycobacterium tuberculosis. The proposed system is having five different phases, which is shown in the following Figure 2.

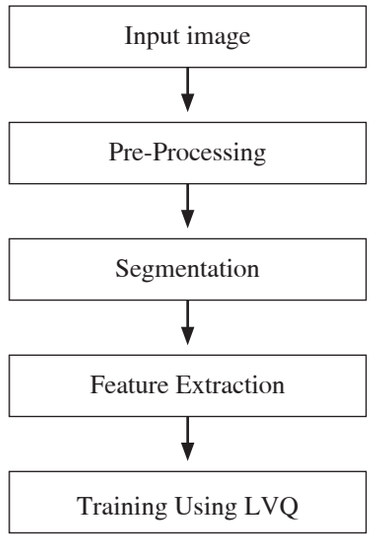


Figure 2. Design System

Training algorithm for LVQ

Steps:

1. Set the initial value of weight matrix and learning rate.
2. Execute steps from 3 to7 until end condition is true.
3. Execute steps from 4 to 5 for each 'V', where 'V' is the input vector.
4. Using squared Euclidian Distance calculate J as follows
   a. D(j) = ý(*wij* Σ *Vi*), where D(j) is squared Euclidian Distance wij is the element of weight matrix Vi is the element of input vector
   b. Find J with D(j) is minimum
5. Wj is updated as follows:
   a. if (T = Cj) then
      T is the target and Cj is winner index
      – wj(N) = wj(O) + α (V-wj(O))
   b. if (T! = Cj) then
      – wj(N) = wj(O) - α (V-wj(O))

   where,wj(O) is old value of wj wj(N) is new value of wj
6. Decrement the learning rate
7. Check for end condition, which may be fixed number of iterations.

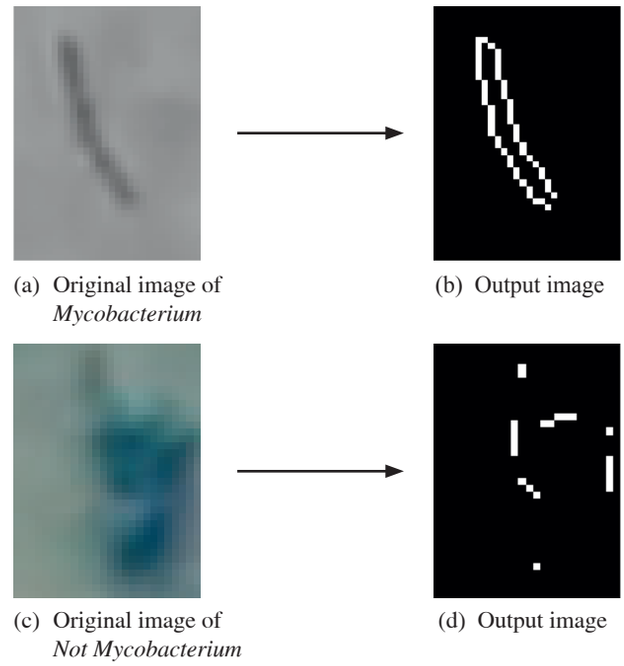
The weights are updated on each step for the process of learning. The weights will move closer to the class which will be winning class or else it will move away from the class. Once the training is finished, the LVQ will be able to recognize any unknown features which are not trained already.

RESULT

We used the 60 images of sputum in learning vector quantization system. The data was divided into a training set and a test set. A training set is 20 image of sputum which consist of 10 images *Mycobacterium* and 10 images are not. A test set of 40 objects (bacilli and non-bacilli) were used to test the system.

The input images are first passed through preprocessing stages. Watershed method is used for segmentation which gave a better result than region growing. The output of segmentation process are shown in the Figure. 3. Feature extraction process is a very important step where in proposed method for our application.

In the training phase, the learning vector quantization was applied on training data. Then, for data in the testing set, classification process seaches in this LVQ for finding the class that is closest to be attached with the object presented for categorization. Figure 4 present the experimental result for LVQ classifier. The figure show that the LVQ classification was successful for identification of Mycobacterium tuberculosis. The evaluation of the learning vector quantization (LVQ) was carried out on Tuberculosis dataset show that average accuracy is 91,33%.



(a) Original image of *Mycobacterium*

(b) Output image

(c) Original image of *Not Mycobacterium*

(d) Output image

Figure 3. The sample output of segmentation process

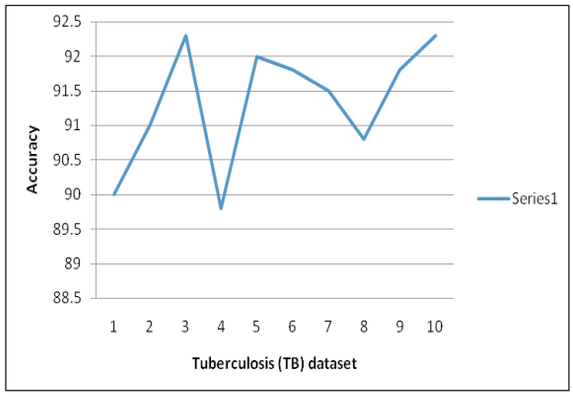


Figure 4. Accuracy LVQ classifier

CONCLUSIONS

In this paper, we presented learning vector quantization method applied to automatically identify TB bacilli. The evaluation of the learning vector quantization (LVQ) was carried out on Tuberculosis dataset show that average of accuracy is 91,33%.

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Literature Review

THE UVEITIS – PERIODONTAL DISEASE CONNECTION IN PREGNANCY: CONTROVERSY BETWEEN MYTH AND REALITY

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ABSTRACT

Background: *Recently, It had been recognized that oral infection, especially periodontal disease are potential contributing factors to a variety of systemic diseases, such as cardiovascular and cerebrovascular diseases, pregnancy problem, diabetes mellitus type 2, etc. However, the adverse effect of periodontal disease toward uveitis still not clearly understood especially if happens during pregnancy. Interestingly, in Indonesia, there is still a myth that pregnant women should not get any dental treatment, therefore, it may deteriorate periodontal disease during pregnancy.* ***Purpose:*** *to explain the possible connection between periodontal disease and uveitis and increase the awareness of these problems during pregnancy that could be understood by doctor and laymen.* ***Reviews:*** *literatures revealed that dental infection can caused uveitis via metastatic spread of toxin and inflammatory mediators. Additionaly, more recent investigation reported that the neural system may also stimulated by oral infection. In the orofacial regions there's trigeminal nerve complex that also related to the orbital region, thus may also involved in the uveitis pathogenesis. The effects of periodonto pathogens toxins toward immunocompetent cell and nerves had also been reported by researcher. Moreover, pregnant women are more susceptible to periodontal disease, therefore maintaining oral hygiene and dental monitoring is a mandatory.* ***Conclusion:*** *in woman who susceptible to uveitis, periodontal disease may exacerbate the symptoms especially in pregnancy. Therefore simple explanation about connection of oral infection-systemic diseases especially in pregnancy should be widespread among Indonesian people.*

Key words: *periodontal disease, uveitis connection, Indonesian, myth*

INTRODUCTION

In recent years there has been a reawakening of the dangers of oral infections and their potential disastrous effects on systemic health. Gingivitis and periodontitis is the potential sources of this oral infection. In modern dentistry by performing a treatment called scalling root planning, curretage or Assisted Drainage Treatment (ADT) must surely be one of the prime candidates for this reassessment. As dentists we are indoctrinated that it is better to keep the oral hygiene to prevent from any other diseases by teaching how to keep the oral hygiene.

Historically, periodontal disease was regarded as an infection caused by bacterial species that colonize the periodontal pocket. Microbial products trigger the release of proinflammatory cytokines and host derived enzymes, the excessive and/or dysregulated production may results in tissue breakdown. The impact of microbial products such as lipopolysaccharide (LPS) on induction of immune responses, toll like receptor (TLR) signaling and cytokine networks is crucial to inflammatory changes that develop in the tissues. Elevated levels of tissue-destructive enzymes such as matrix metalloproteinase's (MMPs) and proinflammatory cytokines can be detected in the gingival crevicular fluid (GCF) and saliva of patients with periodontitis. The pathogenic inflammatory mechanisms may lead to the development and progression of disease.[1]

A possible correlation of focal infection with uveitis could be predicted regarding to an object observation of a phenomenon that related to the uveitissymptoms.

Periodontal treatment that had been conducted to a patient suffered from symptoms uveitis was able to relief all of the symptoms.

A 30 year old female patient come to the clinic. She is a housewife and suffered from several symptoms such as headache, neck pain and spasm, eye redness; blurring of vision; watery; pain and sensitivity to light. The illnesses started 1 year earlier and the treatment and medications had already been conducted by general practitioner and ophthalmologist. Her doctor said that she got the uveitis.

There were a lot of prescribed drugs such as, Medison (corticosteroid) and Sandimun (corticosteroid). But she is not getting better and she come to the dentist. From physical examination, despite her moonface (because of the usage of corticosteroid long term), extra oral were normal, intra orally there were a lot of calculus deposits and gingivitis noted in all regions. Probing revealed that deep periodontal pockets (5 mm) existed in left and right posterior teeth maxilla and right posterior teeth mandible especially over M1 and M2. No caries was found.

Periodontal treatment in several literatures were able to reduce or eliminate several symptoms such as headache, sinusitis, fatigue, muscle pain or spasms.[2,3,4] The same result also occurred in this patient, who had no more headache, redness of the eye, blurring of vision and everything were normal again.

The purpose of this article review is to reveal the possibility of the periodontal disease involvement in the etiopathogenesis of Uveitis, based on the remarkable result of periodontal treatment to a patient suffered from uveitis. However, further researches should be done to support the validity of this successful clinical evidence-based case treatment.

LITERATURE REVIEWS

What is uveitis? Uveitis is an inflammation of the uveal tract, The middle layer between the sclera and the retina is called the uvea. The uvea contains many of the blood vessels which nourish the eye. Inflammation of the uvea can affect the cornea, the retina, the sclera, and other vital parts of the eye. Uveitis can also be related to diseases in other parts of the body, such as arthritis or may be caused by infectious agents (e.g., *Pneumocystis carinii*), may be idiopathic (e.g., sarcoidosis), or may be autoimmune in origin (sympathetic ophthalmia).[5]

The Symptoms of Uveitis include light sensitivity, blurring of vision, pain, redness of the eye and headache. [5]Nevertheless, there are several theories related to the etiopathogenesis of headache, such as the increase of pro-inflammatory cytokines level,[6,7,8] NO[6] involvement of the trigeminal nerve (V2) associated with the sphenopalatine ganglion (SPG)[9,10] and the "neurogenic switching" mechanism.[11]

Systemic Effects of Periodontal Disease. In abundant literatures reported the effect of periodontal diseases to systemic diseases such as cerebrovascular, cardiovascular diseases, diabetes mellitus type 2, etc. Several researchers also revealed the effect of periodontopathic bacteria part i.e. lipopolysaccharides, fimbriae, whole bacteria to systemic condition including allergy. According to a research by Utomo in 2009, by injection $LPS_{1435/1450}$ *Porphyromonas gingivalis* (*P*g $LPS_{1435/1450}$) with low dosage on a gingival sulcus maxillawistar rat. On the 14th day, had found the increases of mRNA SP and CGRP in the bronkus.[12] Moreover on the research by Abd El-Aleem et al., 2004 who injected *Salmonella typhimurium* intragingival on the papil interdental between first and second molars of wistar rat mandible. On examination with the hybridizatio in situ on days 3, 7 and 10 found an increase level of SP and CGRP mRNA in various branches of the n. Trigeminal, namely n. Mandible, n. Maxilla and n. ophthalmicus. In the study of LPS used and injected in the upper jaw Pg $LPS_{1435/1450}$.[13] And it is possible that periodontal disease can cause uveitis.

Host immune response and periodontal disease is a common, complex, inflammatory disease characterized by the destruction of tooth-supporting soft and hard tissues of the periodontium, including alveolar bone and periodontal ligament (PDL). Although the inflammation is initiated by bacteria, the tissue breakdown events that lead to the clinical signs of disease result from the host inflammatory response that develops to combat the challenge presented by the subgingival biofilm.[1]

DISCUSSION

Researches done by Li et al. revealed the possibility of the relationship between oral focal infection and non-oral diseases. Metastatic spread of infection from oral cavity which may be done in several ways were shown in Table 1 (Li et al, 2000).[14]

One of the systemic effects of infection is sickness behavior; it refers to the coordinated set of behavioral changes that develop in sick individuals during an infection. At the molecular level, these changes are due to the effects of local proinflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) which may also affected the brain if produced in sufficient concentration.[16,17]

The cytokine-induced sickness behavior symptoms such as fatigue, malaise, headache, sleep disturbances, inability to concentrate and other symptoms are due to the brain action of pro-inflammatory cytokines[7,17] and nitric oxide (NO)which is produced by inflammation and infection.[6] In addition, CFS is closely related with cytokine-induced sickness behavior.[16,17]

There is a possibility that Uveitis also related to cytokine-induced behavior. Bacterial endotoxins (lipopolysaccharides, LPS) are part of outer cell wall of Gram-negative bacteria. Lipopolysaccharide challenge upregulates the expression of endothelial cells adhesion

Pathway for oral infection	Possible nonoral disease
Metastatic infection from oral cavity via transient bacteremia.....	Subacute infective endocarditis, acute bacterial myocarditis, brain abscess, cavernous sinus thrombosis, sinusitis, lung abscess/infection, Ludwig's angina, orbital cellulitis, skin ulcer, osteomyelitis, prosthetic joint infection
Metastatic injury from circulation of oral microbial toxins.....	Cerebral infarction, acute myocardial infarction, abnormal pregnancy outcome, persistent pyrexia, idiopathic trigeminal neuralgia, toxic shock syndrome, systemic granulocytic cell defect, chronic meningitis
Metastatic inflammation caused by immunological injury from oral organism.....	Behcet's syndrome, chronic urticaria, uveitis, inflammatory bowel disease, Crohn's disease

(Adapted from Li et al., 2000[14])

molecules-1 and stimulate the release of high levels pro-inflammatory mediators by macrophages or monocytes such as IL-1β, IL-6, TNF-α, prostaglandin E2 (PGE2)[14,18] and NO.[18] Other effects are mast cell degranulation[19] and indirectly stimulate afferent nerve endings.[20]

In order to recognize the effect of stress to immune response, the study of psychoneuroimmunology should also be understood.[21] Stress consisted of stress perception and stress response.[22] Stress, mediated by CNS, activates the hypothalamic-pituitary-adrenal axis (HPA-axis) and increases the cortisol secretion.[16,21,23] At the same time, stress also activates the sympathetic-adrenal medullary axis (SAM-axis) to produce more catecholamines (noradrenalin and adrenalin).[21] Upon stressful condition, high-stress perception individuals also produce IL-1β, TNF-α and IL-6 that significantly higher compared to low-perception individuals. [24]

Pro-inflammatory cytokines are also capable of stimulating glucorticoid synthesis through the HPA axis.[16,21,23] Interleukin-6 which is also elevated by stress and adrenaline[25] is a potential stimulator of HPA axis resulting in cortisol secretion to help control the inflammation.[16] Unfortunately, high cortisol level depresses immune function.[21]

In this patient who had Uveitis, the stress in his work was suspected as the main trigger of the existing symptoms. Stress impaired body defense reaction to local infection. Altered mood and emotional condition may be involved in the periodontal disease, stress is suggested to affect periodontal health by increasing thelevel IL-1β, TNF-α and IL-6.[25]

As a consequence of unsuccessful elimination of oral focal infection, in this case periodontal infection, may perpetuate the systemic infection and the cytokine induced-sickness behavior did not come to an end. These never ending sickness behavior may be related to the debilitating symptoms.[16]

Oral inflammation may propagate to distant targets could be through the interplay of immunogenic and neurogenic inflammation.[20] Interplay between immunogenic and neurogenic inflammation is termed "neurogenic switching".[9,26]

Immunogenic inflammation may initiated by mast cell degranulation which induced by antigens, bacteria, proteoglycans, LPS, neuropeptides (i.e. substance P, SP), chemokines, calcium ionophores and physical factors.[27] Degranulated mast cells release histamine and tryptase which may stimulate neurogenic inflammation by binding to a protease activated receptor (PAR) in afferent nerve fibers.[20]

Additionally, pro-inflammatory cytokines and NO released by LPS-induced macrophage or monocytes, and bradykinin from damaged tissue are able to stimulate neuropeptides release from local afferent sensory fibers in the periodontal tissue. Stimulated nerve fibers release neuropeptides i.e SP, calcitonin gene-related peptide (CGRP), vasoactive intestinal peptides (VIP) and neuropeptide Y (NPY).[20]

There was a plausible explanation regarding to the instant disappearing of the symptoms which related to the oozed blood that occurred during the periodontal treatment. It was supposed to be an assisted drainage to the existing pro-inflammatory mediators (cytokines, PGE2, bradykinin, NO) in the periodontal disease which then may immediately "cut off" the neurogenic switching mechanism.[4]

There are several theories related to the etiopathogenesis of headache, such as the increase of pro-inflammatory cytokines level,[6,7,8] NO[6]; involvement of the trigeminal nerve (V2) associated with the sphenopalatine ganglion (SPG)[9,10] and the "neurogenic switching" mechanism.[11]

Headache symptoms in this case which accompanied by neck pain or spasm suffered by the patient according to several literatures are diagnosed as migraine.[28,29] Activated primary afferent neurons of trigeminal nerve sends impulses via trigeminus nucleus caudalis which acts as sensory relay center. Neck pain may resulted from the excitation of trigeminus nucleus caudalis which may extend to dorsal horn for stimulation of C2, C3 and C4.[28]

Periodontal ligament in the maxilla is also innervated by V2. Stimulated C fibers from maxillary periodontal ligaments (V2) may antidromically release SP and CGRP, this mechanism is proposed to be the etiology of sinusitis and migraine.[9,10] Therefore, through the neurogenic switching mechanism[20], periodontal inflammation may also directly affects sinus inflammation (mucosa and artery) through the neuropeptides release of SP and CGRP by afferent nerve of nasal mucosa via the sphenopalatine ganglion.[9]

The trigeminovascular reflex, which is related to intracranial arterial vasodilatation due to increase NO concentration or inflammation is a normal mechanism. Neurons of the first division of trigeminal nerve (V1) reported this condition to the trigeminal sensory nucleus. However, in certain individuals with elevated sympathetic tone or pre-sensitized afferent nerves may trigger headache.[9]

CONCLUSIONS

Periodontal disease is the source of LPS, proinflammatory mediators[14] including PGE2, NO and bradykinin[18] that were able to lower pain threshold of the afferent nerve fibers of the trigeminal nerve[30] (figure 1). The release of *Gingipains R*, a proteolytic enzyme from *P gingivalis* which triggers decreasedof blood flow, especially in micr ovasculatures,Gingipains R in the bloodstream can active factor IX, factor X, prothrombin, and C reactive protein, thus promoting a thrombotic tendency through the release of thrombin, subsequent platelet aggregation, conversion of fibrinogen to fibrin and intravascular clot formation.[14] Visual disturbances such as blurred vision and posterior uveitis,[14] may be induced by proinflammatory cytokines or LPS originated from the periodontal infection via the blood stream.[14] Another possibility is by neurogenic switchig mechanism related to afferent nerves of V I (ophthalmic division of trigeminal nerve).[33,34] Palpitation may be caused by noradrenaline or adrenaline, released in the state of stress to stimulate the body defense system, especially increase of heart rate and force heart contraction.[35]

The instant relief of headache, improve of eyesight and other symptoms after scaling procedures may be caused by decreasing of the "neurogenic switching" mechanism. The oozed blood during scaling should contain pro-inflammatory mediators, bacteria and LPS which may directly "cut off" the "neurogenic switching" mechanism.[4]

Gradual remission of pain and spasm in muscles should be caused from the diminish of hyperalgesia and sensitization of afferent nerve fibers which formerly caused by high concentration of PGE2, bradykinin and NO.

This review article base on an evidence based case of patient suffered from uveitis according to the patient's medical history and examined by a dental practitioner. Further studies with the true uveitis should be done in collaboration with competent medical practitioners and comprehensive medical diagnostic procedures.

Based on the remarkable result of the periodontal treatment and supported by literature reviews in case reported, it is concluded that a correlation oral focal infection, especially periodontal disease with uveitis symptoms should be exist. Further investigation should be done about the etiopathogenesis of periodontal – systemic related illnesses and increase the multidisciplinary approach in the scope of dentistry and general medicine to explore new interrelated cases.

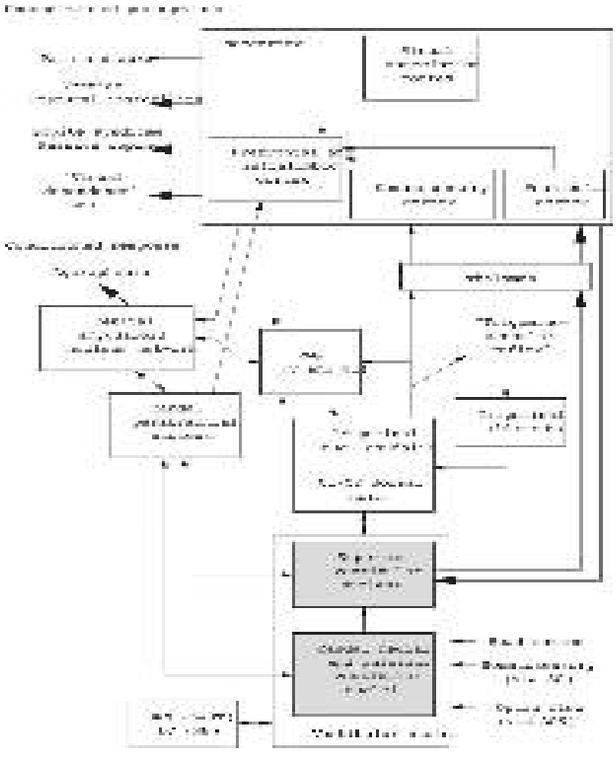

Figure 1. Pathogenetic model for uveitis and the relationship with periodontal disease (adapted from Furman et al., 2005).

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Research Report

DIGITAL DETECTION SYSTEM DESIGN OF MYCOBACTERIUM TUBERCULOSIS THROUGH EXTRACTION OF SPUTUM IMAGE USING NEURAL NETWORK METHOD

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ABSTRACT

Tuberculosis (TBC) is an dangerous disease and many people has been infected. One of many important steps to control TBC effectively and efficiently is by increasing case finding using right method and accurate diagnostic. One of them is to detect Mycobacterium Tuberculosis inside sputum. Conventional detection of Mycobacterium Tuberculosis inside sputum can need a lot of time, so digitally detection method of Mycobacterium Tuberculosis was designed as an effort to get better result of detection. This method was designed by using combination between digital image processing method and Neural Network method. From testing report that was done, Mycobacterium can be detected with successful value reach 77.5% and training error less than 5%.

Key words: *mycobacterium tuberculosis, digital image processing, neural network*

INTRODUCTION

Tuberculosis (TBC) is an dangerous disease and many people has been infected. Accurate and fast detection of *Mycobacterium Tuberculosis* is needed and very important to prevent TB before getting worse.

One of detection method that has been developed to detect *Mycobacterium Tuberculosis* is by sputum examination. Conventional detection method of *Mycobacterium Tuberculosis* through sputum examination using microscope can need a lot of time, so digitally detection method of *Mycobacterium Tuberculosis* was designed as an effort to get better result of detection.

The advantage of computation technology is in digital imaging and image detection. Computation technologies that can be used in medical research are Digital Image Processing and Neural Network (NN).

THEORY

Tuberculosis

Tuberculosis (TBC) is an dangerous disease, most of *Mycobacterium* infect lungs but it can infect another part of body. *Tuberculosis* can infect by cough, patient bring *Mycobacterium* to the air in sputum drop (droplet nuclei). Once cough can result about 3000 sputum drops. Usually, *Tuberculosis* can infect in a long time. Ventilation can reduce number of drop. Quantity of *Mycobacterium* in lungs can increase infection quality of *Tuberculosis*.

Digital Image Processing

Digital image processing is used in many kind of aim. One of them is to convert 24 bit true color image to binary format through some steps character extraction, noise filtering, grayscale, and threshold.

Grayscale

By using representation of RGB (Red, Green, Blue) value, a true color image is converted to white color and gradation of black color that is usually called by grayscale image.

Threshold

Threshold is data conversion of image in refer to the image just has two value 0 and 1. This step is done to get an information of pixel "High" or "Low".

Neural Network

Neural Network (NN) is a method than can useful in many goals, for detection, identification, and control. NN was designed to solve some problem through learning technique. NN algorithm is easy to learned and can be used in medical research is to identify medical image. Basic configuration of Multilayer Neural Network is like Figure 1.

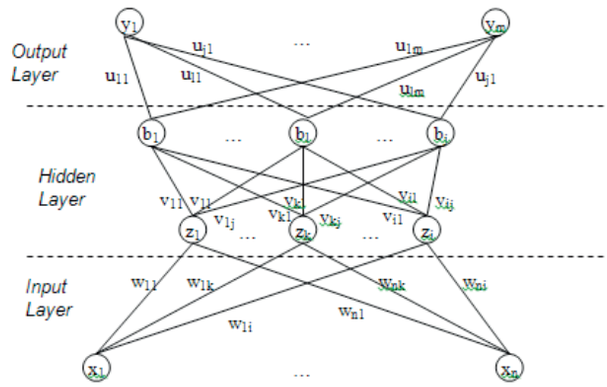


Figure 1. Basic Configuration of Multilayer Neural Network

Back propagation is one of some architectures of Neural Network. This architecture consist input layer, hidden layer and output layer, and every layer consists one or more artificial neuron. The name of this architecture is Multilayer Neural Network.

METHODOLOGY

This research was done by preparing digital image of sputum. Analog image of sputum was converted to digital using computer. 24 bit digital image of sputum was extracted to binary through digital image processing method. Pixel value at digital image was used as input of detection program. Program to detect digital image of *Mycobacterium Tuberculosis* was designed using Neural Network method. Neural Network method was tested by using sputum image and was compared to database that was recognized. Digital image of *Mycobacterium Tuberculosis* or not was used as input data of NN learning. If results of error compared to database, the value less than 5 percent and the conclucion should be done, but if error more than 5 percent then the design of program need to be repaired. Designed of research that was done was like at Figure 2.

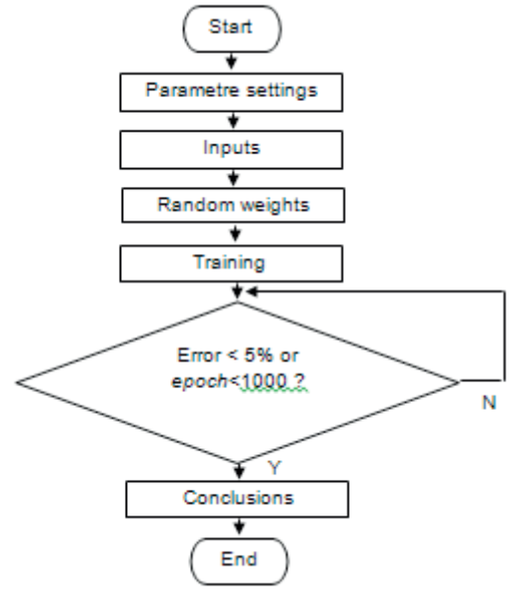
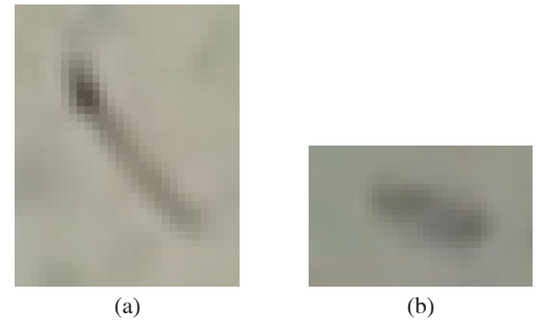


Figure 2. Algorithm of Detection using Neural Network

Detection method of *Mycobacterium Tuberculosis* through image extraction using Neural Network method was designed by using Back propagation type. Neural Network was build using 3 layer objects (one input layer, one hidden layer and one output layer).

RESULT

Input data of Neural Network in Bitmap format with dimension is 70 pixels x 70 pixels. Training data of Neural Network is digital image of sputum which consist *Mycobacterium* and not that was already detected. 20 images data was used as inputs in Neural Network training process with specifications 10 images consists *Mycobacterium* and 10 images are not. Example of Neural Network image data inputs is like in Figure 3.



(a) (b)

Figure 3. Input Image of Neural Network (a)*Mycobacterium* (b) Not *Mycobacterium*

40 images data was used as inputs in testing of Neural Network with specifications 10 images consists *Mycobacterium* and 20 images are not. Input image that was used in Neural Network testing process was not input image that was used in Neural Network training process.

Neural Network training was used to classify sputum image according to type of object that had been researched, sputum images which consists *Mycobacterium* and sputum images which not consists *Mycobacterium*.

Training of Neural Network was done by using Delphi 6.0 software. Program that was designed is shown in Figure 4.

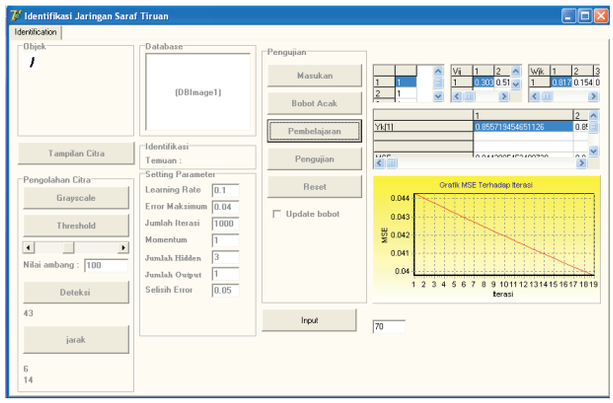


Figure 4. Training Process of Neural Network

In training process of Neural Network, some parameters value was set according to the aim. Parameters value that was set are dimension of input image, Hidden number, Output number, learning rate value, momentum value, limit of epoch, and limit of maximum error. Parameters value of Neural Network was shown in Table 1.

Table 1. Parameters Value Specification of Neural Network

Parameters	Value
Number of Input (pixel)	4900
Number of Hidden	3
Number of Output	1
Learning Rate	0,1
Momentum	1
Number of Epoch	269
Output value	0,1399
Target:	
– *Mycobacterium*	0,1
– Not *Mycobacterium*	0,9
Error	0,0399

Parameters value in Table 1 was used in Neural Network testing process. From the result of Neural Network testing that had been done using training parameter from Table 1, Neural Network training can be done well and error value of training reach criteria less than 5 percent.

Table 2. Successful Level of Neural Network Testing

No.	Image Type	Number of Input	Successful Detection	Successful Level
1.	*Mycobacterium*	20	16	80%
2.	*Not Mycobacterium*	20	15	75%

According to data in Table 2, mean of successful to detect *Mycobacterium* and not *Mycobacterium* image is about 77.5 percent. Result of Neural Network testing by using 20 input images of *Mycobacterium* and 20 input images of not *Mycobacterium*, 16 input images of *Mycobacterium* and 15 input images of not *Mycobacterium* was successfully detected according to the target. Some results does not match because the dimension of *Mycobacterium* is not suitable with the dimension of *Mycobacterium* in program setting. That is caused by *Mycobacterium* which were grow up a become longer than limit of dimension. This condition as difficulty in identification. There were noise of image so it can be affected performance of *Mycobacterium*. The resolution of camera was low so the contrast of *Mycobacterium* image was low too. It can caused by position of *Mycobacterium* and another image and it can influenced value, and result of detection. Coloring at sputum image can affected different illumination so comparison of image value can be different. Cropping position was not suitable, it can affected pixel value because maybe some part of image was out of cropping area. Threshold value was not suitable and it can be affected input of Neural Network. Successful level of *Mycobacterium* detection is like Table 2 that is 80 percent of *Mycobacterium* images and 75 percent of not *Mycobacterium* images or mean of successful level was reach 77,5 percent by using 40 inputs of data. This result is still need to be repaired by choosing better method so detection result will be more accurate, some efforts to get better result are by adding inputs of training data with many variations, repairing characteristic detection or characteristic extraction of *Mycobacterium tuberculosis* and not *Mycobacterium tuberculosis* images, modification value of Neural Network parameters so success level of Mycobacterium will higher than before, repairing digital image processing method, then performance of *Mycobacterium* image could be contrast, or need modification of detection method by adding good method.

CONCLUSIONS

The result of digital detection system of *Mycobacterium Tuberculosis* has been reached 80 percents succesful level of identification and less than 5 percents error value of Neural Network training.

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Case Report

THE UNUSUAL MANIFESTATION AND THE UPDATE MANAGEMENT OF DENGUE VIRAL INFECTION

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ABSTRACT

Dengue virus infection is one of the important health problem in Indonesia, although the mortality rate has been decreased but many dengue shock syndrome and unusual manifestation of dengue infection cases are difficult to predict of the earlier time for getting a good management. ***The Aim:*** *To make update management of unusual manifestation in dengue infection for getting a better health.* ***Material and Method:*** *Data were compiled from Dr.Soetomo Surabaya and Soerya Hospital Sepanjang Sidoarjo in 2009,2010 and 2011.The diagnosis of all cases were based on criteria WHO examination in Institute Tropical Disease for identified PCR and 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.* ***Result:*** *In 2009, three report cases with unusual manifestation of dengue virus infection: a. DHF grade III with liver involvement and had bilateral pleural effusion. b.DHF grade III with liver involvement and encephalopathy. c. DHF grade III with liver involvement acute kidney injury, myocardial involvement and encephalopathy. In 2010, two report cases with unusual manifestation of dengue virus infection: An eight years old girl with obesity suffered from dengue virus infection with liver involvement and bilateral pleural effusion. An eight months old girl with undernutrition, suffered from dengue virus infection with bilateral pleural effusion and liver involvement. In 2011, two report cases with unusual manifestation of dengue virus infection: On the first day admission ten years old girl with obesity suffered from dengue virus infection with bloody diarrhea. Unfortunately coincident with bilateral pleural effusion and ascites, recurrent shock occurred and making the doctor in charge could not help her, due to attack of Cardio Respiratory Arrest that immediately occurred. Ten months old Girl suffered from dengue virus infection with a right unilateral pleural efussion and liver involvement showed an attack of Cardio Respiratory Arrest making the doctor in charge could not help him.* ***Conclusion:*** *If we found unusual manifestation of dengue infection we should be thought a new method management with monitoring carefully.*

Key word: *Dengue, update management, revise criteria diagnosis & treatment*

INTRODUCTION

Dengue virus infection is one of the important heath problem 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 SOERYA hospital Sepanjang promoting update management.

MATERIAL AND METHOD

Data were compiled from Dr. Soetomo Surabaya and Soerya Hospital Sepanjang Sidoarjo in 2009, 2010 and 2011. The diagnosis of all cases were based on criteria WHO examination in Institute Tropical Disease for identified PCR and 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.

RESULT

There were seven cases reported in 2009, 2010 and 2011.

In 2009 there were three cases with unsual manifestation of dengue infection:

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 DHF 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 DHF 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 DHF grade III with liver involvement Acute Kidney Injury (AKI) and encephalopathy.

In 2010 there were two cases with unusual manifestation of dengue virus infection:

1. An eight years old girl with obesity on April 20,2010 suffered from DHF grade III which liver involment and had bilateral effusion. On the first day admission, she suffered from high fever 39º C, nausea, poor apetite, head pain. Laboratory examination revealed hemoglobin level 10,3g/dl, leukocyte count 10.000/mm^3 platelet count 180.000/mm^3. The working diagnosis was dengue fever. The following this admission, the clinical manifestation showed as severe case due to dyspneu with supine X-ray showed bilateral pleural effusion and ascites. Laboratory examination revealed hemoglobin level 11,5 g/dl, leukocyte count 8000/mm^3 and platelet 150.000/mm^3, IgM and IgG anti dengue test showed positive result. Therefore she had been managed as DHF grade III, On the tens days admission she recovered and discharge from SOERYA hospital sepanjang.
2. An eight months old girl with under nutrition,on April 4,2010 suffered from mild dengue fever with NS1 positive result.Laboratory examination showed hemoglobin level 10.5g/dl, leukocyte count 9000/mm^3, hematocryte 32% and platelet count 200.000/mm^3. But on the following day April 11,2010 she became severe clinical manifestation of dengue infection with bilateral effusion and liver involment. The laboratory examination showed hemoglobin level 9g/dl, leukocyte count 7500/mm^3 hematocryte count 30% and platelet 50.000/mm^3. After getting care in ICU she could be help and recovered.

In 2011 there were two cases with unusual manifestation of dengue virus infection:

1. Ten years old girl with obesity on August 19, 2011 suffered from DHF grade III with bloody diarrhea and showed an unusual clinical manifestation of dengue infection on the first day admission, the physical examination showed systole/diastole 120/70mm, pulse rate 120/minute, respiratory rate 32/minutes, temperature 36,5º C, Laboratory examination showed hemoglobin 9,0 g/dl, leukocyte 4.200/ml, platelet count 252.000/ul. Hematocryte 27,8%. On the following day the clinical manifestation became more severe. The laboratory examination showed hemoglobin 10g/dl, trombocyte decrease to 54.000/µl. hematocryte 36,5%. IgM and IgG anti dengue positive. The diagnosis of this case as dengue shock syndrome. Beside it she showed bilateral pleural effusion and ascites. Unfortunately the doctor in charge could not help her she died with showed cardio respiratory arrest.
2. Ten months old girl came from Siti Aisyah hospital Surabaya on August 13,2011 with DHF grade III showed clinical manifestation of fever 38,9º C. dyspneu, malaise, bloody vomite and looked severe illness. Laboratory examination showed, hemoglobin 10,6 g/dl, leukocyte 3.100/mm^3. platelet 100.000/µl. hematocryte 35%. The following day hemoglobin 11,7/mm^3 platelet 46.000/µl. hematocryte 36,6%.Therefore she was sent to ICU pediatric Dr. Soetomo.On the first day admission she looked pale appearance with unilateral pleural effusion and liver involment; on the following day August 16,2011 the doctor in charge failure to improve her and the patient death with showed cardio respiratory arrest syndrome.

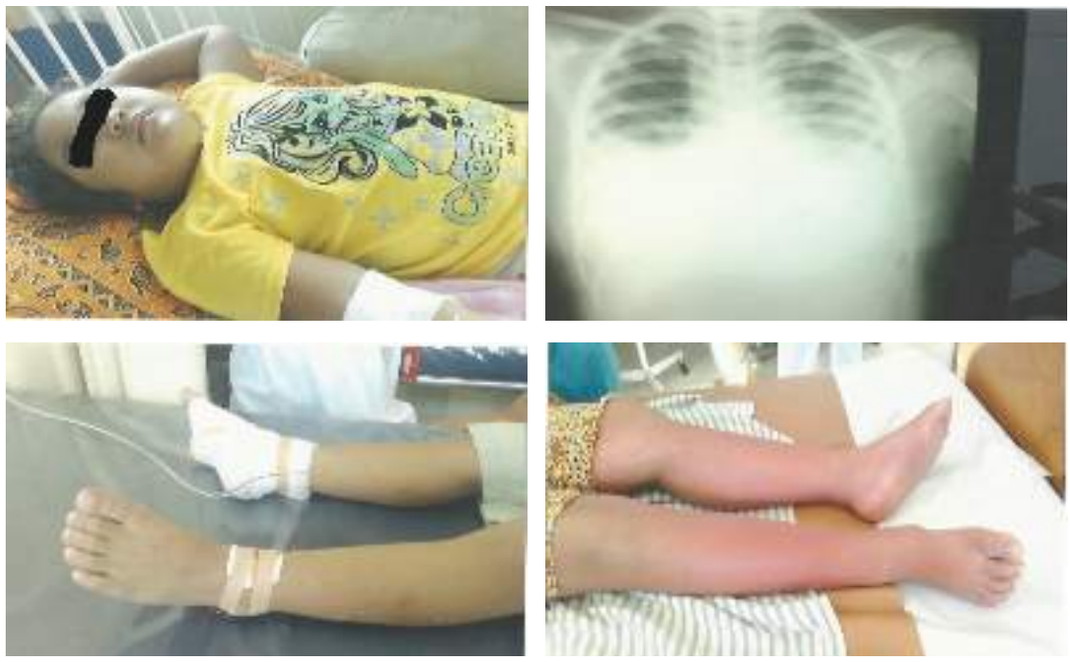


Figure 1. An eight years old girl with obesity suffered from dengue virus infection with liver involvement and had bilateral pleural effusion. She found in April, 2010 at Soerya Hospital Sepanjang.

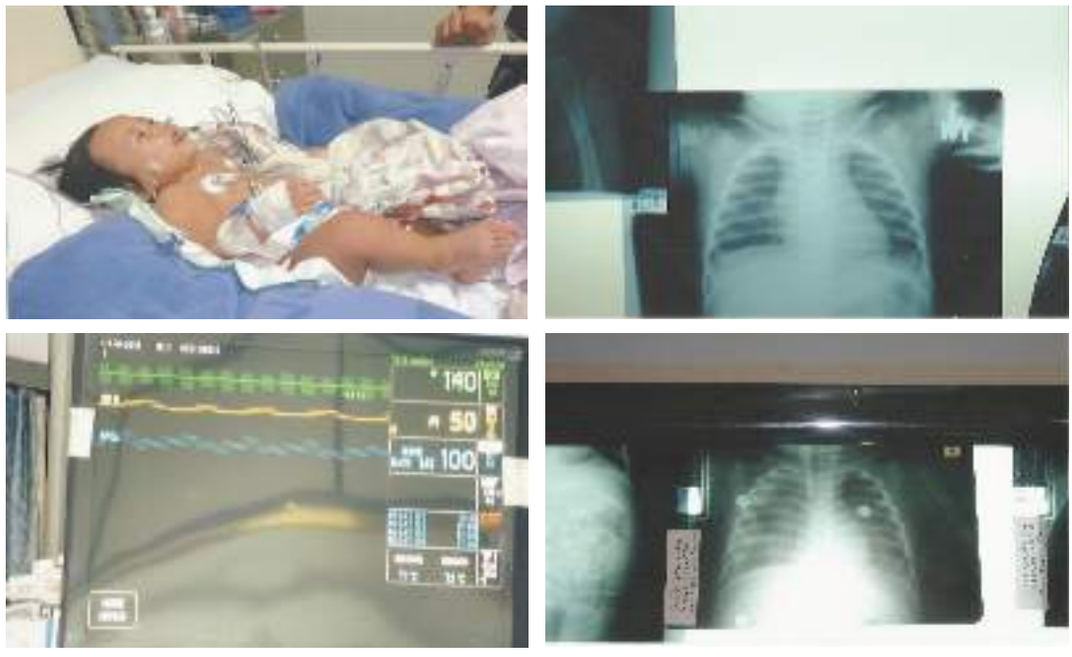


Figure 2. Under nutrition girl with dengue virus infection with bilateral pleural effusion and liver involvement.She found as a mild case of dengue virus infection on April 4, 2010 and than becoming severe and need ICU management on April 11, 2010.

DISCUSSION

What is an Unusual Manifestation of Dengue Infection?

It is a clinical manifestation that occure in a patient which don't follow the usual natural course of dengue infection:such as high fever with the axillar temperature show more than 38.5C: petechiea and, rumple lead positive with,abdominal distention, gastric pain head pain, vomite and malaise.This event due to a mild dengue virus. Therefore the host will make antibody for neutralizing infection dengue virus at the fifth until seventh days of clinical manifestation and the patient become recovery.

But in unusual condition the virus may become multiple and make dengue virus to spread in to some vital organs:

such as cardio vascular organ (heart), liver, lung, brain, and bone marrow. It can cause an increasing permeable vascular wall and plasma leakage occur to promote dengue shock syndrome: if the clinical manifestation is involving liver, lung and brain the patient will be looked as severe case and need a special monitoring. If we want to making clearly understanding for all; please focusing on up date pathogenesis of dengue hemorraghic fever that has been published in 2009.

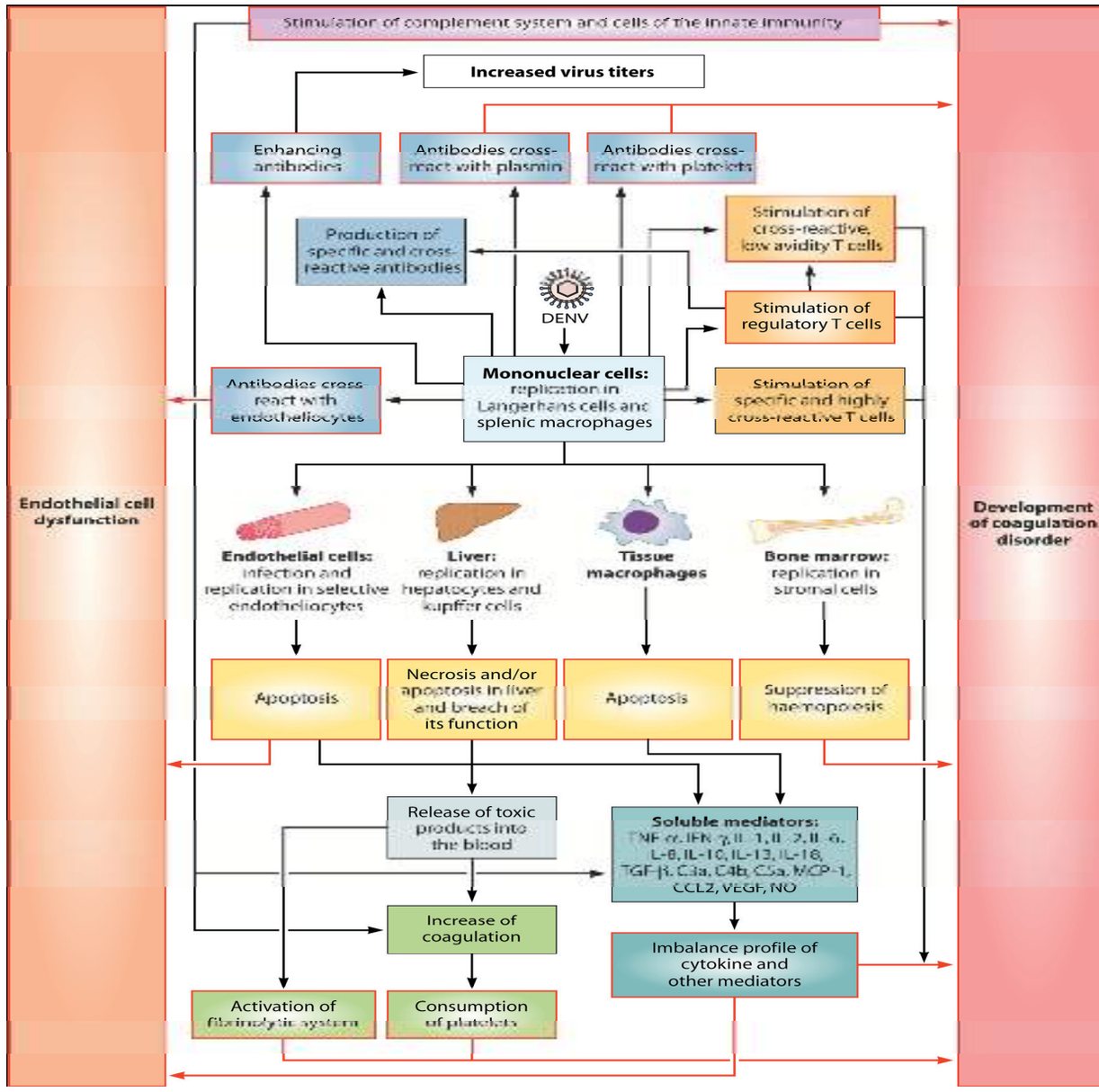
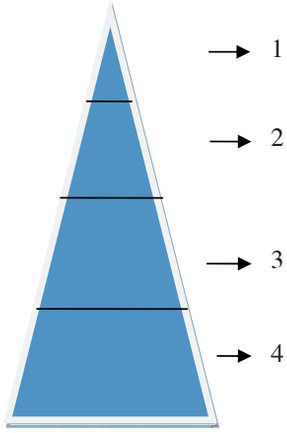


Figure 3. Up date pathogenesis of dengue fever in 2009

In 2009, 2010 and 2011, my team of dengue found many cases in usual manifestation of dengue, there fore I agree with a new idea WHO team which want to revise criteria diagnostic and management of dengue virus infection supporting the new idea WHO team in the International Congress Dengue in Jakarta; I present some sample unusual manifestation of dengue infection in 2009 (3 cases), in 2010 (2 cases), and 2011 (2 cases) I hope this information will give more perfect new idea for management dengue virus infection in the community and will get better result.

Based on criteria WHO 1979, two kinds clinical manifestation of dengue virus infection found in the communities. Those are dengue fever and DHF. The diagnosis has been done based on plasma leakage, that has been occurred in some patients. It is supported by virology examination in institute tropical disease center UNAIR Surabaya Indonesia. PCR examination and citopathogenesis of virus in cell culture of C6/36 cell is usually been done. Based on epidemiology study in community we found pyramid diagram as follow:



1. In the top of pyramide, we found a severe cases of DHF shock cases.
2. In the second part after the top of pyramide,we found many cases with shock syndrome of dengue hemmorrhagic fever, if we evaluated these cases are a) DHF grade III & DHF grade IV in this group we found more severe cases of dengue virus infection, which some of them can not be help, especially if the patient come late to doctor or health center.
3. The third part, we will found some mild cases of dengue fever and dengue hemorrhagic fever (Grade I & II).
4. In the fourth there are many cases with diagnosis of asymptomatic dengue fever.

Focusing in the year 2009, 2010, and 2011 we have many experiences to care severe performance of Dengue Virus Infection with unusual 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 for managing the unusual dengue virus infection we agree with new idea WHO team making new criteria diagnosis and classification of cases as followed:

During three decades, the World Helath Organization (WHO) has recommended the classification of dengue virus infection in: dengue fever (DF) and DHF (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

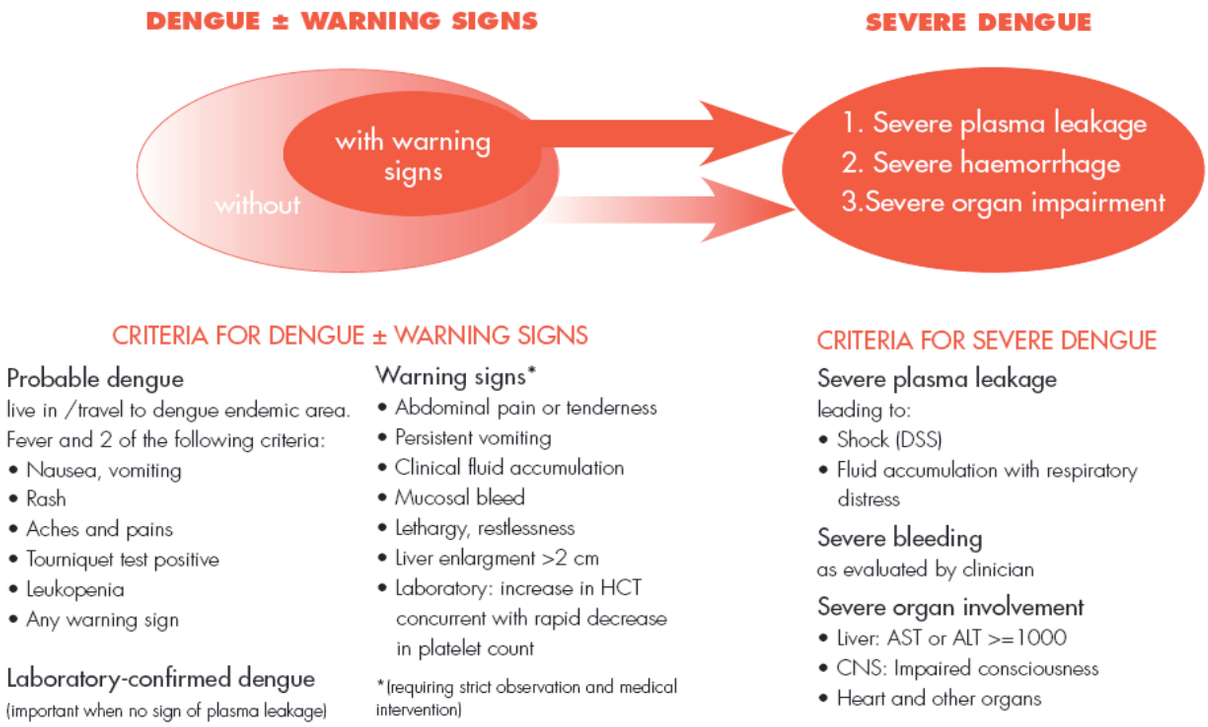


Figure 4. Suggested dengue case classification and levels of severity
Source: WHO, 2009. Dengue: guidelines of diagnosis, treatment, prevention and control – new edition. Genewa: WHO, p. 23.

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.

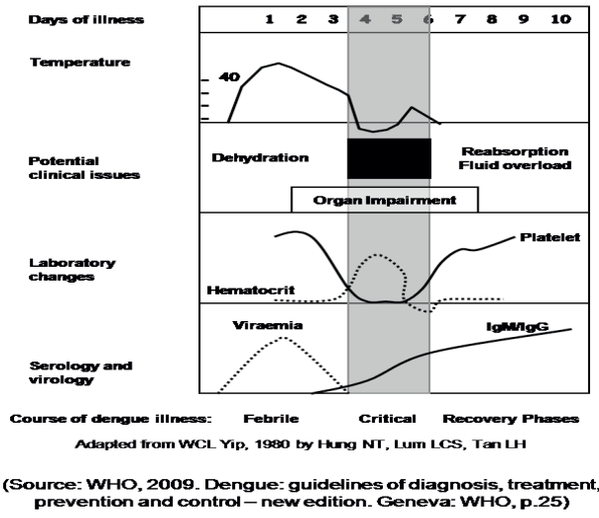


Figure 5. The Course of Dengue illness

In our cases, all the patients in 2009 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 was 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 DHF (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. To improve the leakage of plasma, new finding of 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. The reason is the ringer lactate crystalloid can not be metabolized in liver damage organ and the result could promote the severe liver dysfunction and the complication such DIC, bleeding can occurs. 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 involvements 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, and 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 but severe case had potentially fatal complication of DHF.

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; after using ringer acetat to change ringer lactate in all cases with had liver involvement were improved along their disease's improvements.

In 2009, all the cases showed liver involvement of DHF it mean we should be carefully to manage, don't use drug and fluid resuscitation that could cause liver damage, for example; acetyl salicylic acid, and ibuprovent and

crystalloid of ringer lactate.Why the solution ringer lactate can not be use in patient which has in creasing AST & LAT? As we know that solution ringer lactate usually can be used in normal liver organ, but if the liver organ damage the ringer lactate cannot be used, because the ringer lactate solution can make a bad physiology function of the liver organ, especially the blood cannot be coagulated and DIC can be occurred. Based on this information, we should change from using crystalloid ringer lactate to ringer acetate that usually metabolized in muscle of extremity.

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).

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. The reason is the ringer lactate crystalloid can not be metabolized in liver damage organ and the result could promote the severe liver dysfunction and the complication such DIC, bleeding can occurs. In a subgroup of predominantly DHF/DSS patients, severe liver dysfunction occurs and is a marker of poor prognosis.[8]

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 first resuscitation of dengue shock syndrome cases which had liver damage due to dengue virus infection. Based on this experiences please choose other crystalloid to change it if you found a case with liver damage. 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 sequelae 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 have 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 were improved along with the disease's improvement.

In 2010. We found a ten years old girl with obesity and an eight months old girl case with undernutrition, to manage these cases we should know that all of them had a decreasing respond immune reaction making recovery period longer, usually 5–7 day recovered to delay until 7–10 days. It means the doctor in charge should monitor carefully and taking more time.The second case firstly should a mild clinical manifestation of dengue fever but the following day became more severe and need ICU management and recovered.

In 2011 the first case showed as an unusual manifestation of dengue infection; on the first and second day on admission the doctor in charge did not thought as dengue hemmoraghic fever but always thought as gastro entero colitis and them patient was becoming severe and showed bloody diarrhea and shock.After getting information about laboratory examination the doctor in charge aware that this case was a patient of DHF grade IV: therefore she needed resuscitation as soon as possible but unfortunately the doctor in charge cannot do carefully and then patient

showed cardio respiratory syndrome and the patient cannot be help.

The second case ten months old girl suffered from dengue virus infection with a right unilateral pleural effusion and liver involvement, she came as a referal case from Siti Aisyah Hospital Surabaya with dengue fever grade IV,she looked pale appearance dyspneu and encephalopathy.After the doctor in charge had evaluated the resuscitation had been done identified that the fluid requirement by the patient had been given was over and the docter in charge cannot to improve her and the patient death with showed cardio respiratory syndrome.

For managing these severe cases who needed resuscitation,we should be carrefuly to manage; don't be too fast dropping solution in a minute. Please monitor carefully don't make overload fluid intake. If the doctor incharge cannot do carefully the cardio-respiratory arrest syndrome will occur and the patient cannot be help.

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.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 survive 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 patient. 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 ≥ 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 5).

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).

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 6).

Initiate intravenous fluid resuscitation with crystalloid or colloid solution (if available) at 20 ml/kg as a bolus given over 15minutes to bring the patient out of shock as quickly as possible. 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,

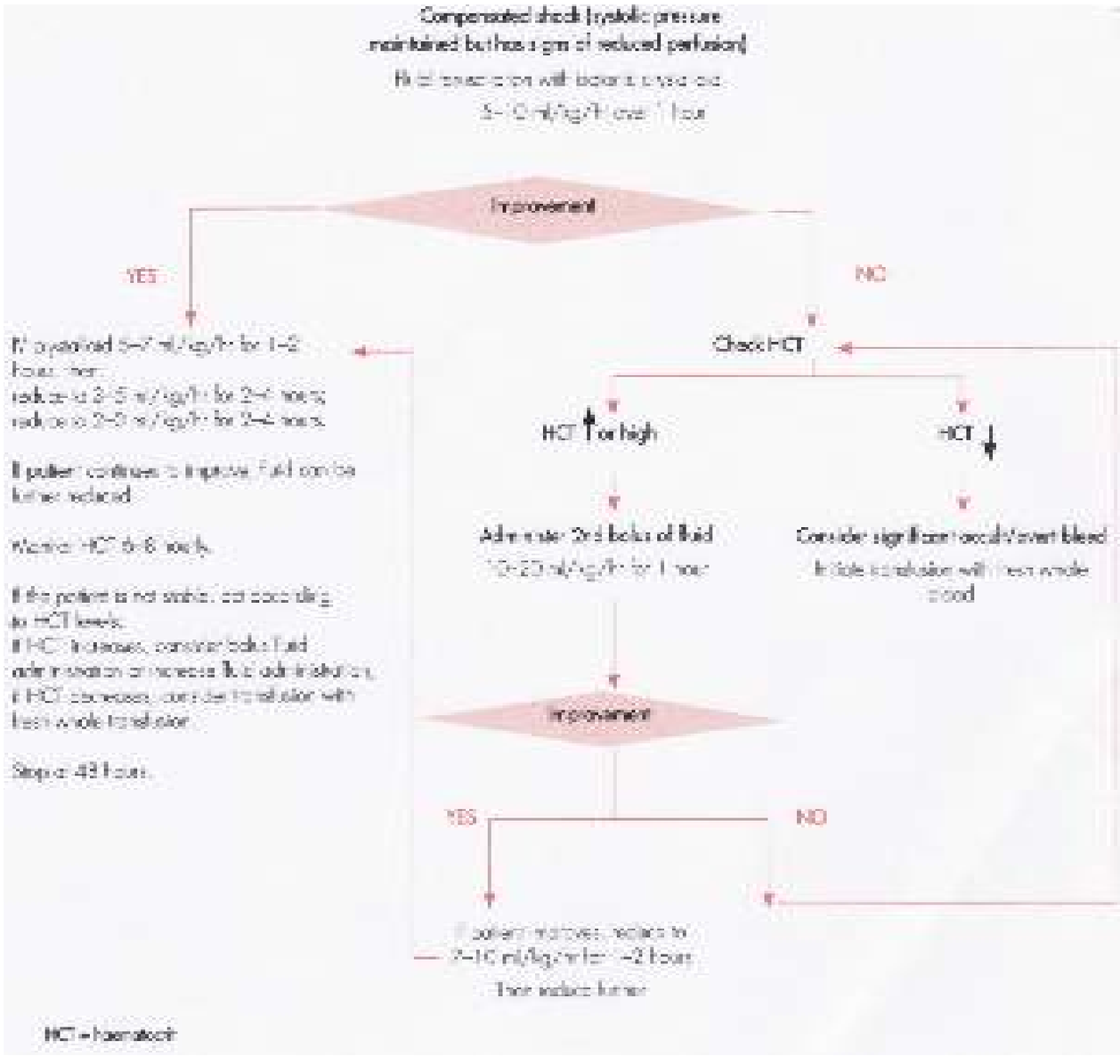


Figure 5. Algorithm for fluid management in compensated shock

< 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 haemorrhagic 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 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 to avoid 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 occur 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

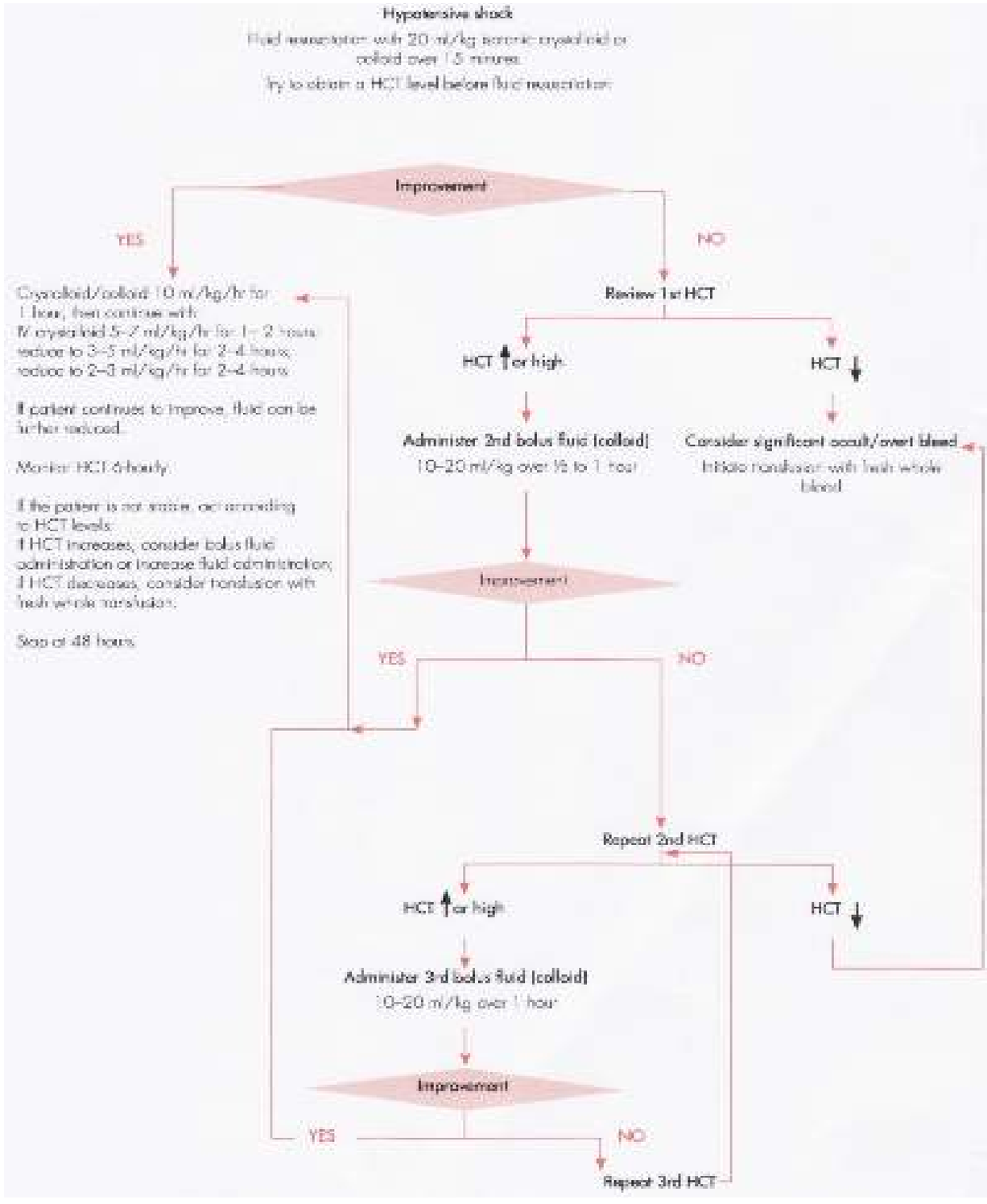


Figure 6. Algorithm for fluid management in hypotensive shock

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.

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).

Cause 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 pH $\geq$ 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

Seven 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 5 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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Literature Review

MODERN WOUND DRESSING FOR WOUND INFECTION: AN OVERVIEW

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ABSTRACT

When the tissue of skin is break means a wound is happens. To seal it, many choices of wound healing are available. Moist wound dressing can be better optional than the conservative ones. A bioactive agent that being added at the dressing in fact can increase healing rate of wound, moreover can subjugate wound infection caused by the pathogens, and also capable to prevent it. In this review, there are summary of modern moist wound healing, the wound pathogens, and some of sturdy bioactive agent for wound dressing. The treatment of wound infections using impregnated wound dressing by bioactive agent can also by antimicrobial agent that will striven against bacteria colonial.

Key words: *moist wound healing, wound infection, wound pathogen, antimicrobial*

INTRODUCTION

Skin and Wound

The skin serves as the body's interactive surface with the environment. As such, it is vulnerable to a variety of wounds, which differ by the type and severity of skin injury.[10] A ***wound*** is a break in the continuity of a tissue of the body, either internal or external. Wounds are classified as open or closed. An open wound is a break in the skin or in a mucous membrane. A closed wound involves underlying tissues without a break in the skin or a mucous membrane.

Any injury, unless it is very minor, may be harmful not only to the tissues directly involved but also to the functions of the entire body. Wounds that threaten life include those that produce cassation of breathing, severe bleeding shock, or damage to the brain, heart, or other vital organ.

The local effects of an open or closed wound may include loss of blood, interference with blood supply, destruction of tissues, nerve injury, functional disturbances, and contamination with foreign material. These effects often involve nearby uninjured tissues. Even superficial wounds sometimes take a week or more to heal. The healing process includes absorption of blood and serum that have seeped into the area, repair of injured cells, replacement of dead cells with scar tissue, and recovery of the body from functional disturbances, if there were any.[11]

Open wounds are injuries to the body that range from very minor, like a scratch that bleeds very little, to severe, such as complete removal of a body part. Open wounds can also range from those that bleed severely but are relatively free from the danger of infection to those that bleed little but have a greater potential for becoming infected. Treatment options vary according to the nature of the lesion. The two most serious first aid problems caused by open wounds are a large, rapid loss of blood, which may result in shock, and contamination and infection of exposed body tissue. Promotion of wound healing with minimal disfigurement and prevention of infection remain the goals of treatment.[10,11]

MODERN MOIST WOUND DRESSING

Alginates

Alginates are biodegradable dressings derived from seaweed. These highly absorptive dressings are soft, nonwoven, and nonadhesive, and conform to the shape of the wound. When in contact with drainage, they form a gel. Alginates (calcium or calcium/sodium) have an active ion

exchange of calcium ions for sodium ions at the wound surface forms soluble sodium alginate gel that provides a moist wound environment. Calcium dressings need moisture/exudate from the wound to function, therefore they are not suitable for dry wounds or wounds with hardened eschar. Alginates are most useful for wounds with heavy exudate. Don't use them for dry or eschar-covered wounds, because they won't form a gel and may stick to the wound, causing tissue trauma when they are removed. The fibrous nature of most alginates can leave residual fibres in the wound if there is insufficient wound exudate to gel the fibres. This may precipitate an inflammatory reaction as it stimulates a foreign body response. Caution is also needed when using alginate rope dressings in very deep or narrow sinuses, as complete removal can be difficult. Studies have shown that some calcium alginate dressings promote haemostasis in bleeding wounds due to the active release of calcium ions that aid the clotting mechanism.

Some alginates assist with debridement of nonviable tissue, and some contain silver, whose broad-spectrum antimicrobial activity minimizes bio-burden—the number of bacteria present on the product. If a patient has a silver dressing, it may need to be removed before magnetic resonance imaging (MRI).

Alginates come in sheets that can be cut to size. They also come in rope or ribbon form, which is especially good for areas of undermining or tunneling. For large wounds, though, alginates can be an expensive choice.

When using an alginate, it is necessary to cover the wound with a secondary dressing to hold the product in place and to protect the wound from outside contaminants. Leave the alginate in place for one to three days, until it begins to gel and shows evidence of breakthrough drainage. The way alginates absorb exudate is dependant on the make up of the alginate, for example some retain their integrity and can be removed in one piece; others disintegrate and need to be irrigated away from the wound bed. Before reapplying the alginate, the wound must be irrigate thoroughly with sterile normal saline solution.

Foams

Foam dressings are made from polyurethane, which may in some cases have been heat-treated on one side to create a semi-permeable membrane. This allows the passage of exudate through the non-adherent, semi-permeable surface into the insulating foam. This type of dressing is nonadhesive and comes in various sizes, shapes, and degrees of thickness. Foams are available in sheets or cavity filling shapes. Most foam dressings are available in bordered or non-bordered formats, which latter to be used if the patient has a skin sensitivity to adhesives.

Several advantages of foams are highly absorbent, cushioning and protective, insulate and conform well to body surfaces, provide thermal insulation. They facilitate a moist wound environment and absorb excess exudate to decrease the risk of maceration. Some lock fluid within the core of the dressing, others transform into a gelling foam. Foam dressings are also available with charcoal impregnation for malodorous wounds.

Foams may be used as a primary or secondary dressing, to promote autolytic debridement, and to inhibit hypergranulation. When using a foam dressing, make sure it's one to two inches larger than the wound. Depending on the level of exudate, foams can be left in place for up to seven days. They indicate when they need to be changed through the spreading discolouration that appears on the dressing. Foam wound cavity dressings reduce dead space in the wound, conform to wound shape and absorb large amounts of exudate, therefore reducing the need for frequent dressing changes. Cavity foam dressings require secondary dressings or tape/bandage to keep in place. Care is needed when adhesives are used to fix dressings in the elderly, as their skin is often fragile and prone to breakdown. Tubular retention bandages to fix dressing in place are a safer option in the elderly.

Hydrocolloids

Hydrocolloids are moisture-retentive dressings, which contain gel-forming agents such as sodium carboxymethylcellulose and gelatin. Although the name begins with the word hydro, these dressings do not contain moisture, but instead form a 'seal' at the wound surface. This prevents the normal daily evaporation of moisture from the skin. Many dressings combine the gel-forming properties with elastomers and adhesives which are applied to a carrier such as foam or film to form an absorbent, self-adhesive, waterproof wafer. Hydrocolloid dressings contain hydrophilic colloidal particles in an adhesive compound laminated to a flexible wafer. In the presence of wound exudate, hydrocolloids absorb liquid and form a gel, the properties of which are determined by the nature of the formulation. In sheet form the polymer outer layer can be either semi-occlusive or occlusive. Like foams, they come in numerous sizes, shapes, and levels of thickness. Some have tapered edges that are less likely to curl up.

Hydrocolloids have minimal absorptive capabilities. They help keep the wound moist and promote autolysis of necrotic areas. Don't use them on wounds that are infected or have heavy exudate. To avoid damaging fragile skin by removing the dressings too frequently, keep them in place for as long as possible, but no longer than seven days. Hydrocolloid interaction debrides by autolysis and can reduce dressing frequency to up to seven days wear time depending on the amount of exudate and the type of hydrocolloid dressing. Hydrocolloids are also available in paste and powders for increased absorption and to decrease dead space in the wound cavity. Warming the hydrocolloid dressing prior to application (while still in the packet) will make application easier and the dressing more conformable. Hydrocolloid dressings may also be cut to size to help conformability. The dressing should exceed the size of the wound by at least 2 cm. The wound itself will indicate when a dressing change is required by the accumulation of moisture within the dressing. When

removing a hydrocolloid dressing, support the surrounding skin. If the dressing is stubborn then submerge the limb in warm water in a bath or shower.

Generally, hydrocolloids with a waterproof backing are not recommended on clinically infected wounds due to the semi-occlusive nature of the dressing. There have been reports of hypergranulation with prolonged use of hydrocolloids in moderate to highly exudating wounds so wound tissue assessment is paramount when applying hydrocolloids for long periods. Hydrocolloids should be discontinued before hypergranulation occurs.

Hydrogels

Hydrogels are insoluble hydrophilic polymers with few absorptive properties, that expand in water. They are available in sheet, amorphous gel, sheet hydrogel-impregnated dressings, or gauze impregnated with various percentages of water. However, dressings generally contain between 60–70% water, which, with other constituents, is held in a viscous form known as the hydrogel. Alternatively, there are also hydrogel sheet dressings available, which contain less water.

Hidrogels designed to hydrate wounds, rehydrate eschar and aid in autolytic debridement. They add moisture to the wound bed and are nonadherent, provide a moist environment for cell migration and absorb some exudate. They're used mainly for dry and minimally exudative wounds, such as wounds containing necrotic or dead tissue. Dead tissue becomes hard and desiccated due to the loss of a blood supply and the application of a hydrogel dressing donates water to the dead tissue, softening it and aiding the body's process of autolytic debridement.

Hydrogels have marked cooling and soothing effect on the skin, which is valuable in burns and painful wounds. If stored in the refrigerator, they can provide cool relief to painful wound sites. Autolytic debridement without harm to granulation or epithelial cells is another advantage of hydrogel dressings.

Any hydrogel dressing should be large enough to cover the wound and at least 3cm of surrounding skin. It is important not to apply excessive amounts of hydrogel as this may cause skin maceration. The viscosity varies between dressings. The thick gels available which helps them stay in the cavity of the wound, while the thin gels allowing easy spread over a large area. Some amorphous gels contain propylene glycol that can cause allergic reactions in elderly skin. Amorphous hydrogels are applied liberally onto or into a wound and covered with a secondary dressing such as foam or film. Mostly hydrogel dressing require a secondary dressing to hold it close against the wound bed, either a film dressing or a hydrocolloid dressing can be used for this purpose.

If the patient is known to have skin sensitivities, then a hydrogel sheet should be used in place of a hydrogel dressing and this should be covered with padding and a bandage. Hydrogel sheet dressings can also be used in preference to hydrogel dressings if the patient has localised pain and cannot tolerate an adherent dressing. In this case, the hydrogel sheet can be held in situ with padding and bandaging or a film dressing.

Hydrogel dressings may require changing every 2–3 days and care must be taken not to macerate the surrounding skin with excessive amounts of hydrogel. But some of them can remain *in situ* for up to three days. The patient/carers should be cautioned that on removal the dressing may have changed in colour/consistency as a result of it removing debris from the wound bed. For easy removal of hydrogels the wound is irrigated. In addition to their use in wounds the thin hydrogels are helpful in the management of lesions such as chicken pox and shingles.[2,4,8]

Hydrofibre Dressings

Hydrofibre dressings are non-woven sodium carboxymethyl cellulose spun into white fibres and manufactured into sheet or ribbon packing dressings, which is applied dry. Aquacel, a hydrofibre dressing, maintains a moist wound healing environment as fibres convert to form a gel on contact with exudate. The vertical wicking of exudate reduces maceration of surrounding skin. Patients may occasionally mention a 'drawing' sensation as the dressing absorbs the exudate. It is used on moderate to heavily exuding wounds and must be changed when fully saturated with exudate. The dressings are claimed to be more absorbent than alginates and to promote non-traumatic dressing removal. However, these dressings may occasionally stick to the edges of a wound so it is advisable not to overlap onto the surrounding skin.[2,8]

Hydroactive Dressings

These multilayered highly absorbent polymer dressings, some with a surface adhesive and a waterproof outer layer, are similar to hydrocolloids. However, instead of forming a gel in contact with exudate, the fluid is trapped within the dressing, to maintain a moist environment.

Semi-Permeable Film Dressings

Film dressings are adhesive, thin transparent polyurethane, which are permeable to gas but impermeable to liquid and bacteria. Films are elastic, conformable and transparent allowing inspection of the wound. As films are non-absorbent they are not suitable for exudating wounds although island dressings with a central nonstick pad are available and can absorb slightly more exudate that the simple films. Films can also be used as secondary dressings to waterproof a primary dressing such as foam. Incorrect removal of film dressings may cause trauma to surrounding skin.[2]

WOUND INFECTION

A wound infection happens when germs enter break the skin. This germs, called bacteria, attach to tissue causing wound to stop healing, and other signs and symptoms. Wounds can be punctures (holes), lacerations (tears),

incisions (cuts), abrasions (scrape), or burn. Deep uclers (open sores), large burns, or bite wounds are more likely than other wounds to get infected. Wound infection can also happen in small wounds that were not treated.[12]

Terminology

It is important to have a clear understanding of the terms used for wound infection. Since 1985 the most commonly used terms have included wound contamination, wound colonisation, wound infection and, more recently, critical colonisation. These terms can be defined as:

- **Wound contamination -** the presence of bacteria within a wound without any host reaction.
- **Wound colonisation -** the presence of bacteria within the wound which do multiply or initiate a host reaction.
- **Critical colonisation -** multiplication of bacteria causing a delay in wound healing, usually associated with an exacerbation of pain not previously reported but still with no overt host reaction.
- **Wound infection -** the deposition and multiplication of bacteria in tissue with an associated host reaction.

In practice it seems that some experienced tissue viability nurses and medical practitioners use the term 'critical colonisation' to describe wounds that are considered to be moving from colonisation to local infection. The challenge within the clinical setting, however, is to ensure that the majority of practitioners recognise this situation with confidence and for the bacterial bioburden to be reduced as soon as possible, perhaps through the use of topical antimicrobials.[7]

Signs and Symptoms

The signs and symptoms of a wound infection are any of the following: [(12)]

- High or low body temperature, low body prssure, or a fast heart beat.
- Increased discharge (blood or other fluid) or pus coming out of the wound. The discharge or pus may have an odd color or a bad smell.
- Increased swelling that goes past the wound area and does not go away after five days. Swollen areas usually look red, feel painful, and feel warm when it is touched.
- Wounds that do not heal or get better with treatment.
- An old wound that bleeds easily.
- A wound that is painful, even though it does not look like it should be.

WOUND PATHOGENS

When a large number of bacteria get into wound, it can get infected. There are different types of bacteria. More than one type may infect wound at the same time. Normal bacteria that lives on skin often enter a wound first. A break in the skin gives them a chance to enter it and cause infection. Bacteria may also come from the invironment, such as soil, air, or water. If an object such as nail cause the wound, bacteria may come from that. A bit wound by an animal or person can cause infection from their saliva (spit).

The majority of micro-organisms are less than 0.1mm in diameter and can therefore only be seen under a microscope. They can be categorised into different groups, such as bacteria, fungi, protozoa and viruses, depending on their structure and metabolic capabilities.[7,12]

Bacteria

These are relatively simple cells that can be further categorised according to differences in their shape and cell wall. Cocci (spherical shaped cells), bacilli (rods) and sprirochaetes (spirals) can be arranged singly; however cocci and bacilli can also be found in pairs, chains and irregular clusters. They can be visualised using a bacteriological staining process called Gram staining; after Gram staining, Gram-positive bacteria are purple and Gram-negative bacteria are red. Species that fail to stain with the Gram reaction, such as Clostridia, require specialised stains. The growth and survival of all bacteria is dependent upon environmental factors, for example strict aerobes require oxygen whereas anaerobes are rapidly killed by oxygen. It is important to note, however, that both aerobes and anaerobes can survive in close proximity to each other and that some can survive in both conditions by growing aerobically and then switching to anaerobic metabolism in the absence of oxygen; these are known as facultative anaerobes.

Fungi

These are composed of larger more complex cells than bacteria. They are either single-celled yeasts or multi-cellular organisms with a nuclei contained within a cell membrane. Fungi can be responsible for superficial infections of the skin, nails and hair and, although they have been isolated from wounds, they are rarely pathogenic in this setting.

Protozoa

These are single celled organisms within a fragile membrane and without a cell wall. They are most significantly associated with infected skin ulcers.

Viruses

These are composed of genetic material (nucleic acid) enclosed within a protein coat or a membranous envelope. Although viruses do not generally cause wound infections, bacteria can infect skin lesions formed during the course of certain viral diseases.

It is important to remember that different micro-organisms can exist in polymicrobial communities and this is often the case within the margins of a wound.[7]

TREATMENT

Once a diagnosis of wound infection has been confirmed and antibiotic sensitivities identified, appropriate management regimens should be considered, with a high priority given to reducing the risk of cross infection. It is important to treat the patient as a whole and not the infection alone, so management strategies must be based on data derived from an holistic assessment of the needs of the individual. The main treatment objective will be to reduce rather than eradicate the bacterial burden within the wound margins. In addition to antibiotic therapy, there are two main generic groups of wound management products that have the potential to reduce the bacterial burden in the wound, these are compounds containing silver or iodine.[7]

Iodine

Iodine is an element that has antiseptic properties. It is active against a number of pathogens, so that iodine dressings can control bacteria on the surface of the wound. Iodine dressings have a maximum dosage that may be used at any one time and a maximum length of time over which they can be used. The dressing changes colour from deep yellow to white as the iodine is used, clearly showing when the antimicrobial activity is exhausted.

In wound management iodine is used in two forms, they are Cadexomer iodine - a polysaccharide starch lattice containing 0.9% elemental iodine that is released on exposure to wound exudate, and PVP-1 (Povidone iodine) - an iodophor composed of elemental iodine and a synthetic polymer.

Both have different physical characteristics that relate to the component parts and the iodine concentration of available iodine that is released when in use. Clinically iodine is indicated for wound cleansing, wound bed preparation (the stimulation and influence of specific cells involved with the immune system) and the prevention and management of wound infection.

In the past, iodine use has been limited by the fact that elemental it can be absorbed systemically, is almost insoluble and can be an irritant to the skin. Contraindications include patients with thyroid disease, patients with large or deep wounds, and patients with a known allergy to iodine. Iodine products can cause thyroid disruption. If patients have a history of a thyroid disorder, their thyroid function should be checked before and while using the product.

Silver

Recently a number of dressings containing silver have become available, although silver and silver compounds have been routinely used in clinical practice as bactericidals for over a century. Silver have a broad spectrum antimicrobial, effective against a range of aerobic, anaerobic, gram positive and gram negative bacteria as well as filamentous fungi and viruses; *Pseudomonas aeruginosa* and *Staphylococcus aureus* are some of microorganisms that silver effectively can handle. Silver interferes with the bacterial electron transport system and inhibits the multiplication of the bacteria. However, to achieve this, silver ions have to be able to enter a cell. The chemical bonding of silver with a sulphonamide antimicrobial - sulphadiazine - has resulted in the development of a safe broad-spectrum agent for topical use (eg Flamazine). In this formulation silver is released slowly from the transport medium in concentrations that are selectively toxic to micro-organisms such as bacteria and fungi.

This type of silver product has been used successfully in the management of acute and chronic wounds. Products that can sustain the interaction of silver with micro-organisms in the exuding wound are likely to be more effective in preventing/controlling local infection as potentially more silver ions will be available to enter bacterial cells. This assumes that the concentration of silver in the solution is both correct and maintained.

Another advatage of silver is that no resistant strains have been discovered. Despite the absence of resistance in clinical practice, it has been possible to produce resistance in the laboratory setting by using sub-therapeutic levels of silver.

There is controversy about the optimal amount of silver required to achieve a balance between efficacy, toxicity and the potential for resistance. When a silver dressing is selected, the amount of available silver should be considered as well as whether the silver is released from the dressing or the bacteria is drawn into the dressing. The importance of choosing a product with a clinically relevant dose of available silver and recommends that treatment stops once the objective for selecting that dressing has been achieved.

Silver products may cause localised discolouration if they come into contact with the skin surrounding a wound; this should wash/wear off within a few days. A rare but more serious complication of using silver products is argyria, which occurs when cells absorb silver salts; this results in a permanent discolouration of the skin (called Argyria). If the silver is absorbed by internal organs it may impair their function.[1,3,7]

Honey

Most research carried out on honey has focused on the role of manuka honey, which, like most honeys, releases hydrogen peroxide but is also believed to have an additional antimicrobial agent known as the unique manuka factor (UMF). It is widely claimed that honey is able to deodorise and debride wounds and these additional properties may be particularly beneficial in infected wounds. There are no standardised protocols for the frequency with which honey should be applied and the type of secondary dressing that should be used.[3]

Antimicrobial Therapy

Antimicrobial are chemical substances that have the capacity, in dilute solutions, to selectively inhibit the growth of or to kill other micro-organisms. Antimicrobial agents can be as synthetic compunds or natural compounds. Many research has proved that plants can provide strongly

recomended agents as antimicrobial which also have high activity to bear micro-organism.

Antibiotics are a kind of antimicrobial that produced by a micro-organism. Whereas it is now generally accepted that systemic antibiotics are essential for the management of clinically infected wounds, the choice of antibiotic to be used is not always apparent. Only after a comprehensive assessment process including consideration of patient characteristics, the results of microbiological investigations and the identification of both the nature and location of the wound, can the most appropriate antibiotic be identified. The routine use of topical antibiotics is not justified for colonised or infected wounds. Unfortunately, the resistance to antibiotics has become a serious problem in recent years particularly with the rise of epidemic strains of MRSA (Methicillin-resistant *Staphylococcus aureus*). The overuse of broad-spectrum antibiotics will only serve to exacerbate the situation. It could therefore be argued that all antibiotic use should be based on known sensitivities. That is why, many other antimicrobial agents are still been hunted until this seconds, whether they are from synthetic precursors or from isolated substances of natural extract.

In addition, a recent systematic review of antimicrobial agents has concluded that systemic or topical antimicrobials are not generally indicated for the management of chronic wound infections. However, there may be some value in the prophylactic use of topical antimicrobials for the initial management of acute cellulitus, whilst awaiting clarification of antibiotic sensitivity and the establishment of a therapeutic regimen.[7]

ANTIMICROBIAL AGENTS FROM PLANTS

A wide source of bioactivity compounds is plants. Finding healing powers in plants is an ancient idea. Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First, it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians. Second, the public is becoming increasingly aware of problems with the overprescription and misuse of traditional antibiotics.[6]

Garcinia mangostana (mangosteen) is one of plant that have a precious substances, called mangostin, since they are known for having a very high activity as antioxidant and antimicrobial. Mangosteen it self is a small purple fruit about the size of a tangerine, which has traditionally been used to treat inflammation, dysentery, and skin disorders. It was also used to fight infection by boiling up the fleshy part of the rind, also know as the pericarp, into a tea. As early as 1855, a German scientist identified mangostin, a powerful antioxidant, known as a xanthone, which appeared to fight infection, fungus, bacteria, histamines and possibly cancer. Xanthones can be extracted from the root bark, stem bark and sap as well as rind [exocarp]of the Mangosteen fruit.[5]

Among more than 40 mangosteen xanthones found in the whole mangosteen fruit, alpha-mangosteen and gamma-mangostin have been the main subject of many studies by mangosteen researchers around the world. Scientists demonstrated that gamma-mangostin proved to be a stronger antioxidant than other compounds long known for their antioxidant properties. Medical researchers in Thailand and Taiwan discovered that gamma-mangostin was shown to have even more potent antioxidant activity than vitamin E, one of the most powerful antioxidants known to science.

Alpha-mangostin was proven to have strong antibacterial activity against Staphylococcus aureus. Scientists demonstrated alpha-mangostin's activity against vancomycin-resistant Enterococci (VRE) and methicillin-resistant Staphylococcus aureus. They found that alpha-mangostin, alone or in combination with gentamicin, against vancomycin-resistant Enterococci (VRE) and used in combination with vancomycin hydrochloride against methicillin-resistant Staphylococcus aureus (MRSA) might be useful in controlling VRE and MRSA infections. Further studies shown that alpha-mangostin works well with and enhances the effects of other commercially available antibiotics such as ampicillin and minocycline. In another laboratory experiment, scientists studied the antimicrobial activity of alpha-mangostin and found that it possesses strong inhibitory effects against Mycobacterium tuberculosis and *Pseudomonas aeruginosa*. Researchers also found that alpha-mangostin is a histaminergic receptor-blocking agent: alpha-mangostin is effective in preventing or stopping allergies.[9]

IMPREGNATED WOUND DRESSING BY ANTIMICROBIAL

Antimicrobial dressings can be used on acute or chronic wounds which are critically colonised, or when local and/or systemic infection is already compromising the wound or could compromise wound healing. When choosing an appropriate wound dressing it is vital to assess whether the wound is colonised, critically colonised or infected. The presence of microorganisms in a wound does not indicate that wound infection is inevitable; indeed, they may have a protective effect. The presence of multiplying bacteria in a wound with no host reaction is termed colonisation.

Critical colonisation has been defined as the multiplication of bacteria causing a delay in wound healing, usually associated with an exacerbation of pain not previously reported, but still with no overt host reaction. Signs of critical colonisation include: a continued delay in healing despite appropriate treatment, thick slough that does not respond to standard debridement techniques, fast returning slough after sharp or larval debridement, and malodour.

Levels of bacteria in critically colonised wounds need to be reduced to allow the wound to heal. The topical

application of an antimicrobial is probably the most effective way to do this. The development of an infection will be influenced largely by the virulence of the organism and the immune status of the patient. Patients considered most at risk are those being treated with long-term steroids and those receiving chemotherapy.

If a wound appears to be infected, it is important to confirm this and identify the causative organism(s) and possible sensitivities to antimicrobial, as antibiotic. A wound swab should be sent for microbiology, culture and sensitivity. The use of systemic antimicrobial is supported where there are clear signs of infection. This is best achieved by starting a topical antiseptic dressing as soon as the signs of critical colonisation are detected.[1]

CONCLUSIONS

Many of kind modern moist wound dressings can facilitate wound treatment to be more effectively and comfortable. Healing rate also can be faster than using an conservatism dressings. However, threat of wound infection can exacerbate wound conditions and delaying healing process. Wound infection happens because of the presence of pathogens, such as bacterial, fungi, protozoa, and viruses. Some eforts that's been taken to heal and prevent these are by using additional stuff in dressing, like kind of iodine, silver, honey, or many other antimicrobial agents. Unfortunately, MRSA case shows that antimicrobial from kind of antibiotics has decending activity against some micro-organism which already having high resistance toward those agents. Luckly, nature has always provide wide of plenty antimicrobial agent source that can be taken just by extracting and isolating from plants. Mangosteen is one of plants that have a high potential as antimicrobial agent source since they have mangostin which have a high level of antimicrobial activity, as well as antioxidant. Alpha-mangostin in fact has been reported that can wipe off apprehensive about MRSA infections. Impregnation antimicrobial agent in moist mound dressing can increase healing rate and prevent wound infection, by battling microbial colonisation.

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