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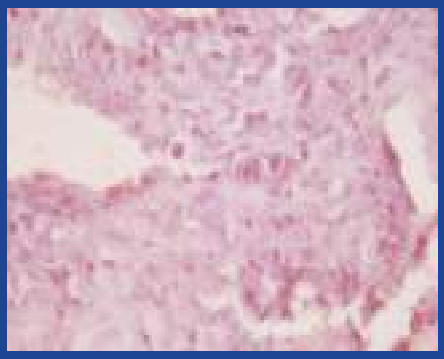

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INDONESIAN JOURNAL of TROPICAL and INFECTIOUS DISEASE

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

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Research Report

COMPARATIVE STUDY ON THE INTENSITY OF *Mycobacterium leprae* EXPOSURE TO CHILDREN WHO LIVE IN LOW AND HIGH ALTITUDE IN LOW LEPROSY ENDEMIC AREA OF SOUTH SULAWESI

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ABSTRACT

Background: The intensity of Mycobacterium leprae exposure to people who live in leprosy endemic area could be measured by serological study and detection of the bacilli in the nose cavity. Different geographical altitude might have some influences to this exposure since the bacilli prefer to live in warm areas. Aim: A combined serological and PCR study of leprosy was conducted in Selayar island, South Sulawesi to 80 school children (40 from low land and 40 from highland altitudes) in order to compare the exposure intensity between the two areas. Method: Anti PGL-1 IgM antibody (ELISA) and PCR study to detect M.leprae in the nasal cavity were performed simultaneously from each person. Result: Seropositive cases were found in 23/40 children from low land compared to 16/40 children from high land, but statistically no significant difference (p>0.05). PCR positive for M.leprae in the nasal cavity only found in 1/40 children, both in low and high altitude. Conclusion: It is concluded that although the existence of M.leprae in nasal cavity is minimal, the intensity of exposure to this bacilli still high as indicated by serological study.

***Key words**: leprosy, serology, PCR, children, low and high land*

ABSTRAK

Latar belakang: Intensitas paparan M.leprae terhadap penduduk yang tinggal di daerah endemik kusta dapat diukur dengan uji serologi dan deteksi kuman M.leprae di mukosa hidung. Tujuan: Perbedaan ketinggian geografis dapat memberikan pengaruh terhadap proses tersebut karena kuman kusta lebih menyukai daerah yang lebih hangat. Metode: Telah dilakukan studi serologi dan PCR di Pulau Selayar, Sulawesi Selatan terhadap 80 murid sekolah (40 anak dari dataran rendah dan 40 anak dari dataran tinggi), dengan tujuan untuk membandingkan intensitas paparan diantara kedua daerah. Hasil: Antibodi IGM anti PGL-1 (ELISA) dan studi PCR untuk mendeteksi M.leprae di mukosa hidung diambil secara bersamaan dari tiap-tiap anak. Ditemukan hasil sero positif pada 23/40 anak dari dataran rendah dibandingkan 16/40 anak dari dataran tinggi, namun tidak ada perbedaan bermakna diantara keduanya (p > 0,05). PCR positif terhadap M.leprae di mukosa hidung hanya ditemukan 1/40 anak baik dari dataran rendah maupun dataran tinggi. Kesimpulan: Hal ini berarti walaupun eksistensi M.leprae di mukosa hidung sedikit, intensitas paparan kuman M.leprae tinggi ditunjukkan dari hasil serologi.

***Kata kunci:** kusta, serologi, PCR, anak-anak, dataran rendah dan dataran tinggi.*

INTRODUCTION

Leprosy is still a public health problem in South East Asia, including Indonesia. Although the elimination target of leprosy in Indonesia has been reached in 2001, some pocket areas of leprosy still exist up till now.[1] The Selayar island district in South Sulawesi is an area with a lower prevalence of leprosy, compared to the surrounding areas which have high prevalence of leprosy.[2] Based on the assumption that this area might has a low transmission of leprosy, a study of leprosy exposure to inhabitants of such area will show a low level. Since *M.leprae* is known as a bacilli who prefer to live in relatively colder area of the body, a different geographical altitude might have also influence the exposure of the bacilli. After the *M.leprae* enter the body, an immunologic response will be developed and specific antibody to *M.leprae* will be produced (anti PGL-1 antibody). The level of this antibody is reflected the antigenic load of the bacilli.[3]. The leprosy bacilli enter the body via respiration tract and the detection of *M.leprae* in the nasal cavity could be performed by PCR study.[4] The level of seropositivity and the presence of *M.leprae* in nasal cavity could be used as an indicator for leprosy exposure intensity in endemic area. The aim of this study is compare the exposure of *M.leprae* to inhabitants who live in low and high lands of Pulau Selayar Area of South Sulawesi, using specific serological indicators for leprosy and the presence of *M.leprae* in the nasal cavity by PCR method.

MATERIAL AND METHODS

Fourty healthy school children from Kahu-Kahu village, Selayar island, who live in low land and another 40 healthy school children from Lembang Matene village (high land) aged 9–12 years old were involved in the study (figure 1). Leprosy contact history was taken to exclude contact individuals and clinical examination was conducted to exclude leprosy patients. Blood and nose swab samples from these children were collected simultaneously.

Serological study

From each children 100ul capillary blood was obtained by a needle puncture to finger tip, dried on a small filter paper and sent to Leprosy lab of Institute of Tropical Disease, Airlangga University, Surabaya. Dried blood on filter papers were dissolved in 1 ml of BSA buffer and 10ul of the solution were taken for indirect ELISA test to measure the level of anti PGL-1 antibody. Using N dioctyl-BSA as an antigen, the level of IgM anti PGL-1 antibody were measured follows the ELISA procedure recommended by Patil.[5] The ELISA results in Optical Density (OD) were converted to unit/ml using the BIOLISE computer software. Three ml of peripheral blood were also collected from cubitous venes in 10 children, to measure the real blood level of anti PGL-1. After conversion to serum level, using cut off level 605 u/ml, sero-positive case was detected.[6]

Figure 2. Indirect ELISA

Figure 1. Geographical area of Selayar Island, South Sulawesi.

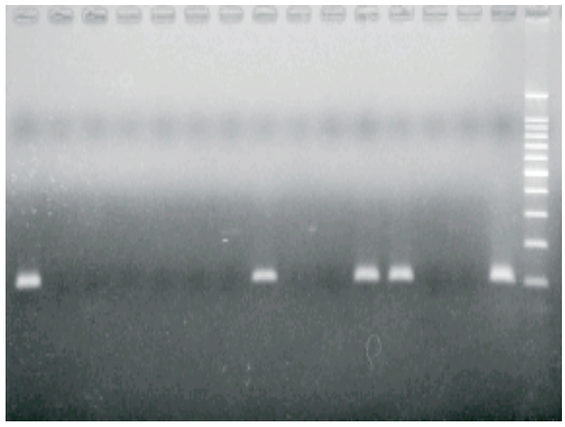

Figure 3. PCR results for detection of *M.leprae* DNA using Lp1 – Lp-4 nested primers
Sample no. : 1 2 3 4 5 6 7 NC PC Ladder (100bp)

PCR study from nose swab samples

Nose swab sample was also taken simultaneously from the same children after collecting finger tips blood for serological sudy. The nose swab samples were kept in freeze condition until ready for PCR study. DNA extraction was performed using the Miniprep Qiagen kit. Using the Lp1-Lp4 nested primers, the *M.leprae* DNA was detected by PCR, following the procedure recommended by Plikaytis.[7] Positive PCR results is indicated by a band of 99 bp in agarose gel field, as pointed by positive control (figure 3)

RESULTS

Clinical examination of all children revealed no sign of leprosy. Based on the cut off value 605 u/ml for IgM anti PGL-1, sero-positive cases was observed in 23 out of 40 (57,5%) children from low land compared to 16 out of 40 (40%) children from highland. Statistically no significant difference between the two groups ($p > 0.05$). After PCR study from the nasal swabs samples, only one out of 40 samples from low land group show PCR positive, similar with the results of nose swabs samples from highland group (1 out of 40 samples or 2.5%). No statistical difference in the PCR results between the two groups.

DISCUSSION

This study was conducted in an area wich is reported as a "low endemic of leprosy" and start with an assumption that the transmission of the disease mainly from the environment. The humoral response to *M.leprae* is represented by specific antibody to cell wall of the bacilli which contains Phenolic Glycolipid-1 (PGL-1). This antibody is not protective to leprosy, but can be used as a marker of immune response to the presence of the bacilli.[9] The level of this specific antibody is corresponded with the amount of antigen or bacilli in the body, which means high level of antibody indicates many bacilli inside the body.[10] In this study, almost half of the school children have already showed sero-positive for leprosy. In this case, the cut off value (> 605 u/ml) used in the study was based on the previous serological study in East Java.[11] A new calculation for measuring the cut off value for South Sulawesi is needed. Although the Selayar island is relatively low endemic for leprosy, it seems that the children who live in this area still exposed to leprosy bacilli quite often. The source of the bacilli might be from leprosy cases who are still not found by surveillance ("the back-log cases") or from non- human resource of *M.leprae* in the environment.[12,13] After tracing the sero-positive children by analyzed the history of contact with leprosy cases, it revealed that most of the children have no contact history with leprosy patients. High percentage of seropositivity to leprosy in South Sulawesi area seems to be common in many parts of this province. Since the island has a long coastal area, it would be possible also that many inhabitants use to go to other surrounding area in South Sulawesi as a sailor and contact with many leprosy patients from other area. Nasal swabs examination have been used to detect the port of entry of the lepra bacilli from inside or from outside or of the body.[14,15] The PCR study for detection of *M.leprae* in the nasal cavity only showed positive in single case from both groups. This minimal results could be correlated with the season during collecting nose swab samples. The study was conducted during rainy season, probably this cause many lepra bacilli were cleaned up from the air and less bacilli aspirated by these children. This reason might explains why the nasal swabs mostly negative in the PCR study, beside the technical eror during sample collection or laboratory work. It is recommended to repeat the nasal swabs collection during the dry season, when the air might be more contaminated with lepra bacilli. The percentage of PCR positivity from nose swabs samples from this study was 2.5%, lower than previous study in other area of South Sulawesi.[16] The serological results of this survey shows that transmission of *M.leprae* is still intense, but PCR results from nose swabs does not support the hypothesis that transmission is occurred via the nasal cavity. Why the percentage of leprosy sero-positivity among these children were high is still a question and more environmental investigation are needed.

CONCLUSION

Low endemic of leprosy does not means less intensity of exposure of *M.leprae* and need more environmental study of leprosy to find out the source of the transmission of the disease in this area.

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Case Report

PROBLEM OF ANTIBIOTIC USE AND ANTIMICROBIAL RESISTANCE IN INDONESIA: ARE WE REALLY MAKING PROGRESS?

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ABSTRACT

Background: Based on the results Antimicrobial Resistance in Indonesia: prevalence and prevention-study (AMRIN-study), the Ministry of Health of Indonesia in 2005 began a program antibiotic resistance control (PPRA) in some government hospitals, and is currently developing to all government teaching hospitals in Indonesia. Aim: The core activities of the PPRA are to implement standardized surveillance emergence of antibiotic resistant bacteria, and the surveillance of antibiotic use in terms of quantity and quality. Method: Our research in the years 2003 showed the proportion of antibiotic use 84% of patients in a hospital. The use of inappropriate antibiotics was very high, 42% no indication. Result: In 2012 the results of surveillance showed decline of inappropriate use of antibiotic, but prevalence extended-spectrum β-lactamase (ESBL)-producing K.pneumoniae (58%), and E.coli (52%) and methicillin-resistant S.aures (MRSA) (24%) were increasing. Conclusion: It was needed to implement the most appropriate programs to prevent the growth and development of bacteria resistant to antibiotics.

Key words: *Indonesia, K.pneumoniae, E.coli, methicillin-resistance S.aureus, surveillance*

ABSTRAK

Latar belakang: Berdasarkan hasil penelitian Antimicrobial Resistance di Indonesia: prevalensi dan pencegahan (AMRIN-studi), Kementerian Kesehatan Republik Indonesia tahun 2005 memulai program pengendalian resistensi terhadap antibiotik (PPRA) di beberapa rumah sakit pemerintah, dan saat ini diperluas untuk semua rumah sakit pendidikan pemerintah di Indonesia. Tujuan: Kegiatan inti dari PPRA adalah untuk melakukan surveilance kuman kebal antibiotik, dan monitoring penggunaan antibiotik dalam hal kuantitas dan kualitas secara terstandar. Metode: Penelitian kami di tahun 2003 menunjukkan proporsi penggunaan antibiotik dari pasien di rumah sakit sebanyak 84%. Penggunaan antibiotik yang tidak tepat sangat tinggi, 42% tidak ada indikasi pemberian antibiotik. Hasil: Hasil surveilance tahun 2012 menunjukkan penurunan penggunaan dari antibiotik yang tidak tepat, tetapi prevalensi extended-spectrum β-laktamase (ESBL) K.pneumoniae (58%), dan E.coli (52%) dan methicillin-resistant S.aures (MRSA) (24%) meningkat. Kesimpulan: Untuk memperbaiki kondisi ini diperlukan program lain yang paling tepat untuk mencegah pertumbuhan dan perkembangan bakteri resisten terhadap antibiotik.

Kata kunci: *Indonesia, K.pneumoniae, E.coli, methicillin-resistance S.aureus, survailans*

INTRODUCTION

World Health Organization has announced that the issue of antibiotic-resistant bacteria is a global problem that threatens human being. This happens because of a deficiency in 6 issues: 1) lack of research, 2) lack of commitment, 3) lack of infection control, 4) Irrational use of antimicrobials, 5) poor quality antibiotics, 6) weak oversight. To cope with these conditions, all countries should participate actively.

Efforts to suppress the development of antibiotic-resistant bacteria have been implemented throughout the world including Indonesia. A scientific research on Antimicrobial Resistance in Indonesia: Prevalence and

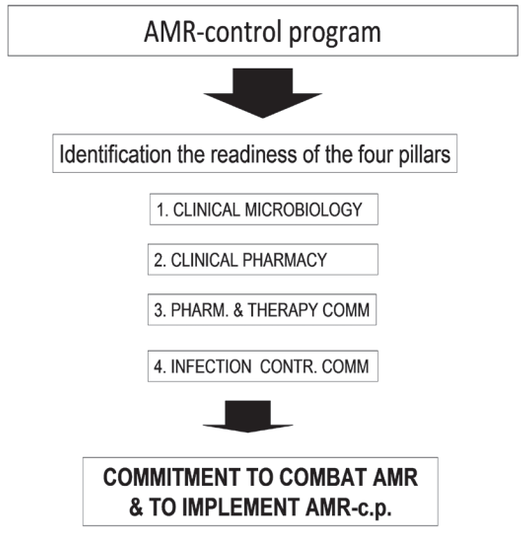
Prevention (AMRIN) study was conducted between 2001 and 2005. It aimed to create a program of scientifically based guidelines for the assessment of antimicrobial resistance, the pattern of antibiotic use, infection control and the implementation of interventions in the home hospital in Indonesia. The results of AMRIN study show that the problems also occur in Indonesia. It means that integrated handling of the various stakeholders in the hospital is required (AMRIN study group, 2005). To overcome this problem, the Ministry of Health has taken an action by developing a program of antimicrobial resistance control program (AMR-control program) for Hospital in Indonesia. The program was first implemented in teaching hospitals in Indonesia and it is expected to further evolve to all hospitals in Indonesia (AMRIN-study, 2005).

The problem in Indonesia is that not all hospitals have the facilities to conduct microbiological examination or culturing of bacteria, antibiotic resistant bacteria so that the surveillance can not be performed. AMR-control program is expected to be able to make the use of antibiotic more rational, to improve the implementation of infection control measures, and to inhibit the development of antibiotic-resistant bacteria. Hospitals that do not have a complete facility are expected to realize it because without good microbiology laboratory, the control of antimicrobial resistance is not possible to be done.

This paper describes the implementation AMR-control program so as to give an idea whether the program works well or whether it is still necessary to make some improvements to the program in order to achieve the program objectives.

Implementation Method of AMR-control Program

AMR-control program was implemented in stages. First, identifying the readiness of the hospital management to implement this program. Hospital is considered ready to execute this program when it has four supporting pillars, namely: 1) clinical microbiology, 2) clinical pharmacy, 3) pharmacy and therapy committee, 4) infection control committee. Once the four pillars had been identified, they were then assessed to know whether they were already running as they should. It was then followed by performing training to the 4 pillars so as they had the same understanding of this program as shown in Scheme 1.



Scheme 1. The concept of AMR-control program (AMRIN study group, 2005)

Second, creating a team consisting of the four pillars responsible for the execution of AMR-control program.

Each pillar has a function in accordance with its respective field.

The Role of Clinical Microbiology in the Management of Infectious Diseases

In Indonesia most of clinical microbiology laboratories are not yet well developed. Hence, the hospital management has to upgrade microbiology laboratory to meet the actual standard.

The clinicians usually treat infectious disease patients with a clinical diagnosis and they give antibiotic empirically. To improve collaboration between clinicians and the clinical microbiologists, the two sides should enhance the quality of their work.

We do the following recommendations to improve the cooperation between clinicians and microbiologists.

1. Holding regular (weekly) meetings between clinicians and microbiologists to discuss current infectious disease cases in hospital.
2. Holding routine evaluation of adherence to clinical guidelines by clinicians and giving feed back of adherence figures.
3. Enhancing the involvement of the clinical microbiologist in patient care such as by instituting a 24 hour service for advice concerning diagnostics and treatment of patients with infectious diseases.

Clinical Pharmacy

Clinical pharmacy has an important role to control antibiotic prescribed in the hospital. In addition, clinical pharmacists have to meet the needs of antibiotics prescribed by a clinician. They should have the signs made by the pharmacy and therapeutics committee to control the excessive use of antibiotic.

Pharmacy and Therapeutics Committee

Pharmacy and Therapeutics Committee is obliged to make the antibiotic guidelines and policies for the use of antibiotics.

They have to revise guidelines for the use of antibiotics and antibiotic use policies when needed.

Another task of this committee is to conduct surveillance of antibiotic use quantitatively and qualitatively particularly in the ward where antibiotic-resistant bacteria emerge.

Infection control committee

This committee is responsible for the prevention of AMR organism spreading. Besides, this committee also has to increase infection control implementation in hospital consisting of:

- Standard precautions
- Isolation of patients
- Source control
- Surveillance of AMR organisms

Implementation steps of AMR-control program

1. Forming a team of AMR-c.p. in hospital.
2. Choosing one department or unit as a pilot project, e.g. department of Internal Medicine or pediatric.
3. Updating or developing antibiotics guideline.
4. Socializing antibiotics guideline.
5. Conducting baseline data collection for 1–3 months respectively.
6. Implementing the antibiotics guideline.
7. Conducting surveillance data collection post intervention.
8. Evaluating and discussing.
9. Presenting the result: e.g. workshop
10. Getting antibiotics guideline updates.
11. Conducting surveillance (monitoring and evaluating).
12. Getting back to point number 3.

Results of AMRIN-study (period of study 2002–2005)

Our study of quantity and quality of antibiotic usw showed that the percentage of antibiotic prescribed in hospitalized patients was quite high (85%). As many as 90% of patients staying for 5 or more days in the department of Surgery and Pediatrics used antibiotics while in the Gynecology & Obstetrics Department and Internal Medicine Department, there were as many as 87% and 67% of the patients respectively using antibiotics (Hadi, 2008). 53% out of 2058 prescriptions was classified as therapy, 15% as prophylaxis, and 32% as unknown indication.

The quality of antibiotic use was assessed by two Indonesian reviewers and one foreign reviewer. Almost 60% of the assessed prescriptions was classified as incorrect either unjustified (42%) or inappropriate (15%) by at least two of the three reviewers (Hadi, 2008).

Our study of bacterial resistance from normal flora gastro-intestinal tract showed that Escherichia coli had been isolated from 781 hospitalized patients and all data were available for analysis. Eighty-one percent of the hospitalized patients carried Escherichia coli resistant to one or more antibiotics. Ampicillin resistance was seen most frequently (570 isolates, 73%), followed by trimethoprim/sulfamethoxazole resistance in 434 isolates (56%), chloramphenicol resistance in 334 isolates (43%), ciprofloxacin resistance in 173 isolates (22%) and gentamicin resistance in 141 isolates (18%) (Duerink, 2007).

In the community group of 2996 individuals, 2494 information cases regarding carriage of Escherichia coli were available. Forty-three percents of the population carried resistant Escherichia coli. Ampicillin resistance was observed in 851 (34%) isolates, trimethoprim/sulfamethoxazole resistance in 716 (29%) isolates and chloramphenicol resistance in 369 isolates (15%) (Duerink, 2007).

Surveillance of Post Implementation of AMR-control Program

Because of limited funds and facilities, surveillance could not be implemented fully in all hospitals in Indonesia. We only present the results of surveillance in a hospital in Surabaya.

The results of surveillance of antibiotic use in the department of internal medicine in 2012 showed that 50.22% of patients was treated with antibiotics. It was lower compared to the percentage of antibiotic use in 2005 which was 67%.

In terms of the quality of antibiotic use, there was also an improvement in which a more rational usage of antibiotics was found compared to the use of antibiotics in 2005. On the one hand, there was a decline regarding no indication of antibiotic therapy from 42% to 30.6%, and inappropriate antibiotic therapy from 15% to 7.3%. On the other hand, there was a change in the pattern of antibiotic use between 2005 and 2012. In 2005 the use of ampicillin was dominant whereas in 2012 3rd generation cephalosporin was more widely used.

We conducted AMR surveillance in the period from January to June 2010 by looking back to the medical records performed in the microbiology laboratory. 4359 bacteria were found consisting of 3115 negative gram and 1244 positive gram bacteria isolates. Among these bacteria, 456 (22%) were ESBL positive isolates and 45 (18%) were MRSA isolates from total of 250 *S aureus* isolates. Of these ESBL isolates, 107 specimens of *E.coli* ESBL(+) (17%) were obtained from a total of 633 *E. Coli* isolates and 196 isolates of *K. pneumonia* ESBL(+) (23%) were obtained from a total of 196 *K pneumonia*.

While the surveillance conducted in the period from July to December 2012 showed that there were *K.pneumoni* ESBL(+) 202 (58%) specimens from a total of 351 *K pneumonia* specimens and *E. coli* ESBL(+) 327 (52%) specimens from a total of 629 *E.coli* specimens.

63 (24%) MRSA specimens were obtained from a total of 259 *S.aureus* specimens while the prevalence of ESBL was (53%).

Table 1. Comparison of prevalence of ESBL and MRSA between 2010 and 1012

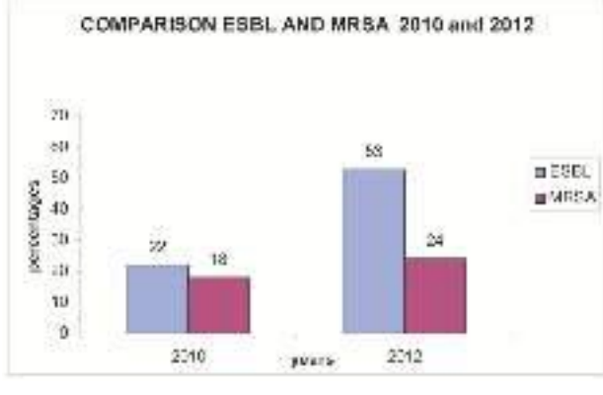


Table 2. Comparison between *K pneumonia* and *E.coli* ESBL producing bacteria's

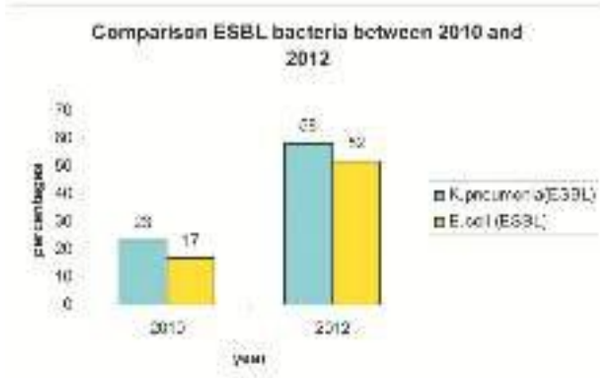


Table 3. Comparison of quality of antibiotic use between 2005 and 2012

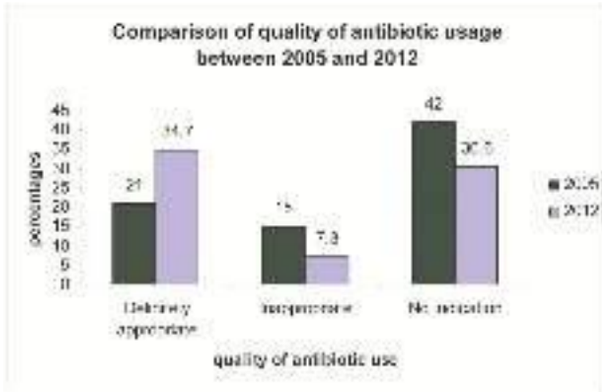


Table 2 shows that the quality of antibiotic use is improved in which there is an increase in the appropriate use of antibiotics and a decrease in the inappropriate use and no indication.

CONCLUSION

This program could not be implemented fully because there were many limitations in many hospitals in Indonesia. Our study in Surabaya showed that the results of surveillance of antibiotic use demonstrated an improvement compared to that between 2005 and 2012. Yet, in terms of the development of antibiotic-resistant bacteria e.g. ESBL producing bacteria, the prevalence increased. In addition, MRSA also increased. This could occur because the possibility of the use of antibiotics in the community outside the hospital was still very high or excessive.

Both improvement of health facilities especially microbiology laboratory and the addition of experts in the field of microbiology are required.

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Research Report

UPDATE MANAGEMENT DENGUE SHOCK SYNDROME IN PEDIATRIC CASES

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ABSTRACT

Background: Since 1968 Dengue Virus Infection has been found in Indonesia, especially at Surabaya and Jakarta city. Firstly management of dengue virus infection very difficult to improve, therefore the higher mortality nearly 41,4 % had been found but on the following years in five decades the mortality rates was becoming to decrease until 1,27 % on 2011. Aim: To find the new management of Dengue Shock Syndrome to reach the lower fatality rate below 1%. Method: Until now to manage Dengue Shock Syndrome is very difficult, some cases can be improved but the other lost due to the late coming in the hospital and not involved in criteria diagnosis base on WHO 1997. To solve this problem WHO 2009 had made new criteria diagnosis Dengue Virus Infection focusing on early detection of severe Dengue Virus Infection especially Dengue Shock Syndrome. Result: On 2011 WHO had made an integrated criteria diagnosis base on WHO 2009 and WHO 1997. These criteria was focusing in Update Management of Dengue Shock Syndrome in Pediatric Cases. Based on this action, this paper will improve clinical management to reach the lower mortality of Dengue Shock Syndrome in Community until CFR < 1%. Conclusion: By using integrated criteria of WHO 2009 and 1997, update management of Dengue Shock Syndrome in Pediatric cases, can improve clinical management to reach the lower mortality in community until CFR < 1%.

Key words: *Dengue Virus Infection; Criteria diagnosis WHO; Update Management, Shock, Pediatric cases*

ABSTRAK

Latar belakang: Sejak tahun 1968, virus infeksi demam berdarah telah ditemukan di Indonesia, khususnya di kota Surabaya dan Jakarta. Pada awalnya, manajemen virus infeksi demam berdarah ini sangat sulit untuk dikembangkan, maka dari itu, ditemukan tingkat kematian hampir sebesar 41,4%; namun dalam beberapa tahun di 5 abad terakhir, tingkat kematian telah menurun sampai 1,27% di tahun 2011. Tujuan: Untuk menemukan manajemen baru dari sindrom shock demam berdarah untuk mencapai tingkat kematian yang lebih rendah yaitu dibawah 1%. Metode: Sampai saat ini pengendalian sindrom shock demam berdarah masih sangat sulit untuk dilakukan, beberapa kasus dapat dikembanganken namun lainnya tidak tertata akibat keterlambatan penanganan di rumah sakit dan tidak masuk dalam kriteria diagnosis berdasarkan pada WHO 1997. Untuk memecahkan masalah ini, WHO 2009 teolag membuat kriteria diagnosis infeksi virus demam berdarah baru yang berfokus pada deteksi awal pada beberapa infeksi virus demam berdarah khususnya sindrom shock karena demam berdarah. Hasil: Pada tahun 2011, WHO telah membuat kriteria diagnosis yang terintegrasi berdasarkan pada WHO 2009 dan WHO 1997; Kriteria ini berfokus pada manajemen terbaru di sindrom shock karena demam berdarah pada ilmu kedokteran anak. Berdasarkan pada tindakan tersebut, penelitian ini akan memotivasi kita untuk mencapai tingkat kematian untuk menurunkan kurs dari 1% menjadi 0%. Kesimpulan: Dengan menggunakan kriteria dari WHO 2009 yang telah terintegrasi, pembaharuan manajemen dari sindrom shock akibat demam berdarah diharapkan dapat memotivasi kita untuk mencapai tingkat kematian di masyarakat lebih rendah kurang dari 1%.

Kata kunci: *Infeksi virus demam berdarah, kriteria diagnosis WHO, pembaharuan manajemen, syok, kasus anak-anak*

INTRODUCTION

Dengue is the most rapidly spreading mosquito borne disease in the world. In last 50 years, incidence has increased 30-fold with increasing geographic expansion to new countries and, in the present decade, from urban to rural settings. In Indonesia, where more than 35% of the country's population lives in urban areas, 150,000 cases were reported in 2007 (the highest on record) with over 25,000 cases reported from both Jakarta and West Java. The case-fatality rate was approximately 1%. Reported case in fatality rates for the region approximately 1%, but in India, Indonesia and Myanmar, focal outbreaks away from the urban areas have reported case-fatality rate of 3–5%.

The mechanisms leading to the severe manifestations of Dengue virus (DENV) infections are still not completely understood but are likely to be multifactorial. The genetic background of the host influences the way that the immune response reacts to DENV infection. Upon inoculation of DENV into the dermis, Langerhans cell and keratinocytes will primarily be infected. The virus subsequently spreads via the blood (primary viremia) and infects tissue macrophages in several organs, especially the machrophages in the spleen. The replication efficiency of DENV in dendritic cells (DC), monocytes and macrophage, as well as its tropism for and replication efficiency in endothelial cells (EC), bone marrow, stromal cells and liver cells, collectively determine the viral load measured in blood. This viral load represents an important risk factor for development of severe disease. Essestially, infection of machrophages, hepatocytes and EC influence the hemostatic and the immune responses to DENV. Infected cells die predominantly through apoptosis and to a lesser extent trough necrosis. Necrosis results in release of toxic products, which activate the coagulation and fibrinolystic systems, depending on the extent of infection of bone marrow stromal cells and the levels of IL-6, IL-8, IL-10 and IL-18, hemopoiesis is suppressed, resulting in decrease blood thrombogenicity. Platelets interact closely with EC and a normal number of functioning platelets is necessary to maintain vascular stability.

A high viral load in blood and possibly viral tropism for EC, severe thrombocytopenia and platelet dysfunction may results in increased vascular permeability and coagulopathy is amplified. In addition, enhancing IgG antibodies bind heterologous virus during secondary infection and enhance infection of APCs, thereby contributing to the increased viral load that is in during secondary viremia in some patients. Furthermore, a high viral load overstimulates both low and high-avidity cross reactive T cells. In the context of certain HLA haplotypes, cross-reactive T cells delay virus clearance, while producing high levels of proinflamatory cytokines and other mediators. Ultimately, these high levels of soluble factors, many of which still remain to be identified, induces changes in EC leading to the coagulopathy and plasma leakage characteristic of DSS.

Dengue infection is a systemic and dynamic disease. It has a wide clinical spectrum that includes both severe and non-severe clinical manifestations. After the incubation period, the illness begins abruptly and is followed by the three phases, febrile, critical and recovery. Laboratory diagnosis methods for confirming dengue virus infection may involve detection of the virus, viral nucleic acid, antigens or antibodies or a combination of these techniques. After the onset of illness, the virus can be detected in serum, plasma, circulating blood cells and other tissues for 4–5 days. During the early stages of the disease, virus isolation, nucleic acid or antigen detection can be used to diagnose the infection. At the end of the acute phase of infection, serology is the method of choice for diagnosis.

Until now to manage Dengue Shock Syndrome is very difficult, some cases can be improved but the other lost due to the late coming in the hospital and not involved in criteria diagnosis base on WHO 1997. To solve this problem, WHO 2009 had made new criteria diagnosis Dengue Virus Infection focusing on early detection of severe Dengue Virus Infection especially Dengue Shock Syndrome. On 2011 WHO had made an integrated criteria diagnosis base on WHO 2009 and WHO 1997. These criteria was focusing in Update Management of Dengue Shock Syndrome in Pediatric Cases. Based on this action, this paper will motivate us to reach the lower mortality of Dengue Shock Syndrome in Community until CFR < 1%.

EPIDEMIOLOGY

Dengue is the most rapidly spreading mosquito-borne viral disease in the world. In the last 50 years, incidence has increased 30-fold with increasing geographic expansion to new countries and, in the present decade, from urban to rural settings (Figure 1).

The countries of the region have been divided into four distinct climatic zones with different dengue transmission potential. Epidemic dengue is a major public health problem in Indonesia, Myanmar, Sri Lanka, Thailand and Timor-Leste which are in the tropical monsoon and equatorial zone where Aedes aegypti is widespread in both urban and rural areas, where multiple virus serotypes are circulating, and where dengue is a leading cause of hospitalization and death in children. Cyclic epidemics are increasing in frequency and in-country geographic expansion is occurring in Bangladesh, India and Maldives – countries in the deciduous dry and wet climatic zone with multiple virus serotypes circulating. Over the past four years, epidemic dengue activity has spread to Bhutan and Nepal in the sub-Himalayan foothills.

Reported case fatality rates for the region are approximately 1%, but in India, Indonesia and Myanmar, focal outbreaks away from the urban areas have reported case-fatality rates of 3–5%.

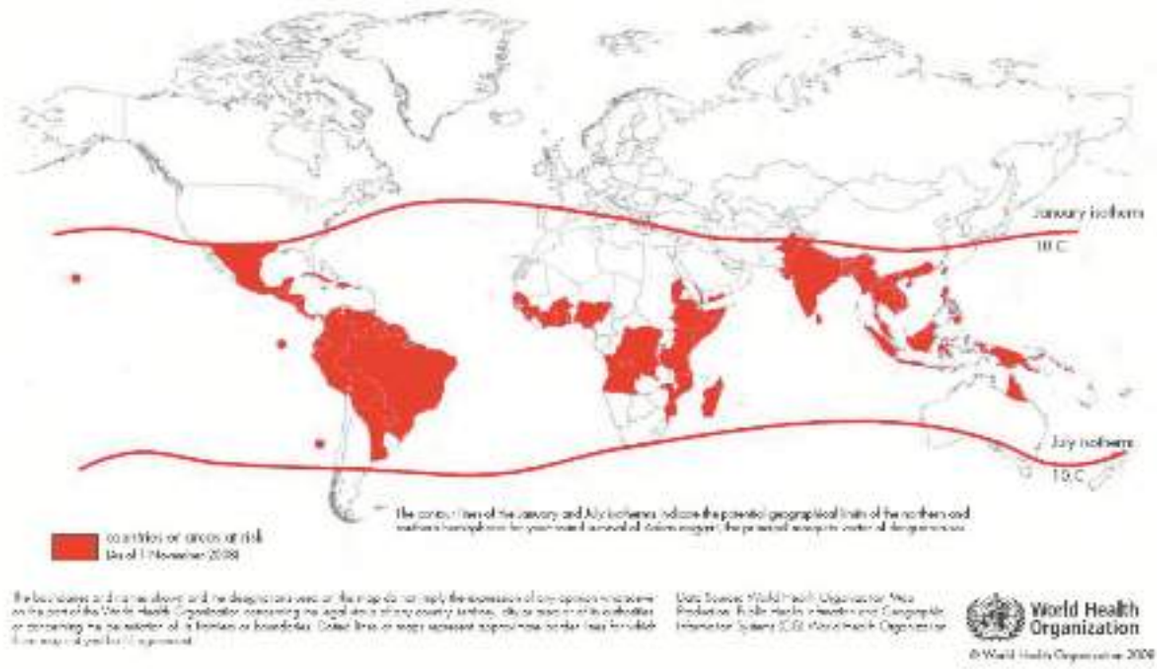
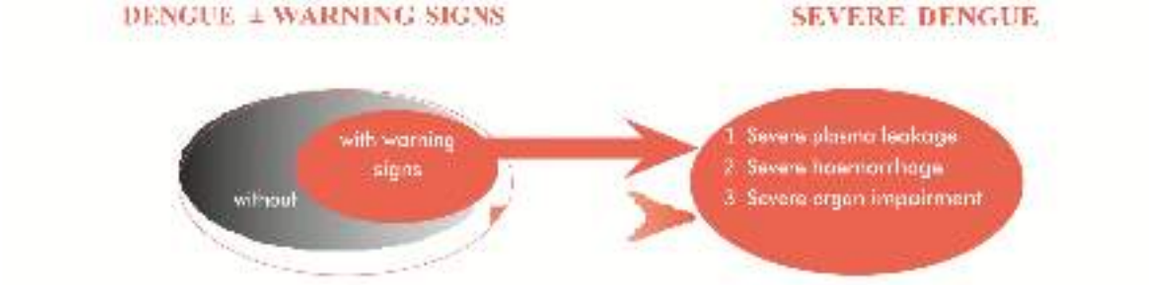


Figure 1. Countries/areas at risk of dengue transmission (WHO, 2008)

In Indonesia, where more than 35% of the country's population lives in urban areas, 50,000 cases were reported in 2007 (the highest on record) with over 25,000 cases reported from both Jakarta and West Java. The case-fatality rate was approximately 1%.

Criteria for diagnosing dengue (with or without warning signs) and severe dengue are presented in Figure 2. It must be kept in mind that even dengue patients without warning signs may develop severe dengue.

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



Probable dengue

live in /travel to dengue endemic area. Fever and 2 of the following criteria:
- Nausea, vomiting
- Rash
- Aches and pains
- Tourniquet test positive
- Leukopenia
- Any warning sign

Laboratory-confirmed dengue (important when no sign of plasma leakage)

signs*
- Abdominal pain or tenderness
- Persistent vomiting
- Clinical fluid accumulation
- Mucosal bleed
- Lethargy, restlessness
- Liver enlargment > 2 cm
- Laboratory: increase in HCT concurrent with rapid decrease in platelet count

* (requiring strict observation and medical intervention)

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 organs

Figure 2. Suggested dengue case classification and levels of severity (WHO, 2009)

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

This model for classifying dengue has been suggested by an expert group (Geneva, Switzerland, 2008) and is currently being tested in 18 countries by comparing its performance in practical settings to the existing WHO case classification. The process will be finalized in 2010. For practical reasons this guide adapts the distinction between dengue and severe dengue.

Dengue inflicts a significant health, economic and social burden on the populations of endemic areas. Globally the estimated number of disability-adjusted life years (DALYs) lost to dengue in 2001 was 528.[1]

The number of cases reported annually to WHO ranged from 0.4 to 1.3 million in the decade 1996–2005. As an infectious disease, the number of cases varies substantially

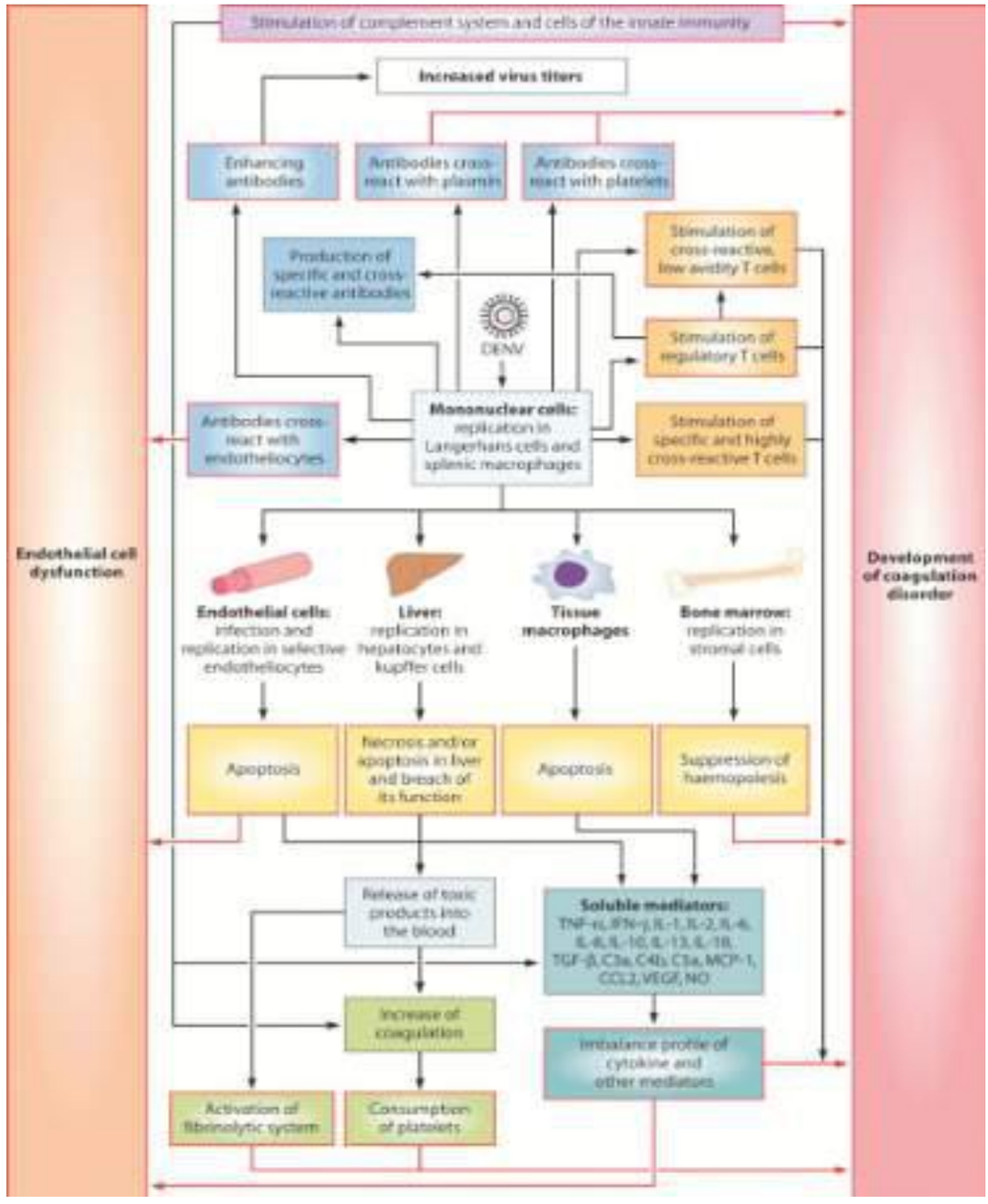


Figure 3. Proposed Model for the pathogenesis of DF, DHF and DSS based on an integrated view of the data presented (see section The Integrated View in the text). Black arrows, processes leading to the indicated event, colored boxes with white centers, pathological events. Each event will ultimately affect the EC or the haemostatic system (purple arrows). (WHO, 2009)

from year to year. Underreporting and misdiagnoses are major obstacles to understanding the full burden of dengue.[2]

On average, a hospitalized case of dengue cost three times what an ambulatory case costs. Combining the ambulatory and hospitalized patients and factoring in the risk of death, the overall cost of a dengue case is US$ 828. Merging this number with the average annual number of officially reported dengue cases from the eight countries studied in the period 2001–2005 (532.000 cases) gives a cost of officially reported dengue of US$ 440 million.

Children are at a higher risk of severe dengue.[3] Intensive care is required for severely ill patients, including intravenous fluids, blood or plasma transfusion and medicines.

Dengue afflicts all levels of society but the burden may be higher among the poorest who grow up in communities with inadequate water supply and solid waste infrastructure, and where conditions are most favourable for multiplication of the main vector, Ae. aegypti.

Travellers play an essential role in the global epidemiology of dengue infections, as viraemic travellers carry various dengue serotypes and strains into areas with mosquitoes that can transmit infection.[4]

PATHOGENESIS

The mechanisms leading to the severe manifestations of DENV infections are still not completely understood but are likely to be multifactorial (Figure 3). The genetic background of the host influences the way that the immune response reacts to DENV infection. Upon inoculation of DENV into the dermis, Langerhans cells and keratinocytes will primarily be infected. The virus subsequently spreads via the blood (primary viremia) and infects tissue macrophages in several organs, especially the macrophages in the spleen. The replication efficiency of DENV in DC, monocytes, and macrophages, as well as its tropism for and replication efficiency in EC, bone marrow stromal cells, and liver cells, collectively determine the viral load measured in blood. This viral load represents an important risk factor for development of severe disease. Essentially, infection of macrophages, hepatocytes, and EC influences the hemostatic and the immune responses to DENV. Infected cells die predominantly through apoptosis and to a lesser extent through necrosis. Necrosis results in release of toxic products, which activate the coagulation and fibrinolytic systems. Depending on the extent of infection of bone marrow stromal cells and the levels of IL-6, IL-8, IL-10, and IL-18, hemopoiesis is suppressed, resulting in decreased blood thrombogenicity. Platelets interact closely with EC, and a normal number of functioning platelets is necessary to maintain vascular stability. A high viral load in blood and possibly viral tropism for EC, severe thrombocytopenia, and platelet dysfunction may result in increased capillary fragility, clinically manifested as petechiae, easy bruising, and gastrointestinal mucosal bleeding, which is characteristic of DHF. At the same time, infection stimulates development of specific antibody and cellular immune responses to DENV. When IgM antibodies that cross-react with EC, platelets, and plasmin are produced, the loop that results in increased vascular permeability and coagulopathy is amplified. In addition, enhancing IgG antibodies bind heterologous virus during secondary infection and enhance infection of APCs, thereby contributing to the increased viral load that is seen during secondary viremia in some patients. Furthermore, a high viral load overstimulates both low- and high-avidity cross-reactive T cells. In the context of certain HLA haplotypes, cross-reactive T cells delay virus clearance, while producing high levels of proinflammatory cytokines and other mediators. Ultimately, these high levels of soluble factors, many of which still remain to be identified, induce changes in EC leading to the coagulopathy and plasma leakage characteristic of DSS.[5]

CLINICAL MANAGEMENT AND DELIVERY OF CLINICAL SERVICES

Dengue infection is a systemic and dynamic disease. It has a wide clinical spectrum that includes both severe and non-severe clinical manifestations.[6] After the incubation period, the illness begins abrupt and is followed by the three phases – febrile, critical and recovery (Figure 4).

For a disease that is complex in its manifestations, management 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.

Activities (triage and management decisions) at the primary and secondary care levels (where patients are first seen and evaluated) are critical in determining the clinical outcome of dengue. A well-managed front-line response not only reduces the number of unnecessary hospital admissions but also saves the lives of dengue patients. Early notification of dengue cases seen in primary and secondary care is crucial for identifying outbreaks and initiating an early response Differential diagnosis needs to be considered.

Febrile Phase

Patients typically develop high-grade fever suddenly. This acute febrile phase usually lasts 2–7 days and is often accompanied by facial flushing, skin erythema, generalized body ache, myalgia, arthralgia and headache.[6] Some patients may have sore throat, injected pharynx and conjunctival injection. Anorexia, nausea and vomiting are common. It can be difficult to distinguish dengue clinically from non-dengue febrile diseases in the early febrile phase. A positive tourniquet test in this phase increases the probability of dengue.[7,8] In addition, these clinical

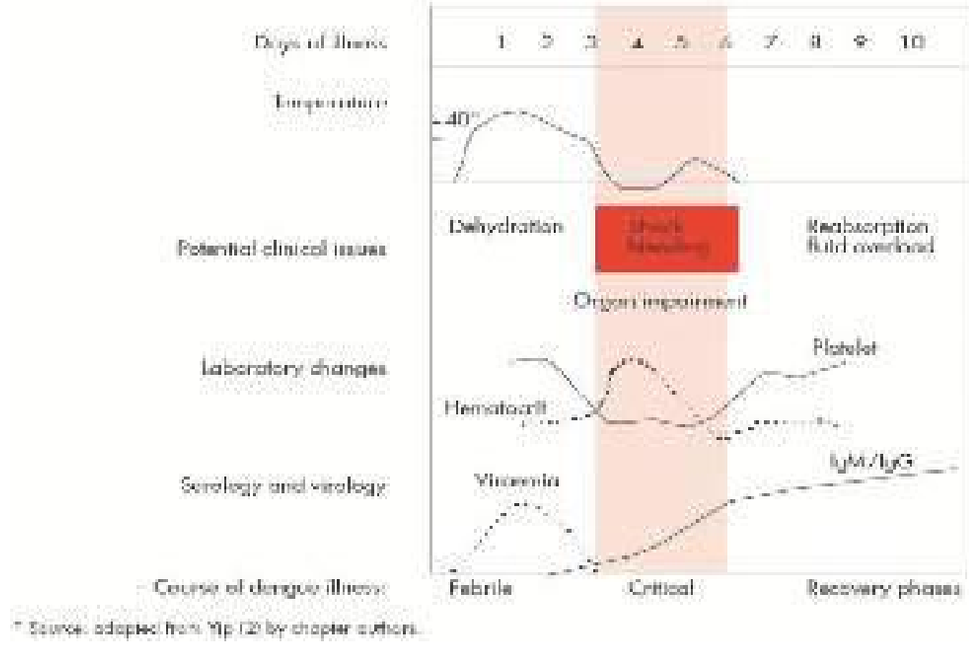


Figure 4. The course of dengue illness (WHO, 2009)

features are indistinguishable between severe and non-severe dengue cases. Therefore monitoring for warning signs and other clinical parameters is crucial to recognizing progression to the critical phase.

Mild haemorrhagic manifestations like petechiae and mucosal membrane bleeding (e.g. nose and gums) may be seen.[7,9] Massive vaginal bleeding (in women of childbearing age) and gastrointestinal bleeding may occur during this phase but is not common.[9] The liver is often enlarged and tender after a few days of fever.[7] The earliest abnormality in the full blood count is a progressive decrease in total white cell count, which should alert the physician to a high probability of dengue.

Critical Phase

Around the time of defervescence, when the temperature drops to 37.5–38° C or less and remains below this level, usually on days 3–7 of illness, an increase in capillary permeability in parallel with increasing haematocrit levels may occur.[10,11] This marks the beginning of the critical phase. The period of clinically significant plasma leakage usually lasts 24–48 hours.

Progressive leukopenia[7] followed by a rapid decrease in platelet count usually precedes plasma leakage. At this point patients without an increase in capillary permeability will improve, while those with increased capillary permeability may become worse as a result of lost plasma volume. The degree of plasma leakage varies. Pleural effusion and ascites may be clinically detectable depending on the degree of plasma leakage and the volume of fluid therapy. Hence chest x-ray and abdominal ultrasound can be useful tools for diagnosis. The degree of increase above the baseline haematocrit often reflects the severity of plasma leakage.

Shock occurs when a critical volume of plasma is lost through leakage. It is often preceded by warning signs. The body temperature may be subnormal when shock occurs. With prolonged shock, the consequent organ hypoperfusion results in progressive organ impairment, metabolic acidosis and disseminated intravascular coagulation. This in turn leads to severe haemorrhage causing the haematocrit to decrease in severe shock. Instead of the leukopenia usually seen during this phase of dengue, the total white cell count may increase in patients with severe bleeding. In addition, severe organ impairment such as severe hepatitis, encephalitis or myocarditis and/or severe bleeding may also develop without obvious plasma leakage or shock.[12]

Those who improve after defervescence are said to have non-severe dengue. Some patients progress to the critical phase of plasma leakage without defervescence and, in these patients, changes in the full blood count should be used to guide the onset of the critical phase and plasma leakage.

Those who deteriorate will manifest with warning signs. This is called dengue with warning signs Cases of dengue with warning signs will probably recover with early intravenous rehydration. Some cases will deteriorate to severe dengue.

Recovery Phase

If the patient survives the 24–48 hour critical phase, a gradual reabsorption of extravascular compartment fluid takes place in the following 48–72 hours. General well-being improves, appetite returns, gastrointestinal symptoms abate, haemodynamic status stabilizes and diuresis ensues. Some patients may have a rash of "isles of white in the sea of red".[13] Some may experience generalized pruritus. Bradycardia and electrocardiographic changes are common during this stage.

Table 1. The various clinical problems during the different phases of dengue

1. Febrile phase	Dehydration; high fever may cause neurological disturbances and febrile seizures in young children
2. Critical phase	Shock from plasma leakage; severe haemorrhage; organ impairment
3. Recovery phase	Hypervolaemia (only if intravenous fluid therapy has been excessivea and/or has extended into this period)

The haematocrit stabilizes or may be lower due to the dilutional effect of reabsorbed fluid. White blood cell count usually starts to rise soon after defervescence but the recovery of platelet count is typically later than that of white blood cell count.

Severe Dengue

Severe dengue is defined by one or more of the following: (i) plasma leakage that may lead to shock (dengue shock) and/or fluid accumulation, with or without respiratory distress, and/or (ii) severe bleeding, and/or (iii) severe organ impairment.

As dengue vascular permeability progresses, hypovolaemia worsens and results in shock. It usually takes place around defervescence, usually on day 4 or 5 (range days 3–7) of illness, preceded by the warning signs. During the initial stage of shock, the compensatory mechanism which maintains a normal systolic blood pressure also produces tachycardia and peripheral vasoconstriction with reduced skin perfusion, resulting in cold extremities and delayed capillary refill time. Uniquely, the diastolic pressure rises towards the systolic pressure and the pulse pressure narrows as the peripheral vascular resistance increases. Patients in dengue shock often remain conscious and lucid. The inexperienced physician may measure a normal systolic pressure and misjudge the critical state of the patient. Finally, there is decompensation and both pressures disappear abruptly. Prolonged hypotensive shock and hypoxia may lead to multi-organ failure and an extremely difficult clinical course.

The patient is considered to have shock if the pulse pressure (i.e. the difference between the systolic and diastolic pressures) is Ý 20 mm Hg in children or he/she has signs of poor capillary perfusion (cold extremities, delayed capillary refill, or rapid pulse rate). In adults, the pulse pressure of Ý 20 mm Hg may indicate a more severe shock. Hypotension is usually associated with prolonged shock which is often complicated by major bleeding.

Patients with severe dengue may have coagulation abnormalities, but these are usually not sufficient to cause major bleeding. When major bleeding does occur, it is almost always associated with profound shock since this, in combination with thrombocytopaenia, hypoxia and acidosis, can lead to multiple organ failure and advanced disseminated intravascular coagulation. Massive bleeding may occur without prolonged shock in instances when acetylsalicylic acid (aspirin), ibuprofen or corticosteroids have been taken.

Unusual manifestations, including acute liver failure and encephalopathy, may be present, even in the absence of severe plasma leakage or shock. Cardiomyopathy and encephalitis are also reported in a few dengue cases. However, most deaths from dengue occur in patients with profound shock, particularly if the situation is complicated by fluid overload.

Severe dengue should be considered if the patient is from an area of dengue risk presenting with fever of 2–7 days plus any of the following features:

1. There is evidence of plasma leakage, such as:
   - high or progressively rising haematocrit;
   - pleural effusions or ascites;
   - circulatory compromise or shock (tachycardia, cold and clammy extremities, capillary refill time greater than three seconds, weak or undetectable pulse, narrow pulse pressure or, in late shock, unrecordable blood pressure).
2. There is significant bleeding.
3. There is an altered level of consciousness (lethargy or restlessness, coma, convulsions).
4. There is severe gastrointestinal involvement (persistent vomiting, increasing or intense abdominal pain, jaundice).
5. There is severe organ impairment (acute liver failure, acute renal failure, encephalopathy or encephalitis, or other unusual manifestations, cardiomyopathy) or other unusual manifestations.

LABORATORY DIAGNOSIS AND DIAGNOSTIC TESTS

Dengue virus infection produces a broad spectrum of symptoms, many of which are non-specific. Thus, a diagnosis based only on clinical symptoms is unreliable. Early laboratory confirmation of clinical diagnosis may be valuable because some patients progress over a short period from mild to severe disease and sometimes to death. Early intervention may be life-saving.

Before day 5 of illness, during the febrile period, dengue infections may be diagnosed by virus isolation in cell culture, by detection of viral RNA by nucleic acid amplification tests (NAAT), or by detection of viral antigens by ELISA or rapid tests. Virus isolation in cell culture is usually performed only in laboratories with the necessary infrastructure and technical expertise. For virus culture, it is important to keep blood samples cooled or frozen to preserve the viability of the virus during transport from the patient to the laboratory. The isolation and identification of dengue viruses in cell cultures usually takes several days. Nucleic acid detection assays with excellent performance characteristics may identify dengue viral RNA within 24–48 hours. However, these tests require expensive equipment and reagents and, in order to avoid contamination, tests must

Table 2. Summary of operating characteristics and comparative costs of dengue diagnostic methods[9]

Diagnostic methods	Diagnostic of acute infection	Time to results	Specimen	Time of collection after onset of symptoms	Facilities	Cost
Viral Isolation and serotype identification	Confirmed	1–2 weeks	Whole blood, serum, tissues	1–5 days	Mosquito or cell culture facilities, BSL-2/BSL-3° laboratory fluorescence microscope or molecular biology equipment	$$$
Nucleic acid detection	Confirmed	1 or 2 days	Tissues, whole blood, serum, plasma	1–5 days	BSL-2 laboratory, equipment for molecular biology	$$$
Antigen detection	Not yet determinated Confirmed	1 day > 1 day	Serum Tissue for immuno-chemistry	1–6 days NA	ELISA facilities Facilities for histology	$$ $$$
IgmELISA IgM rapid test	Probable	1–2 days 30 minutes	Serum, plasma, whole blood	After 5 days	Elisa facilities No additional supplies	$
IgG (paired sera) by ELISA, HI or neutralization test	Confirmed	7 days or more	Serum, plasma, whole blood	Acute sera, 1– days, convalescent after 15 days	ELISA facilities BSL-2 laboratory for neutralization assay	$

observe quality control procedures and must be performed by experienced technicians. NS1 antigen detection kits now becoming commercially available can be used in laboratories with limited equipment and yield results within a few hours. Rapid dengue antigen detection tests can be used in field settings and provide results in less than an hour. Currently, these assays are not type-specific, are expensive and are under evaluation for diagnostic accuracy and cost-effectiveness in multiple settings. Table 2 summarizes various dengue diagnostic methods and their costs.

After day 5, dengue viruses and antigens disappear from the blood coincident with the appearance of specific antibodies. NS1 antigen may be detected in some patients for a few days after defervescence. Dengue serologic tests are more available in dengue- endemic countries than are virological tests. Specimen transport is not a problem as immunoglobulins are stable at tropical room temperatures.

For serology, the time of specimen collection is more flexible than that for virus isolation or RNA detection because an antibody response can be measured by comparing a sample collected during the acute stage of illness with samples collected weeks or months later. Low levels of a detectable dengue IgM response – or the absence of it – in some secondary infections reduces the diagnostic accuracy of IgM ELISA tests. Results of rapid tests may be available within less than one hour. Reliance on rapid tests to diagnose dengue infections should be approached with caution, however, since the performance of all commercial tests has not yet been evaluated by reference laboratories.[16]

A four-fold or greater increase in antibody levels measured by IgG ELISA or by haemagglutination inhibition (HI) test in paired sera indicates an acute or recent flavivirus infection. However, waiting for the convalescent serum collected at the time of patient discharge is not very useful for diagnosis and clinical management and provides only a retrospective result.

Differential Diagnosis

Dengue fever can easily be confused with non-dengue illnesses, particularly in non- epidemic situations. Depending on the geographical origin of the patient, other etiologies – including non-dengue flavivirus infections – should be ruled out. These include yellow fever, Japanese encephalitis, St Louis encephalitis, Zika, and West Nile, alphaviruses (such as Sinbis and chikungunya), and other causes of fever such as malaria, leptospirosis, typhoid, Rickettsial diseases (Rickettsia prowazeki, R. mooseri, R. conori, R. rickettsi, Orientia tsutsugamushi, Coxiella burneti, etc.), measles, enteroviruses, influenza and influenza-like illnesses, haemorrhagic fevers (Arenaviridae: Junin, etc.; Filoviridae: Marburg, Ebola; Bunyaviridae: hantaviruses, Crimean-Congo haemorrhagic fever, etc.).

Both the identification of virus/viral RNA/viral antigen and the detection of an antibody response are preferable for dengue diagnosis to either approach alone (see Table 3).

Unfortunately, an ideal diagnostic test that permits early and rapid diagnosis, is affordable for different health

Table 3. Interpretation of dengue diagnostic tests adapted from Dengue and Control (DENCO) study

Highly suggestive	Confirmed
one of the following 1. IgM + in a single serum sample 2. IgG + in a single serum sample with a HI titre of 1280 or greater	one of the following: 1. PCR + 2. Virus culture + 3. IgM seroconversion in paired sera 4. IgG seroconversion in paired sera or fourfold IgG titer increase in paired sera

systems, is easy to perform, and has a robust performance, is not yet available.

RECOMMENDATIONS FOR TREATMENT

Patients who require emergency treatment and urgent referral when they have severe dengue 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 patients with severe dengue should be admitted to the 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 circulation during the period of plasma leakage. Plasma losses should be replaced immediately and rapidly with isotonic crystalloid solution or, in the case of hypotensive shock, colloid solutions. If possible, obtain haematocrit levels before and after fluid resuscitation.

There should be continued replacement of further plasma losses to maintain effective circulation for 24–48 hours. For overweight or obese patients, the ideal body weight should be used for calculating fluid infusion rates. A group and cross-match should be done for all shock patients. Blood transfusion should be given only in cases with suspected/severe bleeding.

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

The goals of fluid resuscitation include improving central and peripheral circulation (decreasing tachycardia, improving blood pressure, pulse volume, warm and pink extremities, and capillary refill time < 2 seconds) 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.

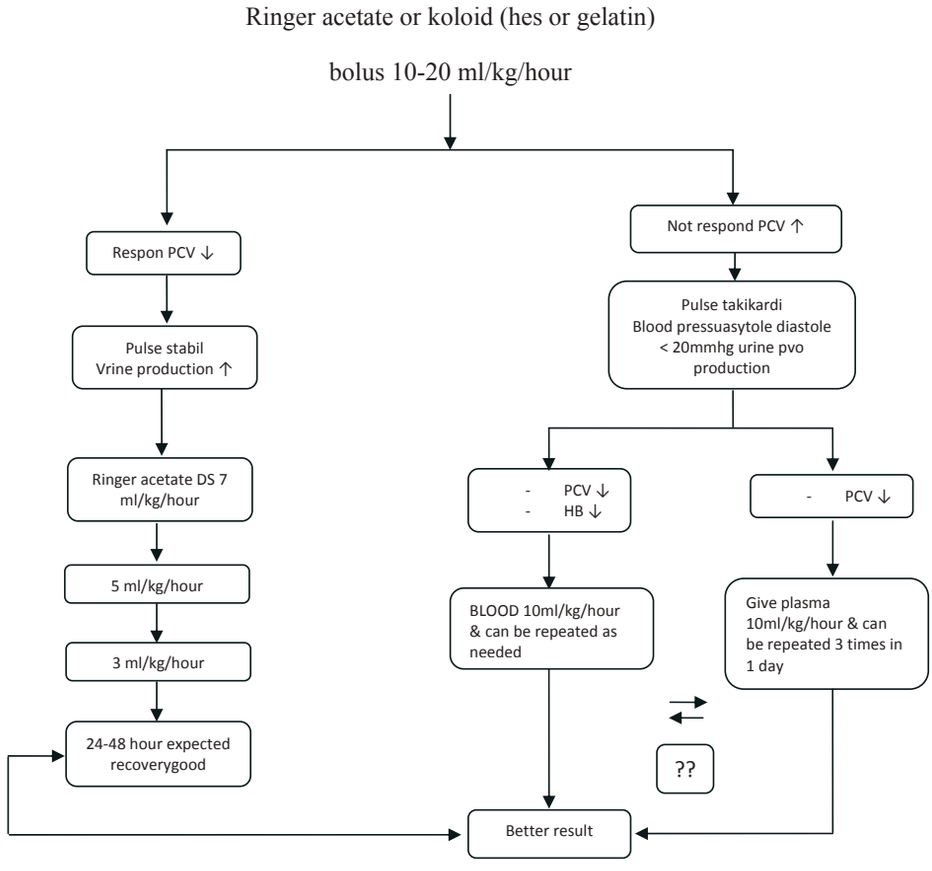


Figure 5 Algorithm for fluid management in compensated shock (WHO, 2009 with modified)

Treatment of Shock

The action plan for treating patients with compensated shock is as follows (Figure 5)

1. 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.
2. 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 to 2–3 ml/kg/hr, and then further depending on haemodynamic status, which can be maintained for up to 24–48 hours.
3. If vital signs are still unstable (i.e. shock persists), check the haematocrit the first bolus. If the haematocrit increases or is 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, reduce 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).
4. Further boluses of crystalloid or colloidal solutions may need to be given during the next 24–48 hours.
5. Awarness of using Ringer Lactat solution in Dengue Virus Infection cases can induce severity.
6. One of indicator not using Ringer Lactat is an increasing liver enzyme, AST and ALT with level more than 100-200 U/L it is marker of Liver damage.
7. Therefore we should choose other solution such as Ringer Acetat or Physiology Salt.
8. Using Ringer Acetat as fluid therapy in Dengue Virus Infection is better to prevent liver damage than using Ringer Lactate.

Patients with hypotensive shock should be managed more vigorously. The action plan for treating patients with hypotensive shock is as follows (Figure 6).

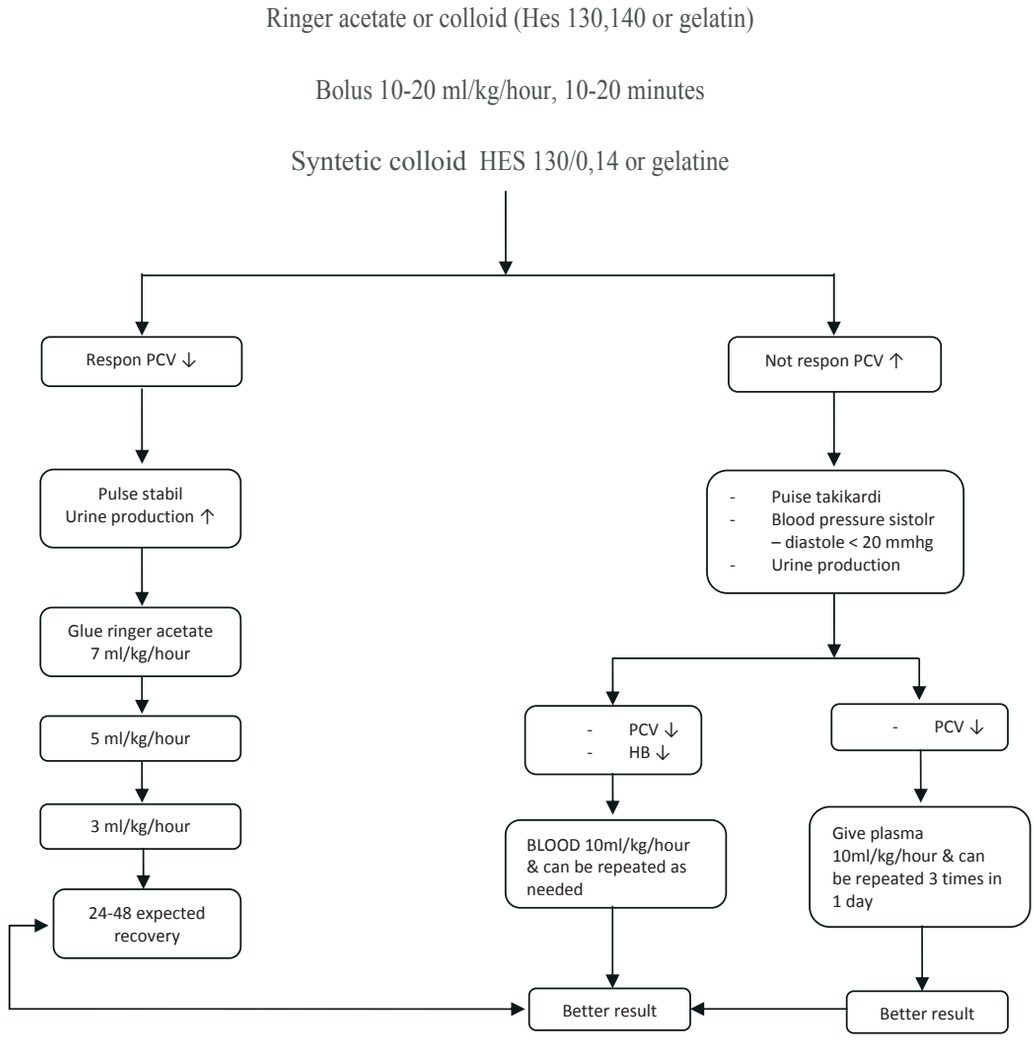


Figure 6. Algorithm for fluid management in hypotensive shock (WHO, 2009 with modification)

1. Initiate intravenous fluid resuscitation with crystalloid or colloid solution (if available) at 20 ml/kg as a bolus given over 15 minutes to bring the patient out of shock as quickly as possible.
2. If the patient's condition improves, give a crystalloid/ colloid infusion of 10 ml/ kg/hr for one hour. Then continue with crystalloid infusion and gradually reduce to 5–7 ml/kg/hr for 1–2 hours, then to 3–5 ml/kg/hr for 2–4 hours, and then to 2–3 ml/kg/hr or less, which can be maintained for up to 24–48 hours.
3. If vital signs are still unstable (i.e. shock persists), review the haematocrit obtained before the first bolus. If the haematocrit was low (<40% in children and adult females, <45% in adult males), this indicates bleeding and the need to cross- match and transfuse blood as soon as possible (see treatment for haemorrhagic complications).
4. 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.
5. If the haematocrit decreases 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 complications). 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.
6. 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 care 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 catheter enables close monitoring of urine output. An acceptable urine output would be about 0.5 ml/kg/ hour. 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, changes 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 discontinued 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 transfusions 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.

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

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

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

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

The action plan for the treatment of haemorrhagic complications is as follows:
- Give 5–10 ml/kg of fresh-packed red cells or 10–20 ml/kg of fresh whole blood at an appropriate rate and observe the clinical response. It is important that fresh whole blood or fresh red cells are given. Oxygen delivery at tissue level is optimal with high levels of 2,3 di-phosphoglycerate (2,3 DPG). Stored blood loses 2,3 DPG, low levels of which impede the oxygen-releasing capacity of haemoglobin, resulting in functional tissue hypoxia. A good clinical response includes improving haemodynamic status and acid-base balance.
- Consider repeating the blood transfusion if there is further blood loss 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 practised when massive bleeding can not be managed with just fresh whole blood/fresh-packed cells, but it may exacerbate the fluid overload.

Treatment of complications and other areas of treatment

Fluid overload

Fluid overload with large pleural effusions and ascites 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 textbook of clinical care for further guidance on management).

Causes of fluid overload are:
1. excessive and/or too rapid intravenous fluids;
2. incorrect use of hypotonic rather than isotonic crystalloid solutions;
3. inappropriate use of large volumes of intravenous fluids in patients with unrecognized severe bleeding;
4. inappropriate transfusion of fresh-frozen plasma, platelet concentrates and cryoprecipitates;
5. continuation of intravenous fluids after plasma leakage has resolved (24–48 hours from defervescence);
6. co-morbid conditions such as congenital or ischaemic heart disease, chronic lung and renal diseases.

Early clinical features of fluid overload are :
1. respiratory distress, difficulty in breathing;
2. rapid breathing;
3. chest wall in-drawing;
4. wheezing (rather than crepitations);
5. large pleural effusions;
6. tense ascites;
7. increased jugular venous pressure (JVP).

Late clinical features are:
1. pulmonary oedema (cough with pink or frothy sputum ± crepitations, cyanosis);
2. irreversible shock (heart failure, often in combination with ongoing hypovolaemia).

Additional investigations are:
1. the chest x-ray which shows cardiomegaly, pleural effusion, upward displacement of the diaphragm by the ascites and varying degrees of "bat's wings" appearance ± Kerley B lines suggestive of fluid overload and pulmonary oedema;
2. ECG to exclude ischaemic changes and arrhythmia;
3. cardiac enzymes.

The action plan for the treatment of fluid overload is as follows:
1. Oxygen therapy should be given immediately.
2. Stopping intravenous fluid therapy during the recovery phase will allow fluid in
3. 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:

» signs 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.

4. 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 continuous infusion of furosemide 0.1 mg/kg/hour. Monitor serum potassium and correct the ensuing hypokalaemia.
5. If the patient has stable haemodynamic status but is still within the critical phase, reduce the intravenous fluid accordingly. Avoid diuretics during the plasma leakage phase because they may lead to intravascular volume depletion.
6. Patients who remain in shock with low or normal haematocrit levels but show signs of fluid overload may have occult haemorrhage. Further infusion of large volumes of intravenous fluids will lead only to a poor outcome. Careful fresh whole blood transfusion should be initiated as soon as possible. If the patient remains in shock and the haematocrit 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 mellitus and/or hypoglycaemic agents. Electrolyte and acid-base imbalances 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 š 7.15) can occur. One should also be alert for co-infections and nosocomial infections. If found cases with Dengue Shock Syndrom with hipotonous heart muscle complication at figure 7.

TO PREVENT LIFE THREATENING HYPOTENSION IN DSS AS FOLLOW

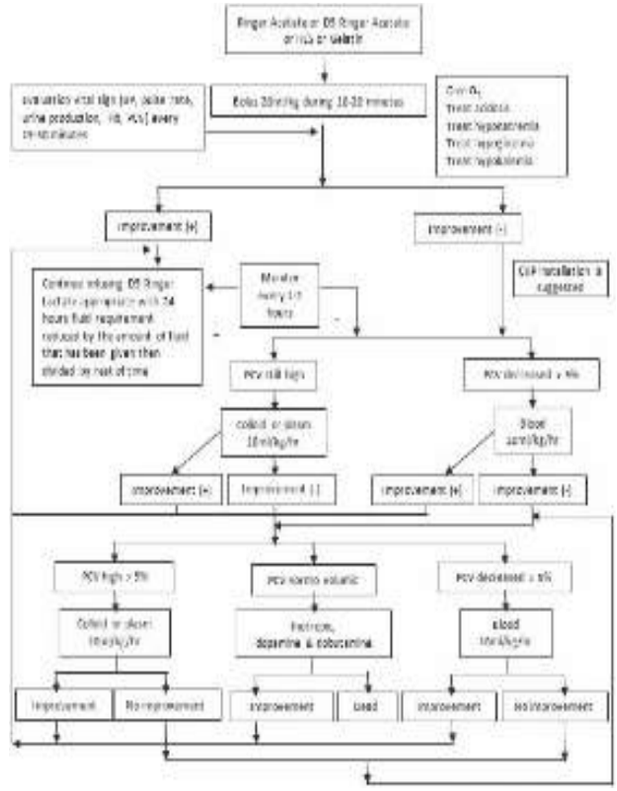


Figure 7. Flow Chart of Dengue Shock Syndrom with hypotonous heart muscle complication(WHO, 2009)

Supportive Care and Adjuvant Therapy

Supportive care and adjuvant therapy may be necessary in severe dengue. This may include:

renal replacement therapy, with a preference to continuous veno-venous haemodialysis (CVVH), since peritoneal dialysis has a risk of bleeding;

vasopressor and inotropic therapies as temporary measures to prevent life-threatening hypotension in dengue shock and during induction for intubation, while correction of intravascular volume is being vigorously carried out;

further treatment of organ impairment, such as severe hepatic involvement or encephalopathy or encephalitis;

further 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 intravenous immunoglobulins, or of recombinant Activated Factor VII.

CONCLUSION

By using integrated criteria of WHO 2009 and 1997, update management of Dengue Shock Syndrome in

Pediatric cases, can improve clinical management to reach the lower mortality in community until CFR < 1%.

Using Ringer Acetat as fluid therapy in Dengue Virus Infection is better to prevent liver damage than using Ringer Lactate.

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Literature Review

THE ROLE OF HYPERBARIC THERAPY IN THE GROWTH OF CANDIDA ALBICANS

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ABSTRACT

Background: Candida albicans is opportunistic pathogen fungi which cause many disease in human such as reccurrent apthous stomatitis, skin lesions, vulvavaginitis, candiduria and gastrointestinal candidiasis. Aim: Infection mechanism of C. albicans is very complex including adhesion and invasion, morphology alteration from khamir form cell to filamen form (hifa), biofilm forming and the avoidance of host immunity. Method: The ability of C. albicans to adhere to the host cell which is act as important factor in the early colonization and infection. Result: The phenotype alteration to be filament form let the C. albicans to penetrate to the epithelium and play important role in infection and separation C. Albicans to the host cell. Hyperbaric oxygen is the inhalation of 100 percent oxygen inside hyperbaric chamber that is pressurized to greater than 1 atmosphere (atm). Conclusion: The organism was found to be inhibited within a pressure/time range well tolerated by human subjects, suggesting that hyperbaric oxygen might be used successfully in treating human candidiasis.

Key words: *Hyperbaric Oxygen, candida albicans, infection, host cell, immunity*

ABSTRAK

Latar belakang: Candida albicans merupakan jamur patogen yang berpotensi menyebabkan beberapa penyakit di manusia seperti reccurrent apthous stomatitis, lesi kulit, vulvavaginitis, candiduria dan candidiasis pada gastrointestinal. Tujuan: Mekanisme infeksi C. Albicans sangat kompleks meliputi adhesi dan invasi, perubahan morfologi dari bentukan khamir sel menjadi bentuk filamen (hifa), pembentukan biofilm dan penghindaran terhadap sistem imun tubuh. Metode: Kemampuan C. albicans untuk melekat di sel tubuh yang merupakan faktor penting pada kolonisasi awal dan infeksi. Hasil: Perubahan fenotip menjadi filamen menyebabkan C. albicans berpenetrasi masuk ke epithelium dan berperan dalam infeksi dan pemisahan C. Albicans ke sel tubuh. Hyperbaric oxygen merupakan terapi dengan menggunakan 100 percent oksigen di dalam ruang udara bertekanan tinggi (RUBT)/hyperbaric chamber yang mendapatkan tekanan lebih dari 1 atmosphere (atm). Kesimpulan: Terjadi penghambatan organisme dalam tekanan dan waktu tertentu pada subyek manusia, menunjukkan bahwa hiperbarik oksigen mungkin dapat digunakan dalam terapi human candidiasis.

Kata kunci: *Oksigen Hiperbarik, candida albicans, infeksi, sel tubuh, imunitas*

INTRODUCTION

Candida albicans is the fourth most common hospital-acquired infection.[1,2] Because C. albicans and other fungal pathogens are eukaryotes and therefore share many of their biological processes with humans, most anti-fungal drugs cause deleterious side effects and, at the doses used, are fungistatic rather than fungicidal. So, it is an important goal of *Candida albicans* research to identify appropriate targets for anti-fungal technologies.

Morphology

Candida albicans can exist in three forms that have distinct shapes: yeast cells (also known as blastospores), pseudohyphal cells and true hyphal cells. Yeast cells are round to ovoid in shape and separate readily from each other. Pseudohyphae resemble elongated, ellipsoid yeast cells that remain attached to one another at the constricted septation site and usually grow in a branching pattern that is thought to facilitate foraging for nutrients away from

the parental cell and colony. True hyphal cells are long and highly polarized, with parallel sides and no obvious constrictions between cells. Actin is always localized at the tip of the growing hypha[3,]A basal septin band (green) forms transiently at the junction of the mother cell and the evaginating germ tube; the first true hyphal septum forms distal to the mother cell and well within the germ tube.[4]

Candidiasis

Candidiasis or thrush is a fungal infection (mycosis) of any species from the genus *Candida* (one genus of yeasts). *Candida albicans* is the most common agent of Candidiasis in humans.[5] Also commonly referred to as a yeast infection, candidiasis is also technically known as candidosis, moniliasis, and oidiomycosis.[6] Candidiasis encompasses infections that range from superficial, such as oral thrush and vaginitis, to systemic and potentially life-threatening diseases. *Candida* infections of the latter category are also referred to as candidemia or invasive candidiasis, and are usually confined to severely immunocompromised persons, such as cancer, transplant, and AIDS patients, as well as nontrauma emergency surgery patients.[4] Superficial infections of skin and mucosal membranes by *Candida* causing local inflammation and discomfort are common in many human populations.[5,6] While clearly attributable to the presence of the opportunistic pathogens of the genus *Candida*, candidiasis describes a number of different disease syndromes that often differ in their causes and outcomes.[7,8]

Hyperbaric Oxygen

Hyperbaric Medicine is the fascinating use of barometric pressure for delivering increased oxygen dissolved in plasma to body tissues. Hyperbaric oxygen therapy (HBO) is a form of treatment in which a patient breathes 100% oxygen at higher than normal atmospheric pressure that is greater than 1 atmosphere absolute (ATA). Therapy is given in special therapeutic chambers, which were earlier used primarily to treat illnesses of deep sea divers. In the sixties HBO went out of practice because of its use without adequate scientific validation. Over the last two decades, animal studies, clinical trials and well-validated clinical experience has proved efficacy of HBO in many indications and there is recently a renewed interest in this field all over the world.[9]

The Effects Of Hyperbaric Oxygen On Fungi

Many effort have been made for a number of years and various reasons to determine oxygen toxicity limits of yeast. Cairney has reviewed this problems associated with studies on the effects of hyperbaric oxygen on the fungi.[10] Oxygen is the most prevalent and most important element for the human body. It passes from the ambient air to the alveolar air and continues through the pulmonary, capillary and venous blood to the systemic arterial and capillary blood. It then moves through the interstitial and intracellular fluids to the microscopic points of oxygen consumption in the perioxomes, endoplasmic reticulum and mitochondria.[10] The interactions between oxygen and antimicrobial agents have important implications fot the therapy of infections, because oxygen tensions could influenced the static and cidal activity of human body against spesific fungies. Increased oxygen tensions can stimulate changes in host tissues (e.g decreased reduction – oxidation potential and increased pH) that might affect the metabolism and/or activation of certain fungies.

Systemic fungal infections generally only occur in patients with other debilitating conditions like diabetes, severe burns or the immunocompromised. Research has shown that there was no response to increase atmospheric pressure alone, but addition of 100% oxygen under pressure led to growth inhibition of pO_2 of 900 mmHg and killing of microorganism at a pO_2 value of 1800 mmHg.[11]

The Effect Of Hyperbaric Oxygen On C.albicans

The effects of hyperbaric oxygen at a steady level of 3 ATA was possesed the same result with McAllister et al who reported total inhibition of C. albicans at 2 ATA oxygen.[12] Gifford and Pitchard describes responses of *Candida utilis* to hyperbaric oxygen.[13] Cultures of organisms in an exponential growth phase did not undergo any further development when exposed to 10 ATA oxygen. When the exposure was continued for several days, all cells died. In the study of Gifford reported that exponentially growing populations were more sensitive than stationary phase populations. The study of Cairney WJ, 1978 showed significant result using 3 ATA oxygen for 4,5 hours of 24 hours period is sufficient to cause inhibition of growth and ability to form pseudohyphae and chlamydospores. This work has confirmed that *C. albicans* is inhibited in vitro within a pressure range readily tolerated by human subjects. This suggests that hyperbaric oxygen treatment might be effective in treating human candidiasis and that exposure tables used for gas gangrene causing by *Clostridium ssp* could be used with some expectation of success.[14]

One study of Gottlieb SF et al, 1964 indicated that exposure of *C. albicans* to 10 ATA of oxygen for 14 dayskilled the organism. It was possible of low oxygen tensions and shorter exposures times a large number of *C. albicans* could have been killed with only a few cells able to survive the exposure to oxygen. In this study they have designed quantitative approach to obtain information regarding fungicidal versus fungistatic effects of HBO on *C. albicans*. They investigated the effects of (i) pressure, (ii) 900 mmHg O_2, (iii)1800 mmHg O_2 on the growth of organisms.[15]

CONCLUSION

Most studies of Hyperbaric oxygen correlated with C. albicans have shown that the effect is inhibitory rather than cidal.

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Research Report

SERO-EPIDEMIOLOGY OF DENGUE VIRUS INFECTION IN 4 CITIES OF INDONESIA

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ABSTRACT

Background: Dengue Virus Infektion is major public health problem in Indonesia. Aedesaegypti is widespread in both urban and rural areas, where multiple virus Serotype are circulating. On 2013 outbreak of dengue virus infection occur in East Java. Therefore study seroepidemiology in Bangkalan and Lombok had been done. Aim: to find a mutated strain of Dengue Virus in 4 cities of Indonesia. Method: On 2011 and 2012 seroepidemiology study had been done in Dr. Soetomo Surabaya and Soerya Sidoarjo Hospital; and on 2013 study had been done in Surabaya, Bangkalan and Lombok Hospital . Diagnosis of Dengue Virus Infection was based on Criteri WHO - 2009. Virus isolation in Surabaya, Sidoarjo, Bangkalan and Lombok had been done. Result: a total of 349 isolate were obtained from dengue patients sera collected in Surabaya and Sidoarjo, 2011–2012 showed that Den V1 (182), Den V2 (20) Den V4 (1) were found in Surabaya on 2011 and Den V 1 (79) , Den V 2 (7) were found in Surabaya on 2012; Den V1 (40), Den V 2 (3) were found in Sidoarjo on 2011 and Den V 1 (17) were found in Sidoarjo on 2012; Virus isolation in Surabaya on 2013, January: 237 serum sample were collected, found Den V 1 (8), Den V 3 (2) and Den V 4 (5). And PCR stereotyping of isolated viruses in Madura found Den V 1 (1) and Den V 4 (23). In Lombok found Den V 4 (4).It is possible to shift predominant strain in Surabaya , Genotype or Serotype shift might increase the number of dengue patients. Conclusion: there were shift predominant strain in Surabaya especially Den V 1. Therefore to continuous surveillance of circulating viruses is required to predict the risk of DHF and DF.

Key words: *Seroepidemiology, Den V, predominant strain, serotype, RNA*

ABSTRAK

Latar belakang: Sampai sekarang infeksi virus dengue masih merupakan penyakit infeksi yang menjadi problematic kesehatan masyarakat Indonesia. Infeksi virus dengue tersebar luas di kota dan desa, dimana banyak serotip virus dengue tersebar dalam darah penderita infeksi virus dengue. Tahun 2013 di Jawa Timur dan Jakarta terjadi penyelidikan kasus infeksi virus dengue secara dekat. Oleh karena itu perlu dilakukan sero epidemiologi. Penderita infeksi virus dengue di Surabaya, Sidoarjo, Bangkalan dan Mataram. Tujuan: Menemukan materi berdasarkan virus dengue di 4 kota di Indonesia. Metode: Pada tahun 2011 dan 2012 penulis sero epodemiologi telah dilakukan di RSUD Dr. Soetomo Surabaya dan RS Soerya Sidoarjo, dan pada tahun 2013 penelitian dilakukan di Surabaya, Sidoarjo, Bangkalan dan Mataram. Diagnose penderita infeksi virus dengue berdasarkan criteria WHO 2009, sekarang dilakukan isolasi virus dengue. Hasil: Jumlah kasus yang diketahui sebanyak 349, berasal dari penderita infeksi virus dengue yang dirawat di Surabaya dan Sidoarjo. Seperti pada tahun 2011–2012 menunjukan hasil sebagai berikut: Den V1 (182), Den V2 (20), Den V4 (1), temuan ini di Sidoarjo pada tahun 2011 dan Den V1 (79), Den V2 (7), ditemukan di Surabaya pada tahun 2012: Den V1 (40), Den 2 (3). Ditemukan di Sidoarjo tahun 2011: Den V1 (17), ditemukan di Sidoarjo tahun 2012. Isolasi virus dengue di Surabaya tahun 2013 pada bulan Januari terkumpul 237 serum, hasil yang positif terdeteksi Den V1 (8), Den V3 (2) dan Den V4 (5). Hasil isolasi virus dengue dari Madura ditemukan Den V1 (1) dan Den V4 (23), dan dari Lombok ditemukan Den 4 (4). Jika temuan ini dicermati perubahannya stain virus dengue terjadi di Surabaya terutama Den V1. Perubahan genotif dan serotif memungkinkan peningkatan

jumlah penderita virus dengue. Kesimpulan: Ditemukan perubahan stain dengue virus di Surabaya terutama Den V1, oleh karena itu servellance virus dengue, perlu di teliti dan di cari hubungannya dengan perjalanan klinis DBD dan demam dengue.

Kata kunci: *Seroepidemiology, Den V, predominant strain, serotype, RNA*

INTRODUCTION

Dengue virus (DENV) infection can make a major public health problem; > 50 million persons are infected each year worldwide,[1] and the incidence of severe, sometimes lethal, forms of the disease is increasing.[2] Dengue viruses are mosquitoborneflaviviruses with a single-stranded, nonsegmented, positive-sense RNA genome −11 kb in length. Four antigenically distinct serotype, DENV types 1 to 4, exist.[3] Infection with any serotype can lead to disease, ranging from mild infection, dengue fever (a generally mild disease with complete recovery), to severe forms (dengue hemorrhagic fever and dengue shock syndrome). Molecular epidemiology studies have investigated the possibility of a link between particular DENV genotype or cluster or particular clinical form of disease.[4,5] Consequently, finding new viral genotypes in areas where they had been absent could be of epidemiologic and clinical interest.

Emerging Infectious Diseases dengue epidemic are expanding rapidly. This emerging diseases continues to baffle and challenge epidemiologists and clinicians to study. Despite endemicity of 3 or more different dengue viruses, why does severe dengue occur in some populations and not in other? Why are children principally affected in some areas and adults in others? How can severe dengue reliably be recognize early enough to permit appropriate therapy to be applied?[6]

During an infection there are 4 dengue viruses, the principal threat to human health resides in the ability of the infecting virus can causes an acute febrile syndrome characterized by clinically significant vascular permeability, dengue hemorrhagic fever (DHF). However, because at onset vascular permeability exhibits only subtle changes, how can a diagnostic be made early enough to begin life-saving intravenous treatment? In person with light skin color, the standard syphygmomanometer cuff tourniquet test has been widely used to screen children in outpatient settings; a positive result in an early warning of incipient DHF. Because of genetic diversity among humans, the tourniquet test as a screening tool requires widespread evaluation and validation.[6]

Human are not uniformly susceptible to the DHF syndrome. HLA gene distribution correlates with increased susceptibility as well as with increased resistance.[7] In addition, a powerful resistance gene is found in blacks.[8] Importantly, susceptibility to vascular permeability during a dengue infection is age-related. The susceptibility of young children to DHF precisely paralleled age-related changes in microvascular permeability measuredin normal children and adult.

Dengue fever syndrome in susceptible adults may be contrasted to the innate susceptibility of children for vascular permeability syndrome during a secondary dengue virus infection.

In Southeast Asia, the epicenter of DHF epidemics, dengue infection rates are falling in children, resulting in changing epidemiologic patters of DHF. In Thailand, for example, the modal age at which children are hospitalized for DHF has steadily increased over the past decades.

In addition, because an increasing number of persons experience their first dengue infection at an older age, dengue fever cases are now appearing in adults.[2]

MATERIALS AND METHODS

Population Study

Seroepidemiology study had been done in Surabaya, Sidoarjo, Bangkalan and Mataram. Surabaya and Sidoarjo are part of East Java including in Java Island, and Bangkalan is part of Madura Island. Mataram city is part of West Nusa Tenggara province. There are 392 serum specimens from patients which had a clinical manifestation of dengue infection based on Criteria WHO-2009 from 2011 until January 2013.

349 isolate were obtained from dengue patients sera in Surabaya (Dr. Soetomo Hospital) and Sidoarjo (Soerya Hospital) on 2011–2012; and 43 serum sample were collected in Surabaya, Bangkalan, and Mataram on January 2013.

Sample Collection and Diagnosis of Dengue

Human sera were obtained from 392 patients presenting clinical manifestations of dengue and tested for anti-dengue IgM antibodies (Becton-Dickinson). The clinical samples corresponded with dengue cases reported during 2011–2013. Diagnosis of dengue virus infected was based on Criteria WHO 2009. Dengue-infected samples were obtained during the first five days of the onset of fever and were processed for anti dengue IgM detection using IgM capture ELISA (MAC-ELISA) as described by Vorndam et al.

As a routine practice and with the idea of recording epidemic data, the suspected dengue sample already clinically diagnosed in community health centers were sent to Institute of Tropical Disease Airlangga University, Surabaya, Indonesia. In the laboratory, the presence of dengue virus was confirmed by MAC-ELISA and RT-PCR.

Dengue Virus Isolates

Dengue virus isolated by using Aedesalbopictus C6/36 cells were grown in 48-well tissue culture plates as described by Irgarashi.[9] Briefly, $2x10^5$ cells were plated in 1 ml of minimum essential medium (Gibco BRL, Grand Island, N.Y.) supplemented with 7% fetal bovine serum (Sigma Chemical Co., St Louis, Mo) and 1% glutamine, vitamins and nonessential amino acids. After 24 hours of cultures, 100 µl of every sera diluted 1:10 was added to the corresponding well. The mixture was then gently shaken and incubated for 60 minutes at room temperature. Cells were then washed with serum-free medium and cultured at 28° C with complete medium for at least 10 days. Cells were harvested for RT-PCR diagnosis.

RNA extraction

Total RNA was extracted either from 100µl of serum or from cultured cells by using Trizol LS (GIBCO BRL, Gaithersburg, MD.) according to the manufacturers recommendations. Ethanol-precipitated RNA was recovered by centrifugation and air-dried. The RNA pellet was re-suspended in 50 µl of Diethyl-pyrocarbonate (Sigma)-treated water (DEPC water) and used as template for RT-PCR.

RT-PCR

Synthetic oligonucleotide primer pairs were designed based on published sequence data for each of the four serotypes of dengue.[10,11] Four fragments of an expected size of 392bp (DEN-1), 392 bp (DEN-4), 290 bp (DEN-3) and 119 bp (DEN-2) were obtained by using the SuperScrip^{TM} One Step RT-PCR kit inconjunction with Platinum^{R}Taq polymerase (Invitrogen, Life Technologies). A mixture of 5 µl of RNA, 25 µM of sencse and anti-sense PCR primers, and DEPC water to a total volume 0f 50 µl was incubated at 85° C for 5 minutes and then chilled on ice. The tubes-reaction mixture containing 2X PCR buffer containing 0.4 mM $MgSO_4$ and SuperScrip^{TM}RT/platinum^{R}Taq Mix, as recommended by the manufacturer (Invitrogen TM Life Technologies), was added to thr RNA and primers-containing tube. The reverse transcription reaction was performed at 50° C for 30 minutes. Thermocycling began with a hot start at 9° C for 2 minutes followed by 40 cycles of annealing at 55° C for 30 seconds, and extension at 72° C for one minutes and denaturing at 94° C for 15 minutes.

The PCR conditions for serotype assessment were as follows: 40 cycles of denaturing at 94° C for 30 seconds, annealing at 55° C for 1 minute, and extension at 72° C for 7 minutes. The reaction mixtures were electrophoresed and visualized under UV light after ethidium bromide staining of the gels.

RESULTS

Project 1. Virus isolation was collected from Surabaya and Sidoarjo on 2011–2012.

Table A total of 349 isolates sera were collected from Surabaya and Sidoarjo, 2011–2012

	2011		2012	
	Surabaya	Sidoarjo	Surabaya	Sidoarjo
D1	182	40	79	17
D2	20	3	7	0
D3	0	0	0	0
D4	1	0	0	0

Project 2. 43 viruses isolation were collected from Surabaya, Bangkalan_Madura and Mataram_Lombok on January 2013

Table PCR serotyping of isolated viruses

Site	D1	D2	D3	D4
Surabaya	8	0	2	5
Bangkalan	1	0	0	23
Mataram	0	0	0	4

DISCUSSION

Seroepidemiology of Dengue Virus Infection is a science of transferring Dengue Virus to other host of human being by biting of Aedesaegypti mosquito which has been supported by biotic and abiotic factor: such as an increasing population of Aedes aegypti in urban and sub urban environment due to the changing of raining season to sunrise.

Dengue virus infection in human being can be found if the interaction among etiologic, host and environment has occurred: (1)The etiologic is dengue virus which usually life in Aedesaegypti mosquito where it like life in clean water. (2)Host is human being who has decreasing immunity due to very tired and getting more virulent virus from biting of Aedesaegyptie mosquito which is supported by (3)changing humadity of environment.

Since 1968 Dengue Virus Infection has been found in Indonesia, especially at Surabaya and Jakarta city . Firstly

management of dengue virus infection very difficult to improve, therefore the higher mortality nearly 41,4% had been found; but on the following years in five decades the mortality rates was becoming to decrease until 1,27 % on 2011.

On January 2013, the outbreak of Dengue Virus Infection has been occurred and many cases of Children under five years has been found and one of them is a 21 days old baby suffered from DSS has been found, as a younger baby case.

Beside it many cases babies below one years old has been found as severe dengue virus infection which shown clinical manifestation of bleeding and encephalopathy, therefore some of them cannot be help. It should be studied!, what kind Serotype of dengue virus was becoming predominant strain as a cause of severity?

As we know in the first decade the Serotype of Dengue 2 and 3 were predominant strain Den 2 tended to be neutralized stronger than other viruses. It was found in Surabaya before 2008 but on the year 2009, 2010, 2011 and 2012 there were changing of Serotype and found Den 1 genotype 1V showed a severity clinical manifestation with primary infection but on 2013 Serotype Den 1 and IV were found at Surabaya, Bangkalan and Mataram; especially Bangkalan and Mataram showed more Den IV.

Phylogenetic analysis on Den V 1 in Surabaya showed that genotype 1 and IV consisted at the same time in 2012 although only genotype IV were isolate in 2011. There were differences between D 1 genotype I and IV. Analyzing the difference of genotype is important for vaccine development.

Based on this result of study, monitoring the emergence of imported or mutated strain of Dengue Virus in human being and mosquito should always be continued. Therefore, continuous surveillance of circulating viruses is required to predict the risk of DHF and DF.

This idea is very important for making procedure Update management of Dengue Virus Infection Cases to decrease Case Mortality in the outbreak situation and try to do new prevention method before Outbreak Occur.

Picture. a 21 days old baby suffered from DSS

CONCLUSSION

Until now, there were changing of serotype of dengue virus based on time, place and humadity.

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Research Report

IDENTIFICATION OF INFLUENZA VIRUSES IN HUMAN AND POULTRY IN THE AREA OF LARANGAN WET MARKET SIDOARJO-EAST JAVA, INDONESIA

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ABSTRACT

Background: Influenza is a viral infection that attacks the respiratory system (nose, throat, and lungs) that commonly known as "flu". There are 3 types of influenza viruses, such as type A, type B, and type C. Influenza virus type A is the type of virus that can infect both human and animals, virus type B are normally found only in human, and Influenza virus type C can cause mild illness in human and not causing any epidemics or pandemics. Among these 3 types of influenza viruses, only influenza A viruses infect birds, particularly wild bird that are the natural host for all subtypes of influenza A virus. Generally, those wild birds do not get sick when they are infected with influenza virus, unlike chickens or ducks which may die from avian influenza. Aim: In this study, we are identifying the influenza viruses among poultry in Larangan wet market. Method: Around 500 kinds of poultry were examined from cloacal swab. Result: Those samples were restrained with symptoms of suspected H5. The people who worked as the poultry-traders intact with the animal everyday were also examined, by taking nasopharyngeal swab and blood serum. Conclusion: Identification of influenza viruses was obtained to define the type and subtype of influenza virus by PCR.

Key words: *subtype of influenza viruses, human, poultry, symptoms, PCR result*

ABSTRAK

Latar belakang: Influenza merupakan infeksi virus yang menyerang sistem pernafasan (hidung, tenggorokan, dan paru-paru), biasa dikenal sebagai "flu". Terdapat 3 tipe virus influenza yaitu tipe A, tipe B, dan tipe C. Virus influenza tipe A adalah jenis virus yang dapat menginfeksi manusia dan hewan, virus influenza tipe B normalnya hanya ditemukan pada manusia, sedangkan virus influenza tipe C dapat menyebabkan sakit ringan pada manusia tanpa mengakibatkan kasus epidemi maupun pandemi. Dari ketiga tipe virus influenza tersebut, hanya tipe A yang dapat menginfeksi hewan jenis burung, terutama burung liar yang secara alami merupakan host dari semua subtipe virus influenza A. Secara umum, burung liar tersebut tidak akan menjadi sakit ketika terinfeksi virus influenza, tidak seperti pada ayam mapun bebek jika terinfeksi kemungkinan besar dapat mati. Tujuan: Dalam penelitian ini, kami mengidentiifikasi virus influenza pada unggas di pasar tradisional di Larangan, Sidoarjo. Metode: Sekitar 500 unggas diidentifikasi berdasarkan hapusan kloaka. Hasil: Pengambilan sampel tersebut disesuaikan dengan gejala yang muncul pada hewan itu terkait dengan gejala virus H5. Selain itu, kami juga mengumpulan sampel dari responden yang merupakan pekerja di pasar Larangan. Sampel responden berupa hapusan hidung dan serum darah. Kesimpulan: Identifikasi virus influenza dilakukan untuk melihat tipe dan subtipe virus berdasarkan PCR.

Kata kunci: *subtipe virus influenza, manusia, unggas, gejala, hasil PCR*

INTRODUCTION

Influenza virus type A can infect humans and animals. Various subtypes of this influenza A virus which usually attack human are H1N1, H1N2, and H3N2 (Rendell et al., 2006). Meanwhile several other influenza A types of attacking animals like H7N9, H5N1, or H3N2. Only this influenza A virus that attacks poultry which actually attacking domestic birds. Human infections with avian influenza (AI or "bird flu") are rare but occur most commonly after exposure to infected poultry (bird to human spread). HIN1 is a flu virus that was first detected in 2009 called as "swine flu", caused a world wide pandemic. Currently the HIN1 is a seasonal influenza virus found in humans and it is now also circulates among pigs. In 2010, even though World Health Organization announced that the pandemic was over, H1N1 flue virus is still circulating (Corzo et al., 2013).

Recently, there is a new type of influenza virus. H7N9 is a new subtype of avian influenza that has been reported to be detected in poultry in China. However no cases of H7N9 outside China have been reported yet and no sustained person-to-person spread of the H7N9 virus has been found at this time.

H5N1 is a highly pathogenic avian flu virus that caused serious outbreaks in domestic poultry in parts of Asia and the Middle East (WHO, 2012). Although H5N1 does not usually infect humans, nearly 600 cases of human cases of H5N1 have been reported from 15 countries since 2003 in Asia, Africa, Europe, and the Near East. About 60% of these people died from their illness. In 2011, 62 human with H5N1 cases and 34 deaths were reported from five countries—Bangladesh, Cambodia, China, Egypt, and Indonesia. Six countries— Bangladesh, China, Egypt, India, Indonesia, and Vietnam—have widespread and ongoing infections in their poultry. In 1997 an outbreak of H5N1 occurred in the farms and traditional markets in Hong Kong. For the first time reported that the H5N1 virus can infect human with the number of deaths of 6 to 18 cases. Poultry outbreaks also happen in other countries recently as well. Most human cases of "highly pathogenic" H5N1 virus infection have occurred in people who had recent contact with sick or dead poultry that were infected with H5N1 viruses. However, unlike other types of flu, H5N1 usually does not spread between people and no further evidence discovers that this virus can spread easily between people. Thus, the symptoms and possible complications of H5N1 in people can include fever, cough, shortness/difficulty breathing leads to respiratory failure, or pneumonia (Iskander et al., 2013).

Markets in Indonesia are the center of social and economic activities, but the market can also be a source of spread of diseases. A number of outbreaks of the disease at this time can even be transmitted through food products and living animals that are sold in the market. Traditional animal market needs special attention due to the occurrence of direct contact between wild birds carrying the virus of avian influenza (AI) in poultry and human (the poultry traders or buyers).

Weak bio-security and poor hygiene and sanitation lead to the spread and transmission of AI virus in poultry markets. Survey on wild birds around the Larangan wet market Sidoarjo has been conducted and showed that the wild birds infected with avian influenza virus H5N1 (Poetranto, 2011). There were highly possibilities of transmission of H5N1 virus from wild birds to poultry sold in the market and to the people works in that market. Therefore the Influenza study group, Institute of Tropical Disease Airlangga University was planning to hold Surveillance Influenza virus in traditional community animal market, the Larangan wet market Sidoarjo.

The aims of the study are to detect any potential transmission of AI virus in the traditional animal Larangan wet market, and also to detect the presence of AI virus in poultry trade and wild birds around the traditional market. This study is only an identification project to obtain early detection of transmission of AI virus among wild birds to poultry and the impact to those who works as poultry traders. Furthermore, the early detection could be useful for surveillance of influenza planning.

MATERIALS AND METHODS

There were 3 sample activities in this study. The first one is the sampling on human. Some general health assessment was carried out by checking the condition of 63 poultry traders. Physical examination and nasal swab sampling was taken during the study. The second sampling was on the poultry. The types of poultry sell in Larangan market was variety, such as chicken and duck. During the study, examination on the poultry in the market was carried out, especially those that showed the symptoms of influenza. Nasal swab and cloacae swab was taken from around 350–400 poultry. And the last sampling was on wild birds and poultry around the market. The same examination was held, and cloacae swab was also taken from approximately 50–100 wild birds and poultry.

The 63 nasal swabs were taken with cotton swab tube. Each of the tube were given 2 ml 5% BSA-BHI (*Bovine Serum Albumin – Brain Heart Infusion*), and mixed by vortex twice. After all samples ready, the next step is filtration which were done inside the Bio-Safety Cabinet (BSC), with 5 ml syringe and sterilized filter, then collect the filtrate in 1.5 ml tube. These filtrates samples were inoculated in monolayer of MDCK cells. These samples were incubated at 33–35° C during 3–7 days. Daily CPE (*Cytopathic Effect*) was observed. MDCK cells were chosen considering their better virus sensitivity (possible positive samples). After that, the fluids were harvested for HA test. The positive samples were submitted to the serologic test such as the Influenza Rapid Test, and also evaluated with Haemagglutination test (HA) as described in WHO Laboratory's method (2007).

Around 500 animal samples with nasal and cloacae swab were collected. The samples were treated similar as human samples with the filtration procedures. After that, those samples were inoculated using 9–10 days old

embryonated hen's eggs. The treatment of animal samples was performed in the BSL3 laboratory according to the CDC guidelines (WHO, 2011).

After the samples has been harvested, and show HA test positive, the samples are extracted into RNA and followed by cDNA synthesis. The final product will be cDNA, and ready to use or storage at -20° C until the Polymerase Chain Reaction (PCR) techniques was performed.

PCR was performed using primer forward (F) and reverse (R). Because this is an identification study, the primers that we applied were: H1(F) – H1(R), H3(F) – H3(R), and H5(F) – H5(R). PCR Reaction for each sample: 2.5 µl cDNA were amplified in a volume of 25 µl containing 12.5 µl premix, 8 µl dilute water (DW), and 1µl primer (10 pmol) for each primer forward and primer reverse. This reaction mixture was then heated in the PCR machine for 3 hours 19 minutes, with the thermal cycler of 40 cycles as follows: 94° C for 2 minutes, 94° C for 30 second, 50° C for 40 second, 72° C for 2 minutes, and 72° C for 10 minutes. The mixture then held at 4° C for indeterminate period until the heat cools down. The amplified PCR product was analyzed by electrophoresis (ELP) on 1.5% agarose gel at 100 V for about 40 minutes. The bands were stained with 2 µl/Ml ethidium bromide, documented by Gel Documentation System.

RESULT AND DISCUSSION

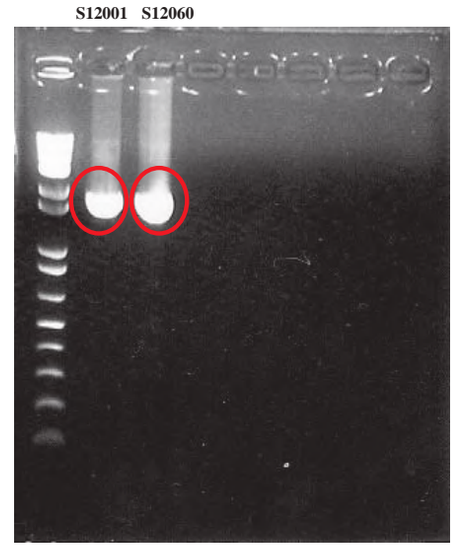
Human Samples

Among 63 people who worked as a poultry traders in Larangan market, several symptoms of influenza-like-illness (ILI) was identified such as cough, heavy breathing, arthritis, and diarrhoea. The samples were categorized by age and symptoms. Approximately around 36.5% samples were in the productive age between 31–40 years old, and only 6.3% were above 60 years old. Several symptoms also have been identified. Those symptoms are showed in the figure below.

The most common symptoms were coughing and heavy breathing, which appeared in all age categories. However, only among people age 51–60 years old that have other symptoms as arthritis and diarrhoea. And surprisingly, those who are above 60 years old did not have any symptoms of ILI even though they also working as poultry traders. This might relate on the length of working and how often they spent the time around the market, as well as how the antibody of the person who might resistant to the influenza virus. Those are the limitation of this study, that the information on the length of working and the antibody serum were not collected.

Table 1. Age category of human sample and the symptoms

Age	Cough	Arthritis	Diarrhoea	Heavy breathing
< 20–30 years old	21%	-	-	21%
31–40 years old	11%	-	-	11%
41–50 years old	9.7%	-	-	9.7%
51–60 years old	9%	4.5%	4.5%	9%
> 60 years old	-	-	-	-



Picture 1. The H3 result of Larangan human sample

Of these human samples, the serologic test has been taken using Influenza rapid test. The results were identified as influenza type B. As the PCR reaction was conducted, only 2 samples (3.17%) were identified of positive H3 (with 1000 base pair), as showed below:

ANIMAL SAMPLE

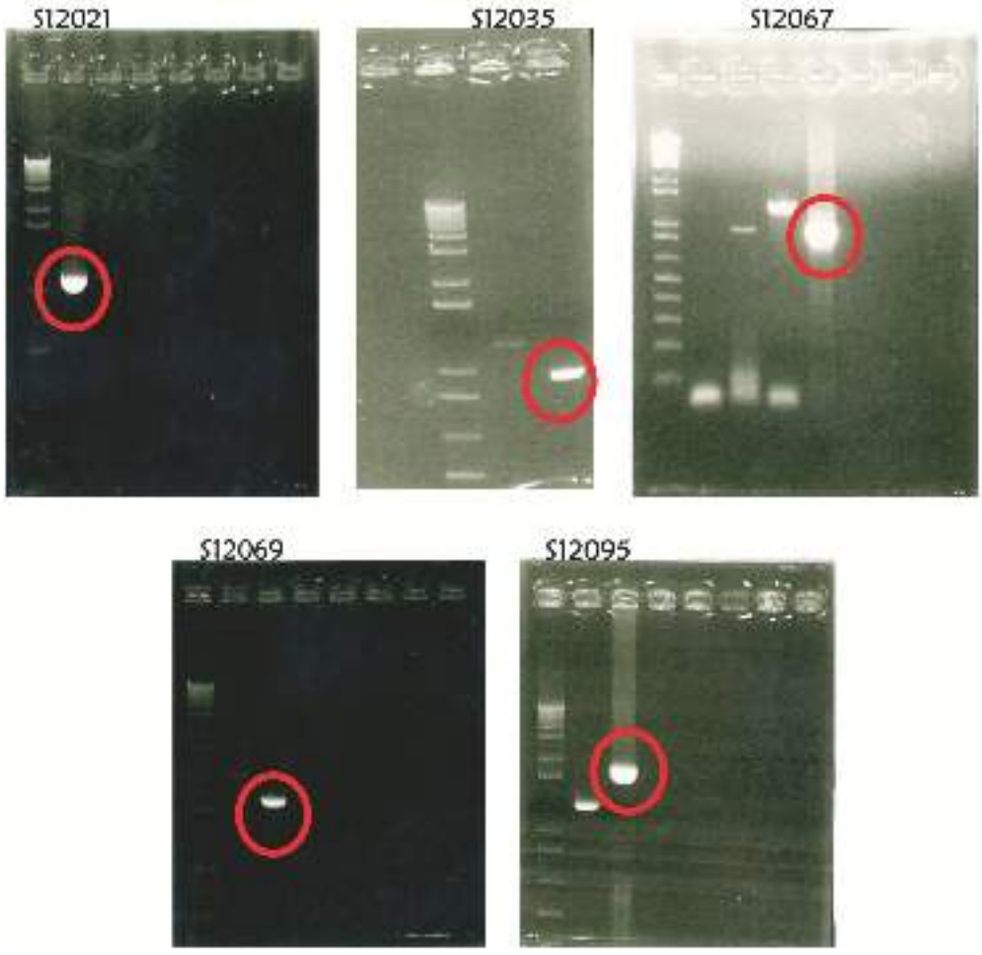
About 500 poultry samples were collected from the market and the area near the market. Several kinds of poultry and wild birds were identified with symptoms of influenza virus (particularly H5) are described below.

The HA test was conducted and the titter results using chicken RBC (HA ck+) and guinea pig RBC (HA gp+), and also using the diagnostic kit (dx +) that showing possible positive outcome. The diagnostic kit result was identified as influenza type A However, based on the assessment on the egg conditions after inoculation, it showed a relatively high number of possible positive results especially on 24 and 32 hpi (post infection).

Further assessments were attained with PCR technique using H5 primer. There were two steps of PCR to identify the types of protein on the surface. The first one is to

Table 2. Poultry category with symptoms

Poultry category	With symptoms
Broiler chicken	45.5%
Backyard chicken	46.5%
Duck	5.9%
Migrant bird	1.0%
Owl	1.0%
Total	100.0%



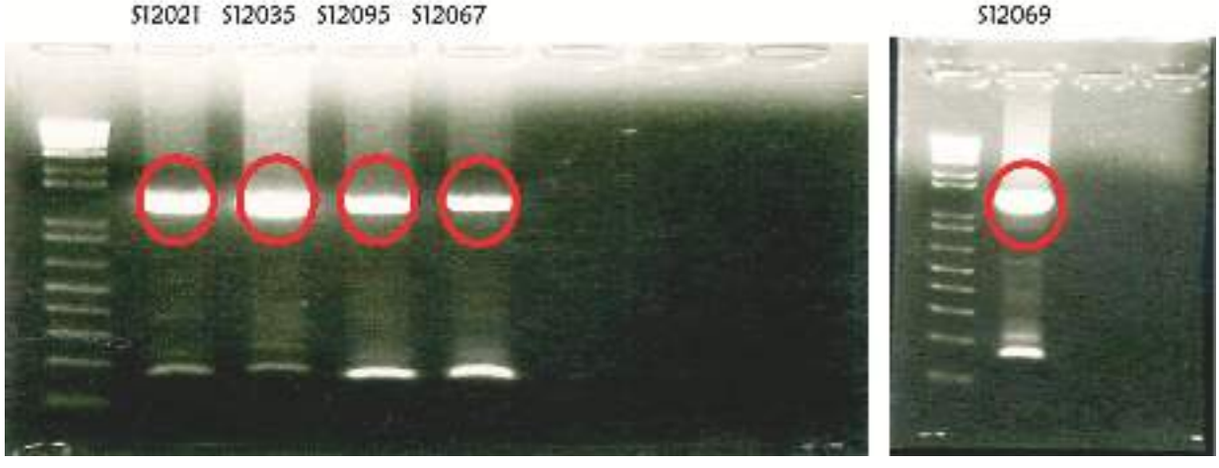
Picture 2. PCR result of HA

identify Hemagglutinin (HA) by using H5 primers. And the second one is to identify Neuraminidase (NA) by using H5N1 primer.

The H5 results are identified positive when the marks showed on 1.500 bp (base-pair). Only 5 samples were identified positive result H5, specifically the types of H5N1. The PCR outcome of HA types are illustrated as follow.

The PCR outcome of NA types are described below.

The NA results are identified positive when the marks showed on 1.500 bp (base-pair). Based on 5 positive sample of H5, we can identify the NA result as positive, specifically the types of H5N1.



Picture 3. PCR result of NA

CONCLUSION

There are some limitations on this study. For human sample, there are no information on the length of a person working in the market, the anthropometry measurement to check the nutrition aspect, the immune serum to identify the immune system and the antibody. As well as human sample, the animal sample also have are no specific assessments held, especially for the phylogenetic analysis to check any mutations occur.

For the next study, it will better if the anybody serum also taken to check the level of immunity. The anthropometry measurement and nutrition assessment also needed to be carried out to identify the nutrition condition related to the immune system. Furthermore, examinations such as sequencing need to be conducted, as well as the phylogenetic analysis. And last but not least, it is better to start developing a pandemic influenza planning as a result of the surveillance activity.

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Case Report

AWARENESS OF USING RINGER LACTAT SOLUTION IN DENGUE VIRUS INFECTION CASES COULD INDUCE SEVERITY

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ABSTRACT

Background: In 2012, serotype of Dengue Virus had changed from Den-2 and Den-3 to Den-1. In 5–10 years ago, serotype of Den-1 case showed a mild clinical manifestation; but now as a primary case it can also show severe clinical manifestation. One of indicator is an increasing liver enzyme, AST and ALT, with level more than 100–200 U/L. Aim: To getting a better solutions for this problem. Method: Obsevasional Study had been done in medical faculty of Airlangga University (Dr. Soetomo and Soerya hospital) Surabaya on Mei–August 2012. There were 10 cases of dengue virus infection were studied, 5 cases got Ringer Acetate solution (Group A) and 5 cases got Ringer Lactate solution (Group B). The diagnosis was based on criteria WHO 2009. Result: Five cases of Dengue Virus Infection had showed a liver damage soon after using Ringer Lactate solution; AST and ALT were increasing more than 100–200 U/L; but the other 5 cases showed better condition. It might be due to use Ringer Acetate that did not have effect for inducing liver damage. By managing carefully, all of the cases had shown full recovery and healthy condition when being discharged. Conclusion: Using Ringer Acetate as fluid therapy in Dengue Virus Infection is better to prevent liver damage than using Ringer Lactate.

Key words: *Ringer Lactate, Ringer Acetate, Dengue Virus Infection, Pediatric Cases, Severity*

ABSTRAK

Latar belakang: Pada tahun 2012 serotype virus dengue telah mengalami perubahan dari Den-2, Den-3 ke Den-1. Dalam kurun waktu 5–10 tahun yang lalu, serotype Den-1 menunjukkan manifestasi klinis yang ringan, tetapi sekarang serotype Den-1 ini walaupun sebagai kasus primer ternyata dapat menunjukkan manifestasi klinis yang berat. Salah satu indikator yang digunakan adalah kenaikan enzym AST dan ALT lebih dari 100–200 U/L. Tujuan: Berusaha menemukan tatalaksana terbaru dengan hasil yang memuaskan. Metode: Studi observasional telah dilakukan di RSUD Dr Soetomo Surabaya & RS Soerya Sepanjang sejak bulan Mei–Agustus 2012. Dari 10 kasus infeksi virus dengue yangditeliti, 5 kasus mendapatkan terapi cairan Ringer Acetate (Kelompok A) dan 5 kasus mendapatkan cairan Ringer Lactate (Kelompok B). Diagnose Infeksi Virus Dengue berdasarkan Kriteria WHO 2009. Hasil: Lima kasus infeksi virus dengue telah menunjukkan gangguan fungsi hati setelah memperoleh infus cairan Ringer Lactate dan terbukti kadar AST dan ALT meningkat lebih dari 100–200 U/L, tetapi 5 kasus yang memperoleh Ringer Acetate menunjukkan keadaan yang lebih baik. Maka dapat dibuat kesimpulan bahwa terapi cairan pada penderita infeksi virus dengue lebih baik memanfaatkan cairan ringer acetate sebab metabolism cairan ini terjadi di otot ekstremitas dan tidak mengganggu fungsi hati. Dan berbeda secara nyata apabila cairan yang digunakan larutan Ringer Lactate yang metabolism di dalam hati. Hal ini sesuai dengan hasil penelitian terdahulu yang mengutamakan bahwa larutan Ringer Acetate yang diberikan kepada penderita infeksi virus dengue tidak meng ganggu fungsi hati penderita. Kesimpulan: Lebih baik memanfaatkan cairan ringer acetate sebagai terapi infeksi virus dengue daripada menggunakan cairan ringer lactate yang dapat mengganggu fungsi hati.

Kata kunci: *Ringer Laktat, Ringer Asetat, Infeksi Virus Dengue, Kasus Anak, Kegawatan*

INTRODUCTION

Since 1968, dengue virus infection has always been found in indonesia. It is due to increasing the population of Aedes Aegypti and Aedes Albopictus that now transfer from one case to another case. It is supported by changing of summer to raining season. Global climate can also affect all the event in the world.

In 2009 some hospital in Surabaya and Sidoarjo had found the changing serotype on dengue virus from Den-2 and Den-3 to Den-1. In 5–10 years ago, serotype of Den-1 showed a mild clinical manifestation, but now, it can also show severe clinical manifestation of dengue virus as a primary infection. It can be predicted by test marker of positive NS-1 Dengue test. This virus can cause liver damage.

Based on this finding, we should be careful to treat dengue virus infection cases. Firstly, we should look for the indicators of dengue virus infection suffering for liver damage, that are AST and ALT titers in every case of Dengue Virus Infection. If we find the indicators of liver damage (increasing AST and ALT titers), please don't use ringer lactate for treating dengue with shock case.

To convince this, researcher want to present this papper to get a better solutions from many aspect.

MATERIALS AND METHODS

Observasional study had been studied in Medical Faculty Airlangga University, Surabaya, Indonesia. On Mei–August 2012, there were 10 cases of DVI, 5 cases were given Ringer Acetate (group A), and the other cases were given Ringer Lactate (group B). The diagnosis was made based on criteria WHO 2009.

Group A

Patient 1

A four years old boy was brought by his parents to Soerya Hospital, in emergency Department with the main complaint of fever since 2 days before admission. She also suffered from nausea and cephalgia.

Table 1. Result of laboratory examination

Day of admission	Hb	Hct	Leukocyte	Trombocyte	AST	ALT
1	12,6	37,5	4860	280000	113,6	49,9
2	10,9	31,7	3020	222000		
3	11,8	35,1	2090	188000		
4	11,9	34,9	4110	134000		
5	11,9	36,1	4110	126000		
6	12,3	37,1	6870	187000	73,5	32,6

Patient 2

A nine years old girl was brought by his parents to Soerya Hospital, in emergency Department with the main complaint of fever since 3 days before admission. She also suffered from nausea, and cephalgia. She lost her appetite and refuse to eat. She was in a weak condition.

Table 2. Result of laboratory examination

Day of admission	Hb	Hct	Leukocyte	Trombocyte	AST	ALT
1	11,7	35,5	2490	134000	36,1	15,8
2	11,4	34,6	4170	139000		
3	11,3	33,9	3650	199000	31,1	16,4
4	11,1	33,5	3230	263000		
5	11,4	34,6	4310	408000		

Patient 3

An eleven years old boy was brought by his parents to Soerya Hospital, in emergency Department with the main complaint of fever since 6 days before admission. The fever had been subsided in the third day of fever, but the temperature raised in the forth day.

Table 3. Result of laboratory examination

Day of admission	Hb	Hct	Leukocyte	Trombocyte	AST	ALT
1	13,3	37,8	4420	34000	106,9	41,7
2	13,9	37,8	4420	30000		
3	12,8	38,5	8230	32000	142,3	59,3
4	13,2	39,4	9200	80000		
5	11,8	34,8	9460	166000		
6	12	35,3	9610	150000	62,9	51,2

Patient 4

A ten years old girl was brought by his parents to Soerya Hospital, with the main complaint of fever since 5 days before admission. She had been brought to the doctor before, but the fever stayed. She also suffered from vomit after eating, myalgia and cephalgia.

Table 4. Result of laboratory examination

Day of admission	Hb	Hct	Leukocyte	Trombocyte	AST	ALT
1	14,3	40,5	3610	36000	91,5	26,2
2	14,3	40,8	3640	36000		
3	13	38,4	10780	35000	68,6	27,7
4	12,6	36,5	8110	46000		
5	12,4	34,8	7770	94000		
6	12,8	36,0	6630	196600	42,8	55,4

Patient 5

A nine years old girl was brought by his parents to Soerya Hospital, in emergency Department with the main

complaint of fever since 2 days before admission. She also suffered from nausea, and cephalgia.

Table 5. Result of laboratory examination

Day of admission	Hb	Hct	Leukocyte	Trombocyte	AST	ALT
1	15,3	44	3140	91000	60,9	27,3
2	14,7	42	5050	60000		
3	13	36,7	4210	42000	57,3	25,2
4	12,5	35,6	4970	53000		
5	12,1	34,6	4840	120000		
6	12,2	35,4	5990	213000	56,9	47,2

Group B

Patient 1

A twelve years old boy was brought by his parents to Dr. Soetomo Hospital, in emergency Department with the main complaint of fever since 2 days before admission. He also suffered from heavy cephalgia.

Table 6. Result of laboratory examination

Day of admission	Hb	Hct	Leukocyte	Trombocyte	AST	ALT
1	12,6	39,5	3660	214000	36,8	18,9
2	11,5	35,9	3300	155000		
3	13,1	40,8	3740	54000	809,1	382,6
4	15,2	46,4	5220	33000		
5	12,9	39,6	8100	56000		
6	11,5	35,1	7540	85000	179,4	178,1
7	12,1	36,2	11100	163000	137,1	147,7

Patient 2

A nine years old boy was brought by his parents to Dr. Soetomo Hospital, Surabaya in emergency Department with the main complaint of clammy extremities since 7 hours before admission. He suffered from acute and continues high grade fever for 5 days and subside suddenly. He also complained of headache, retroorbital pain, melena and myalgia. There was a history of contact to person with dengue hemorrhage fever in his neighborhood.

Table 7. Result of laboratory examination

Day of admission	Hb	Hct	Leukocyte	Trombocyte	AST	ALT
1	17,9	52,1	5400	29000	1360	566
2	16,8	43	2000	32000		
3	10,6	27	7100	20000	7050	3541
4						
5	12,5	35	7400	85000	1367	1037

Patient 3

A three years old boy was reffered from Mojokerto hospital with hepatic comme on Dr. Soetomo hospital in emergency department., with the main complain of fever since 5 days before. One day before admission he had clammy extremities. He also complained of nausea and vomitting and refuse to eat since 1 day before admission. No sign of bleeding was found in this child.

Table 8. Result of laboratory examination

Day of admission	Hb	Hct	Leukocyte	Trombocyte	AST	ALT
1	10,3	31,9	14600	15000	3154	1274
2	10,7	30	20500	85000		
3	9,7	30,9	11000	75000		
4						
5	8,8	28,4	3500	100000	1254	1069

Patient 4

A seven years old boy was brought to his parents in Dr Soetomo Hospital emergency department with main complain of fever. He suffered from fever since 4 days before admission. The fever was suddenly continues and high grade. He also complained of headache, and difficult to sleep. There was no history of bleeding. He felt nausea and vomit which made he lost his appetite. There was no history of contact to person with dengue hemorrhagic fever in his neighborhood.

Table 9. Result of laboratory examination

Day of admission	Hb	Hct	Leukocyte	Trombocyte	AST	ALT
1	15,8	48	7400	26000	2824	1011
2	16,8	43	2000	32000		
3	10,3	36,6	7600	22000		
4	10,4	29,6	9700	73000	1107	537
5	8,8	26,7	9800	1122000		

Patient 5

An overweight-eleven years old boy had been reffered from Bangkalan to emergency room in Soetomo Hospital, with maint complaint fever, cephlagia, headache, vomitting, dyspnea, and restless since 4 days before and had been managed in Bangkalan hospital as dengue Virus Infection, and got Ringer Lactate solution for early recucitation, but the condition became worse, and reffered to Soetomo Hospital.

Table 10. Result of laboratory examination

Day of admission	Hb	Hct	Leukocyte	Trombocyte	AST	ALT
1	13	47	9400	200000		
2						
3						
4	18	55	7000	40000		
5	16	47	9100	80000	275	405
6	15,7	45	9800	29000		

RESULT

In our cases, all of the patient were observed intensively in Pediatric Intensive Care Unit and treated conservatively. Five cases (Group A) had got Ringer Acetate since the first day came to the hospital. They showed naturaly clinical manifestation and recovered well. One case showed ALT,

AST increase more than 100 I/U but in short time showed recovery.

Five cases (Group B) had got Ringer Lactate in an early day of admission in hospital. Three cases showed increasing AST and ALT more than 1000 and showed encephalopaty and become severe. Patient no 4 got transfusion of FFP which indicator were abnormal coagulation profile. Patient no 2 had got PRC transfusion to replace the blood loss from intetinal bleeding. All of the involved organ were recovered along with recovery process of the disease.

DISCUSSION

To manage Dengue Virus Infection cases in our hospital, as a clinician or a doctor in charge should identify the cause of infection by doing the physical and laboratory examination. It should be correlated with criteria or sign & symptoms of the cases:

1. Severe cases have to be handled in emergency room or intensive care unit.
2. Early and mild case could be handled in general practitioner practice or pediatrician clinic.

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 indetectable levels over 2–3 months. Anti dengue virus IgG appears shortly afterwards. Secondary infection with dengue virus results in the earlier appearance of high titers of IgG before or simultaneously with the IgM responses.[2,4]

Everyday, one or two cases could be inpatient in our hospital, for making a proper diagnosis and well recovery.

During three decades, the World Health Organization (WHO 1997) has recommended the classification of dengue virus infection in: dengue fever (DF) and dengue hemorrhagic fever (DHF) with or without dengue shock syndrome (DSS). In order to be regarded as a DF (or classical dengue) case, the patient must present fever and two symptoms out of the following: headache, retroocular pain, osteomyoarticular pains, rash, leucopenia, and some kind of bleeding. The DHF requires the presence of the four following criterias: a) acute sudden onset of high fever for 2 to 7 days; b) some kind of spontaneous bleeding, usually petechiaes, or at least having a positive tourniquette test; c) thrombocytopenia lower than 100,000/mm^3; and d) plasma leakage, evidenced by a 20% elevation of the hematocrite, or by a 20% decrease of the hematocrite after the critical stage, or by the verification of pleural leakage, ascites or pericardial leakage by means of image studies. The course of the dengue disease goes through 3 clinical stages: the febrile stage, the critical stage, and the recovery stage.[6] (*see figure 1*)

WHO 2009 has divided Dengue Virus infection into 3 grade of disease, 1) Dengue virus infection without warning sign (we also can say *probable* dengue infection); 2) Dengue virus infection with warning signs (such as: abdominal pain, persistent vomiting, bleeding, etc) and the worst is 3) Severe dengue, which severe plasma leakage leading to hypovolemic shock and also it could be fluid accumulation leading to respiratory distress. Severe bleeding and severe organ impairment also could be occurred. It could be evaluated by laboratory findings; increasing of AST and ALT more than 1000.[5] (*see figure 2*)

Clinical diagnosis of DHF was based on WHO criteria. Hepatomegaly is a common but not constant finding. Liver involvement in Dengue Virus Infection occurred because dengue virus antigen has been found in liver; hepatocytes and kupffer cells support the viral replication. In some countries, however, hepatomegaly varies from one epidemic to another, suggesting that the strain or serotype of virus may influence liver involvement. Elevated liver enzyme levels are usually happen. Serum AST and ALT were used as a measure of cellular injury. The elevation is usually mild, but in some patients, AST and ALT level reach 500 to 1000 U/L. Leucopenia is common; thrombocytopenia and hemoconcentration are constant findings in Dengue Virus Infection.[5]

Haemorrhagic manifestation in Dengue virus Infection patients are not common, and within mild to severe. Skin hemorrhage, including petechiae and purpura, are the most common, along with gum bleeding, epistaxis, menorrhagia, and gastrointestinal bleeding. The pathogenesis probably derives from vasculopathy, platelet deficiency and dysfunction, and blood coagulation defects.[5]

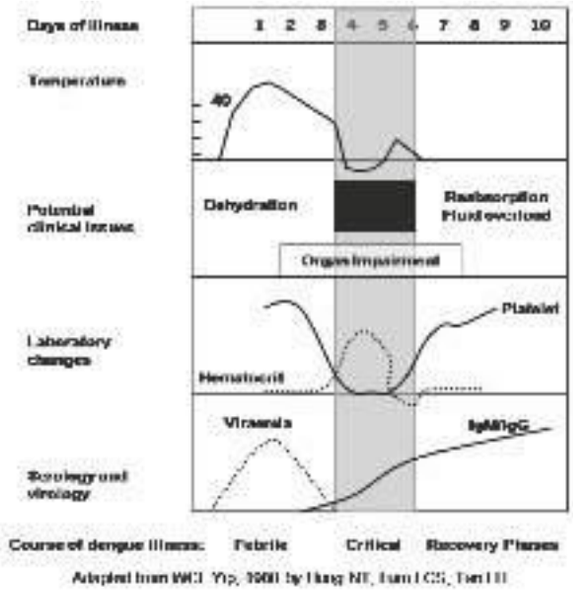

Figure 1. The Course of Dengue virus infection (WHO, 2009)

Figure 2. Level of severity of dengue virus Infection cases (WHO, 2009)

Our study is focused in using Ringer Lactate as a fluid therapy. Ringer Lactate was usually used in health centre and hospital for Dengue Shock Syndrome since 1969. But sometimes it could be found some cases that becoming more severe so that the patient had to be admitted in Dr. Soetomo Hospital Surabaya, as a top referral hospital in East Java, Indonesia. Therefore if there was a severe case with liver damage, we try to evaluate the patient and change the fluid resuscitation with Ringer Acetate.

Since 1968, Ringer lactate had been used for early treatment of cholera disease which the patient suffered from hypovolemic shock due to massive diarrhea. The result was proven good. From this experience, Ringer Lactate was also used to treat Dengue Shock Syndrome, but after 30 years using this protocol for DSS rescucitation, there were more cases become severe and difficult to manage.[3]

In 1997, in WHO course of DHF had been done in Bangkok. I had followed the course as a fellowship doctor from East Java, Indonesia. I got information that don't use Ringer lactate in a case with liver damage. Based on this experience, until now I always remind the statement of our WHO teacher there, that we have to be careful in using Ringer Lactate in a case with liver damage, especially AST and ALT > 100–200. To make sure this information, we try again to study the new pathophysiology of Dengue Virus Infection (*see figure 3*)

Focusing on pathogenesis of hemorrhage in DHF (see figure 3) and update pathogenesis of DHF (see figure 4). Severity can occure due to virus replicate in hepatocyt and kupffer cells and induce necrosis as an apoptosis in liver and it's function damage.[1]

This event can be promoted by using Ringer lactate. It is due to Ringer Lactate is metabolized in liver so if this solution is used, liver damage can be occurred more severe.

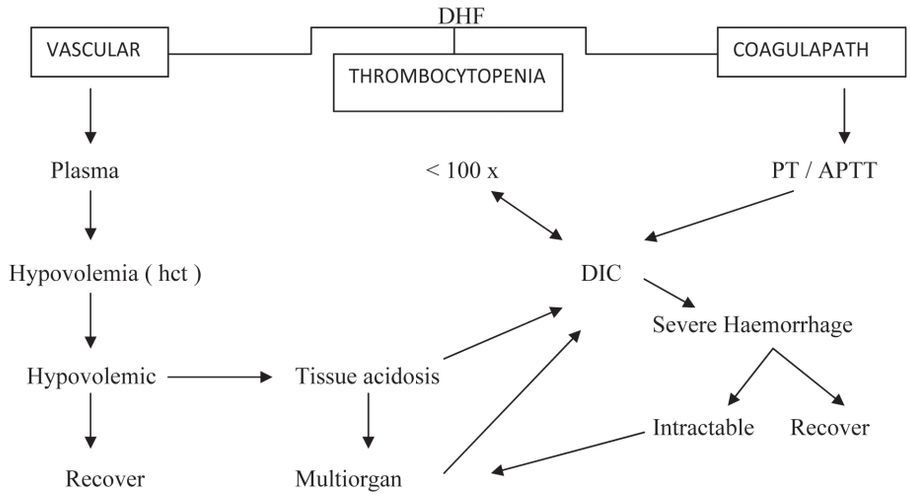

Figure 3. Pathogenesis of haemorrhage in DHF (Malaysia Ministry of Health, 2003)

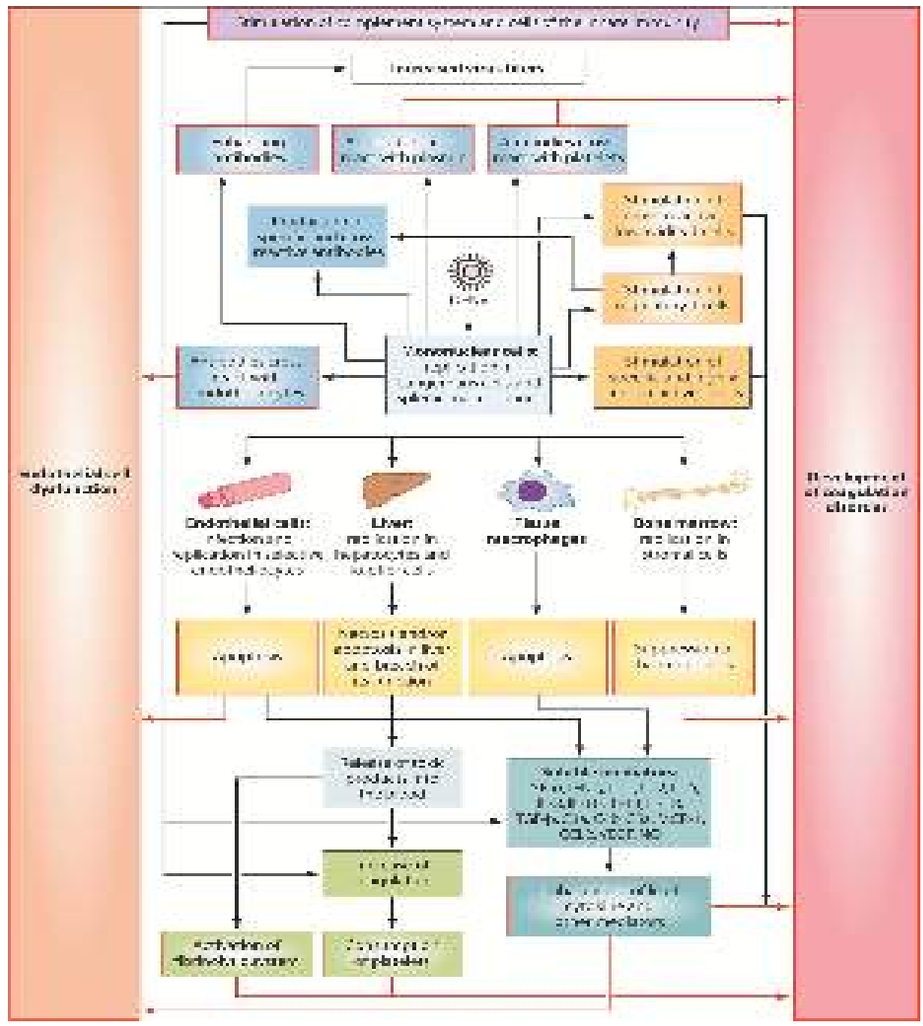

Figure 4. Update pathogenesis of Dengue Virus Infection in 2009 (WHO, 2009)

SUMMARY

Ten Cases of dengue Virus Infection had been observed intensively in pediatric Care Unit and treated conservatively. Five cases as a Group A got Ringer Acetate and 5 cases as Group B got Ringer Lactate before refered or on the first day admission in Dr Soetomo Hospital; recucitation start with 5–7 ml / kg BW / hour for 1–2 hours then reduce 3–5ml / kg BW / hour for 2–4 hours and then reduce to 2–3 ml / kg BW / hour or less. According to the clinical respond, if the hematocrite remains the same or rises only minimmaly continues with the same rate (2–3 ml / kg BW / hour) for another 2–4 hours, but if vital signs are worsening and hematocrit is rising rapidly, increase rate to 5–10 ml / kg BW / hour for 1–2 hours. Intravenous fluids usually needed only 24–48 hours, and should be reduced gradually which haematocrite level decreases.

Patient with warning signs should be monitored by health care provides until the period of critical phase is over.

We prefer using Ringer Acetate to ringer Lactate, due to Ringer Acetate has some benefit, such as:

1. Ringer Acetate is metabolized in muscle and could be tolerated in patient with liver dysfunction.
2. Acetate metabolism is faster than lactate metabolism.

CONCLUSION

Using Ringer Acetate as fluid therapy in Dengue Virus Infection is better to prevent liver damage than using Ringer Lactate.

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Research Report

QUICK DIAGNOSIS OF JAPANESE ENCEPHALITIS FOR NEW DIAGNOSED EMERGING DISEASE USING PCR TECHNIQUE IN SURABAYA, INDONESIA

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ABSTRACT

Background: Japanese enchepalitis (JE) is a viral disease that considered as zoonotic disease, which transmitted through mosquito vectors that had JE virus. Mainly caused by the mosquito C. Tritaeniorhynchus (the most important vector is the mosquito Culex, which feeds on cattle in preference to human). JE virus disease can also cause disturbances in the central nervous system eg. brain, bone marrow, and meninges which has serious impact on public health. This disease has been reported from Japan, Korea, Taiwan, India, Myanmar, Thailand, Western Pacific and Southeast Asia to Indonesia. However, the incidence of this disease in Indonesia has not been well known in various animal species or humans. Aim: The purpose of this study is to develop rapid diagnostic examinations on patient diagnosed JE virus in Surabaya by using PCR (Polymerase Chain Reaction). Because, JE disease can lead to dead-end at the patient if not treated immediately. Method: The research methods, extraction method, PCR (1st RT-PCR and 2nd Nested PCR) are conducted using Japanese encephalitis PCR detection kit. Result: The results of the examination showed that 2 out of 17 people (11,765%) are positive with PCR bands 227 bp (basepair). This diagnostic technique to determine and to deal with early onset of the disease. Solutions for preventive actions can be started from the termination of the cycle vectors to vaccination measures. Conclusion: For his own medical factors given to reduce fever and swelling and reduce the pain.

Key words: *Japanese encephalitis, PCR, New Emerging Disease, preventive, Indonesia*

ABSTRAK

Latar belakang: Japanese encephalitis (JE) adalah penyakit virus yang dianggap sebagai penyakit zoonosis , yang ditularkan melalui vektor nyamuk yang memiliki virus JE. Terutama disebabkan oleh nyamuk Culex C. (vektor yang paling penting adalah nyamuk Culex, yang menyusu pada sapi dalam preferensi untuk manusia). Penyakit virus JE juga dapat menyebabkan gangguan pada sistem saraf pusat misalnya: otak, sumsum tulang, dan meninges yang memiliki dampak serius pada kesehatan masyarakat. Penyakit ini telah dilaporkan dari Jepang, Korea, Taiwan, India, Myanmar, Thailand, Pasifik Barat dan Asia Tenggara ke Indonesia. Namun, kejadian penyakit ini di Indonesia belum dikenal di berbagai spesies hewan atau manusia. Tujuan: Tujuan dari penelitian ini adalah untuk mengembangkan pemeriksaan diagnostik cepat pada pasien didiagnosis virus JE di Surabaya dengan menggunakan PCR (Polymerase Chain Reaction). Karena, penyakit JE dapat menyebabkan buntu pada pasien jika tidak segera diobati. Metode: Metode penelitian, metode ekstraksi, PCR (1st RT -PCR dan 2 Nested PCR) dilakukan menggunakan Japanese ensefalitis PCR deteksi kit . Hasil: Hasil pemeriksaan menunjukkan bahwa 2 dari 17 orang (11.765%) positif dengan PCR band 227 bp (basepair). Teknik diagnostik ini untuk mengetahui dan menangani onset awal disease. Solusi untuk tindakan pencegahan dapat dimulai dari penghentian vektor siklus tindakan vaksinasi. Kesimpulan: Untuk faktor medis sendiri diberikan untuk mengurangi demam dan pembengkakan dan mengurangi rasa sakit .

Key words: *Japanese encephalitis, PCR, New Emerging Disease, pencegahan, Indonesia*

INTRODUCTION

Japanese encephalitis (JE) is a disease can cause brain inflammation in animals and humans which can be transmitted from animals to the human through mosquito bites. This disease has become widespread in parts of East Asia such as Japan, Korea, Siberia, China, Taiwan, Thailand, Laos, Cambodia, Vietnam, Philippines, Malaysia, Indonesia, Myanmar, Bangladesh, India, Sri Lanka, and Nepal. In Indonesia, JE case was first reported in 1960. Japanese encephalitis virus is part of the Flaviviridae family. This virus has the envelope (about 50 nm) with a small lipoprotein that surrounds the nucleocapsid core protein and consists of a single chain of RNA. JE virus is related to West Nile virus and St. Louis encephalitis viruses. On the outer layer are formed by (E) protein and act as protective antigen. This helps in the entry of the virus into the cell[1]. JE disease in humans is a way of ending the cycle of transmission (dead - end), because viraemia in humans occurs only a few hours which is difficult to spread further to other people. Human disease can result in death if not treated properly. Wei and Gautama, in the same year reported that the most vulnerable age among children infected with JE is between 5 to 9 years.[23]

In Asia, with around 50,000 cases and 10,000 deaths per year in children below 15 years of age. The JEV has shown a tendency to extend to other geographic regions. Case fatality averages 30% and a high percentage of the survivors are left with permanent neuropsychiatric sequelae. JE is a disease of public health importance because of its epidemic potential and high fatality rate. In endemic areas, the highest age-specific attack rates occur in children of 3 to 6 years of age. Approximately one third of patients die, and half of the survivors suffer severe neuropsychiatric sequelae from the disease.[10]

The Clinical symptoms commonly shown in the case of Japanese encephalitis is usually a non - specific symptom such as fever, followed by headache, vomiting, and decreased level of consciousness. Because the tissue covering the brain and spinal cord become infected and swollen, the patient will usually experience stiffness in the neck and painful. Then within two or three days, the patient began to experience the effects of swelling on the brain. These effects may include interference with balance and coordination, paralysis on several groups of muscles, tremors, seizures, and disturbances in consciousness.[4] Patients also experience dehydration and weight loss. If the patient can survive with the pain, the fever will drop down about day 7, and the symptoms will begin to rise again approximately on day 14. Meanwhile there are also people who will continue to have a very high fever and get even worse. In this case, the symptoms will usually be followed by coma and then death occurs within 7–14 days. However, the area also quite a few patients who had recovered but was followed by permanent disability due to brain damage[4].

Some reports suggest that children and teenagers are prone to this disease. In Thailand, allegedly 40 of 100,000 children to adolescents aged 5–25 years suffering from this disease. In addition, it was reported also that a lot of JE cases occur in rural areas. By all means this case epidemiology in the Northern Vietnam, northern Thailand, Korea, Japan, Taiwan, China, Nepal and northern India more common in summer sat. Within the area of southern Vietnam, southern Thailand, Indonesia, Malaysia, Philippines, Sri Lanka, and southern India, JE cases occur sporadically throughout the year. This disease also has been reported to cause behavioral abnormalities. In some children the clinical symptoms that appeared to be a single seizure, followed by a rapid recovery of consciousness. The symptoms of seizures are a common cause shaking on digits or mouth, eye deviation, nystagmus, excess salivation, or irregular respiration.[4]

In Indonesia the first time in the case of JE in serological report which occur in humans in 1999 in Bali. Examination of serum specimens from 12 patients with clinical diagnosis of viral encephalitis, meningitis or dengue hemorrhagic fever (DHF) found two of them positively infected with Japanese encephalitis.[11] JE cases in humans were also reported in some areas, namely in West Sumatra, West Kalimantan, Yogyakarta, Central Java, East Java, West Nusa Tenggara, East Nusa Tenggara and Papua.[5]

A recent report there are even reported cases of JE virus infection in tourists who holiday in Bali. The tourists traveling 3 weeks to Java and Bali, including vacation stricken rural to rural. Last week of March was spent in Bali. After returning home, the patient complained of fatigue and 5 days later he fell ill with numbness in both clengan, and can not use a knife and fork while eating. He also vomited and fell to the floor several times, can not stand by itself. When admitted to the hospital on the same night, the patient was febrile (39.18° C), but in general good condition. The next day he became confused and do not understand simple questions or instructions. Test results show the conditions that lead to the condition encephalitis JE.[12]

Based on the background of the above, the study is to conduct a quick diagnostic on Japanese Encephalitis using PCR techniques among patients in Surabaya, Indonesia. Some above incident, due to the lack of knowledge about the disease is accompanied by a rapid diagnostic examination for checking the disease and So far as JE is a viral disease, then there is no treatment to stop or slow the progression of the virus. Treatment can only be done in a way that is symptomatic relieve symptoms seen each patient. The aims of the study are to get a quick diagnose of Japanese encephalitis virus using PCR techniques, and to be able to diagnose JE virus in Surabaya to treat early-infected patients from JE disease. The method is by using PCR technique (Japanese Encephalitis Virus Detection Kit).

Action is one step vaccination is effective in preventing the disease. Generally, vaccines are given to children to adolescents under 17 years USIS in JE endemic areas. For tourists or travelers visiting endemic areas of JE can also take advantage of this vaccine as a precautionary measure. Another preventive measure is to efforts to control mosquito populations.[13] and factors are the main

factors for the prevention of outbreaks of JE virus cope in endemic areas.

MATERIALS AND METHODS

The samples are considered as positive when there is a band that emerged in 227 bp. The samples are mainly from whole blood, serum, CSF. The samples are extracted. First PCR conducted starting with reverse transcription reaction cycle in 45° C for 30 minutes, then continue with 30 cycles of denaturation in 94° C for 45 second, annealing prosess in 72° C for 60 second, extention phase in 72° C for 60 second, and finally 1 cycle of final extension in 72° C for 5 minutes. Second PCR was conducted after the first finish. Starting wih pre-denaturation 1 cycle in 94° C for 2 minutes, continue with denaturation 30 cycle in 94° C for 30 second, annealing in 50° C for 30 second, extension in 72° C for 30 second, and final extension 1 cycle in 72° C for 5 minutes. After the PCR process is completed, we continue to electrophoresis with agarose gel for 30 min (110V).

RESULT AND DISCUSSION

There are total 17 patients has been examined in Institute of Tropical Disease (ITD) Universitas Airlangga. 2 person was found posistive with JE virus. The PCR result showed positive JE virus.

JE virus was first discovered in Indonesia proved by HA and HI antibody tests. In the tropics, JE virus continues to circulate among mosquitoes, birds and pigs.[6]

The mechanism of transmission of JE virus in humans occurs because of mosquito Cx. tritaeniorhynchus were supposed to be zoophilic population becomes heavy or sudden there was an increase of mosquito populations and thus be forced even this mosquito bite humans around him. Also, it can also occur because of the number of pigs suffering from viraemia (virus containing JE) virus became much so that in nature reserves increased and easily transmitted to humans. Age, JE vector, Culex mosquitoes, ranged between 14–21 days and Culex flight distance can reach more than 3 km. Culex generally breed in stagnant water overgrown with plants such as rice fields and irrigation channels, shallow ditches or ponds that are not used. In pigs, viraemia occurs during 2–4 days and is followed by the formation of antibodies in the first period of up to 4 weeks. JE virus can cross the placenta depends on the gestation and JE virus strains. Fetal death and mummification can occur when the JE infection takes place at 40–60 days gestation. While the JE infection after 85 days gestation, abnormalities caused very little. JE incubation period in humans ranges from 4 to 14 days.[13]

Japanese enchephalitis virus is quite a new problem in public health, especially in Indonesia as the cases still not many but could be pandemic based on the availability of the vectors of disease. Precaution may be a good way to

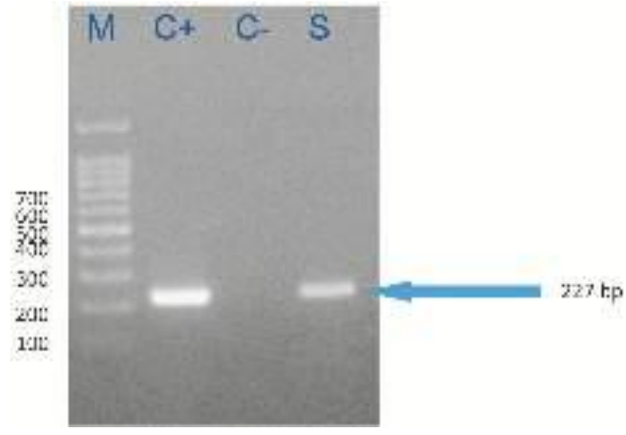

Figure 1. Can be seen as well as the positive control samples are at base pair 227 bp which indicates that the samples was positive JEV.

be applied to both vectors, source of transmission (pigs), human and living environments.

First way is by against the vector (Mosquito), by using insecticides to kill adult mosquitoes and larvae and try to remain the hygiene of water in our home. Second way is against the source of transmission (pig), by giving vaccination to infantile pigs, and make sure that the case of the pigs are surrounded by wire netting, sprayed insecticides, and should be free of mosquitoes. And the third way is prevention to ourself, by getting vaccination. It is an act that should be done 1 month before the time of transmission and addressed to people who have a high risk for getting the infection. Due to the one-month period in an environment of high risk people can take precautions as a whole (the three preventive measures), as well as stop the line of the spread of the virus. Alongside, we could also use mosquito repellent during sleep or during activity.

CONCLUSION

JE disease is a viral disease that is zoonotic and disturbing the public, which causes agents and vectors and animal reservoir potential. The existence of JE disease itself can be seen with the examinee 17 peoples in Surabaya 2 peoples JE disease was detected. Then made an attempt to overcome these problems. Any suggestions with regard to the presence of JE control such depth research on JE in humans in Indonesia in order to know the region spread in Indonesia.

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Case Report

ANALYSIS ON WHOLE BLOOD, SGOT, SGPT, AND TNF-α EXAMINATION IN PATIENTS WITH NON-DENGUE AND POSITIVE DENGUE FEVER (DF/DHF)

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ABSTRACT

Background: In Indonesia has four serotypes, the DEN-1, DEN-2, DEN-3 and DEN-4. The management of Dengue virus becomes difficult because the patients were infected with different clinical profiles depending on the serotypes and genotypes of infecting dengue virus. Consequently, the diagnosis and treatment becomes difficult. Aim: The purpose of this study was to identify the difference between the results of laboratory tests between non-dengue fever and positive dengue fever. Method: This study was an observational cross-sectional study. Fifteen samples were diagnosed with dengue fever and fifteen samples with negative dengue fever on NS1, IgM / IgG-anti-DHF strip test results. Laboratory tests comprising whole blood, SGOT, SGPT, and TNF-α were first examined when the patient came to the hospital. The collected data were analyzed by Chi-Square test SPSS version 13 for Windows. Result: The results of the study in two groups regarding sex, age, days of fever, grade, hemoglobin levels, leukocytes count, platelet count, hematocrit percentage, SGOT levels, and TNF-α level were not significantly different with p > 0.050, whereas the SGPT level in non-dengue increased 3 x of normal value of 66.7%, n = 10/15 and in positive dengue fever the SGPT level was within normal limits, found in 60%, n = 9/15, so there was significant difference with p = 0.022 (p < 0.05). Conclusion: in non-dengue SGOT and SGPT levels increased of 1–3 times the normal value. In positive dengue fever SGOT levels increased 1–3 x normal value, but SGPT levels was within normal value, so SGPT levels can be used as a predictive factor for distinguishing the two types of fever.

Key words: *Dengue Fever, Whole Blood, SGOT, SGPT, TNF-α*

ABSTRAK

Latar belakang: Di Indonesia memiliki empat serotype dengue yaitu DEN-1, DEN-2, DEN-3 dan DEN-4. Aspek manajemen virus dengue menjadi sulit, karena orang terinfeksi hadir dengan profil klinis berbeda tergantung pada serotipe dan genotipe dari virus dengue yang menginfeksi, sebagai konsekuensi diagnosis dan perawatan menjadi sulit. Tujuan: Tujuan penelitian untuk mengetahui perbedaan hasil pemeriksaan laboratorium antara Demam bukan dengue dan Demam positif dengue. Metode: Penelitian ini bersifat observasional dengan bentuk cross sectional. Lima belas sampel didiagnosis demam berdarah dan lima belas sampel negatif demam berdarah dari hasil NS1, IgM/IgG-anti DHF metode Strip. Pemeriksaan laboratorium adalah Darah Lengkap, SGOT, SGPT, dan TNF-α diperiksa saat pertama kali penderita datang ke Rumah Sakit. Data yang terkumpul dianalisis uji Chi-Square dengan SPSS versi 13 for Windows. Hasil: Hasil penelitian pada jenis kelamin (sex), Umur, hari demam, Grade, kadar Hemoglobin, jumlah lekosit, jumlah trombosit, persentase hematokrit, kadar SGOT, dan kadar TNF-α pada kedua kelompok tidak berbeda bermakna dengan nilai P>0,050, sedangkan kadar SGPT pada Demam bukan dengue meningkat 3x nilai normal sebesar 66,7%, n=10/15 dan Demam positif dengue masih dalam batas normal sebesar 60%, n=9/15, sehingga hasil SGPT antara kedua kelompok terdapat perbedaan secara bermakna dengan p=0,022 (P<0,05). Kesimpulan: Pada Demam bukan dengue kadar SGOT dan SGPT meningkat 1–3x nilai normal. Pada Demam positif dengue kadar SGOT meningkat 1–3x nilai normal, namun kadar SGPT masih dalam batas normal, sehingga kadar SGPT dapat sebagai faktor prediksi untuk membedakan kedua tipe Demam tersebut.

Kata kunci: *Demam Dengue, Darah lengkap, SGOT, SGPT, TNF-α.*

INTRODUCTION

Dengue fever has become one of the world's most important diseases caused by arthropods that develop in tropical and subtropical regions. Approximately 100 million cases of dengue infection occur each year, and an estimated 2.5 to 5 billion people worldwide are at the risk of dengue virus infection (Halstead SB, 2002). These four dengue virus serotypes (DEN-1, 2, 3 and 4) can transmit to humans through the bite of female mosquitoes of the genus Aedes (Chen LK, et al 2003). Dengue hemorrhagic fever (DHF) are spread throughout Southeast Asia, Western Pacific, and the Caribbean. Indonesia is an endemic region with widespread distribution throughout the country. The incidence of dengue fever in Indonesia between 6 to 15 per 100,000 population (1989–1995), and has risen sharply to 35 per 100,000 population in 1998, while the DHF mortality tends to decline until it reached 2% in 1999 (WHO, 2000).

Dengue fever is an acute febrile illness for 2–7 days, characterized by two or more clinical manifestations such as headache, retro-orbital pain, myalgia/arthralgia, skin rash, petechiae (hemorrhagic manifestations), and leukopenia. The diagnosis of dengue hemorrhagic fever (DHF) can be established when all above are met, and there is at least one hemorrhagic manifestations, such as petechiae, ecchymosis, purpura, epistaxis, gingival bleeding, melena, hemetemesis, thrombocytopenia (< 100.000/ul), and there is minimally one sign of pleural effusion, ascites, or hypoproteinemia, plasma leakage, such as increased hematocrit > 20% compared to a standard age and sex, decreased hematocrit > 20% after fluid therapy than previously hematocrit value (WHO, 1997).

Differences between DHF and DF is the presence or absence of plasma leakage after a phase of fever, and the patient will experience critical phase for 2–3 days. During this phase, the patient has no fever, but at the risk for seizure/shock if not handled with adequate treatment. Continuous abdominal pain is accompanied by vomiting, loss of consciousness, hypotension, restlessness, rapid and weak pulse and hypothermia are the signs and symptoms of dengue shock syndrome. Dengue virus is a non-hepatotropic virus, but it can damage the liver because dengue infection is often unusual and has been described since the 1960s (Burke T, 1968).

According to HY Lei et al. (2001), dengue virus infection affects immune system, such as the change of the CD4/CD8 ratio, cytokinesoverproduction can infect endothelial cells and hepatocytes with the result of apoptosis and dysfunction of these cells. Coagulation and fibrinolysis systems are also activated during dengue virus infection. Immune response disorder is not only disturbing viral clearing from the body, but cytokines overproduction can affect endothelial cells, monocytes and hepatocytes. Platelet destruction is caused by cross-reaction of anti-platelet autoantibody due to overproduction of IL-6 that plays a major role in the formation of anti-platelet autoantibodies and anti-endothelial cells, and increased levels of tPA and coagulation deficiency.

The level of liver dysfunction in dengue infection varies from mild symptoms, identified through AST and ALT examination, up to severity with jaundice and even fulminant hepatic failure (Seneviratne SL, 2006, Halstead SB, 2002). Liver dysfunction could be a direct effect of viral infection that can be detrimental with consequences of dysregulated immune response to the virus in the host (Seneviratne SL, 2006). However, clinical studies showed that liver involvement in dengue infection, especially in adults, is still rare. With such considerations, the liver function (SGOT and SGPT) examination is worth to be investigated.

MATERIALS AND METHODS

This was an observational cross-sectional study. The sampling process was done at Inpatient Wards, Tropical Infectious Diseases Division, Department of Internal Medicine, Dr. Soetomo Hospital, Faculty of Medicine, Airlangga University, Surabaya from July to December 2011. Inclusion criteria were patients with DF/DHF grade I-IV aged > 15 years, fulfilling the WHO criteria and positive on one of three tests: NS-1 dengue and or IgM/IgG anti-dengue. Patients who showed positive results were classified as dengue positive patients, and patients with a negative result were classified as suffering from non-dengue fever. Patients included in the study were willing to sign informed consent. Sample size was 15 subjects of non-dengue fever and 15 subjects of positive dengue fever.

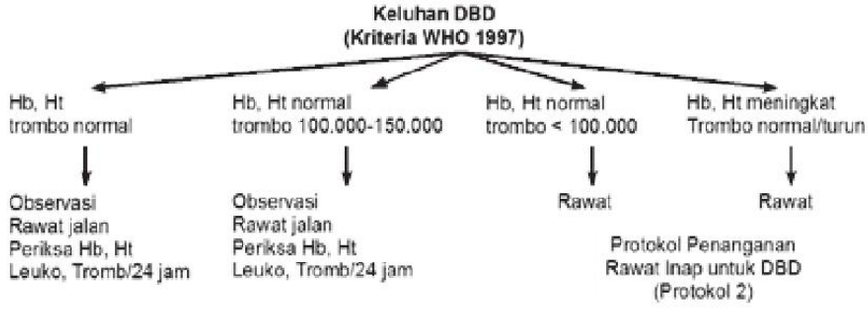

Figure 1. Scheme of DHF symptoms according to 1997 WHO criteria

Research Implementation

Venous blood of the subjects were taken as much as 5 ml, collected in a 2 ml EDTA tubes for whole blood count, and 3 ml was collected in plan tubes to obtain 1 ml serum for SGOT, SGPT, and Tumor Necrosis Factor α (TNF-α). Whole blood count was performed with a Sysmex 4020, while AST and ALT examination was performed with a Hitachi 902. Examination of TNF-α was performed using an ELISA method (Biosource TNF-α EASIA Kit). After all the data collected, the were processed and analyzed using a statistical test (Chi Square test) with SPSS for Window version 13.

RESULTS

1. Sex distribution in groups of non-dengue and positive dengue fever.

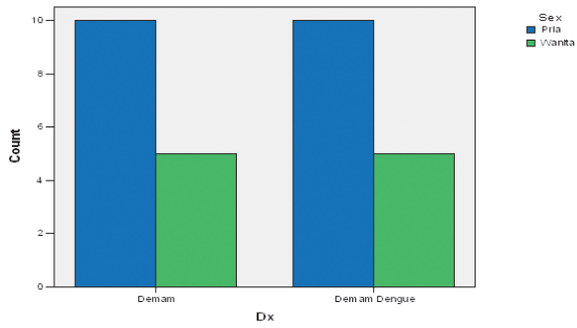

Figure 2. Sex distribution between the groups was not dengue fever and dengue fever positive.

Based on sex distribution, among 30 study subjects in both groups the higher prevalence was found in men (66.7%, n = 10/15). Chi- Square test results showed no significant differences, with P = 0.300 (P > 0.05).

2. Age distribution in groups of non-dengue and positive dengue fever.

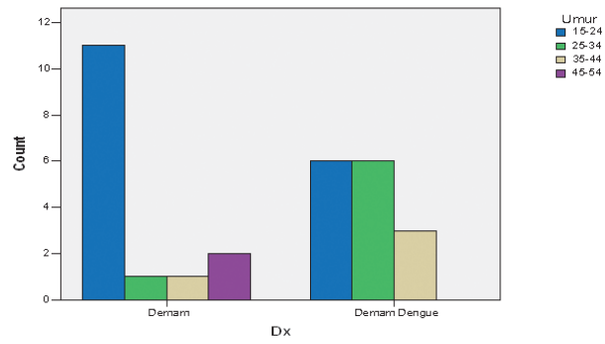

Figure 3. Age distribution between in groups of non-dengue and positive dengue fever.

Based on age distribution, among 30 study subjects, higher prevalence was found between 15–24 years. Chi-Square test results showed no significant differences, with P = 0.128 (P > 0.05).

3. Fever distribution in groups of non-dengue and positive dengue fever.

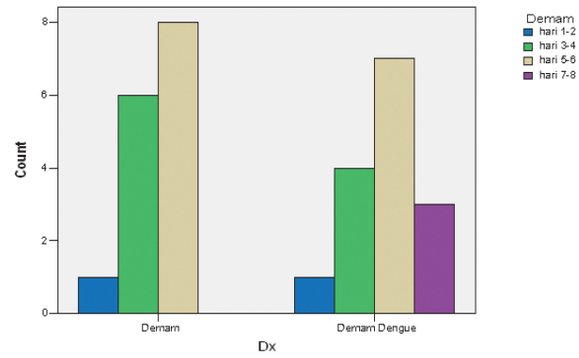

Figure 4. Fever incidence in groups of non-dengue and positive dengue fever.

Based on fever distribution among 30 study subjects, many in both groups come on days 5–6. Chi-Square test results showed no significant correlation, with a value of P = 0.096 (P > 0.050).

4. Grade (disease severity) distribution in groups of non-dengue and positive dengue fever.

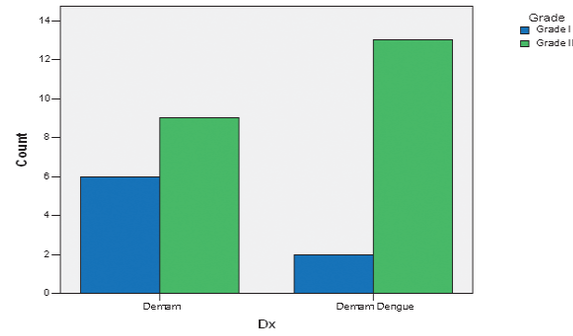

Figure 5. Grade (disease severity) distribution in groups of non-dengue and positive dengue fever.

Based on disease severity among 30 subjects, most visited the hospital in Grade II condition. Chi- Square test results showed no significant relationship with the value of P = 0.090 (P < 0.050).

5. Hemoglobin levels in groups of non-dengue and positive dengue fever.

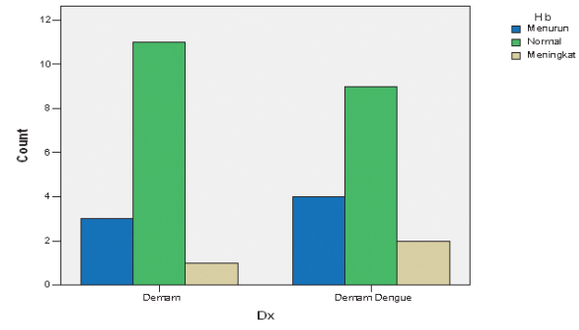

Figure 6. Hemoglobin levels in groups of non-dengue and positive dengue fever.

Based on hemoglobin levels distribution, hemoglobin levels in both groups were still within normal limits. Chi-Square test results showed no significant differences, with P = 0.434 (P > 0.050).

6. Leukocytes count in groups of non-dengue and positive dengue fever.

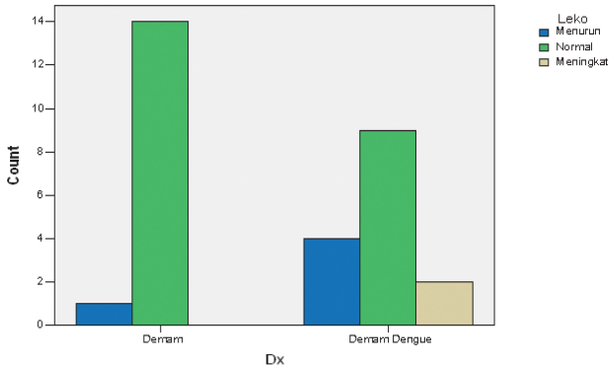

Figure 7. Leukocytes count in groups of non-dengue and positive dengue fever.

The leukocytes count distribution in both groups was still within normal limits. Chi- Square test results showed no significant differences, with P = 0.274 (P < 0.050).

7. Platelet counts distribution in groups of non-dengue and positive dengue fever.

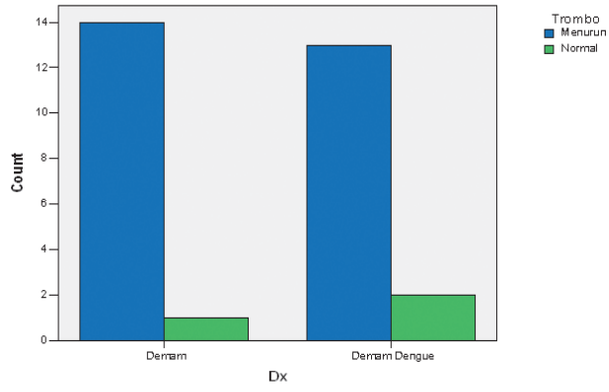

Figure 8. Platelet counts distribution in groups of non-dengue and positive dengue fever.

Based on platelet count distribution, both groups showed reduction less than normal value. Chi-square test results showed no significant differences, with P = 0.338 (P > 0.050).

8. Hematocrit percentage in groups of non-dengue and positive dengue fever.

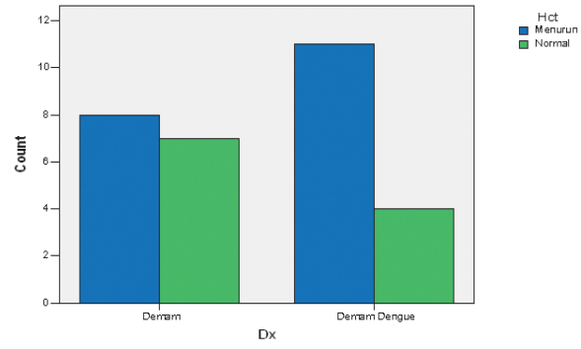

Figure 9. Hematocrit percentage in groups of non-dengue and positive dengue fever.

Based on hematocrit percentage distribution, both groups showed a decrease from normal value. Chi- Square test results showed no significant differences, with P = 0.161 (P > 0.050).

9. SGOT levels in groups of non-dengue and positive dengue fever.

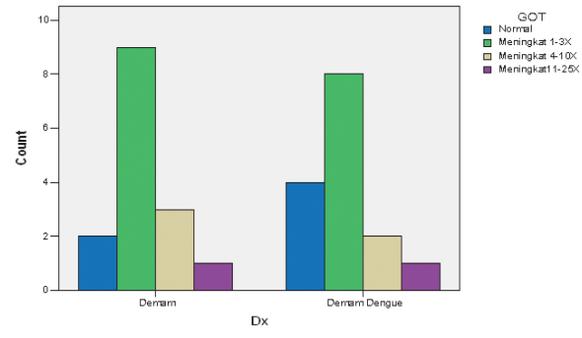

Figure 10. SGOT levels in groups of non-dengue and positive dengue fever.

Based on SGOT levels distribution, both subjects showed increase 1–3 times the normal value. Chi- Square test results showed no significant differences, with P = 0.143 (P > 0.050).

10. SGPT levels in groups of non-dengue and positive dengue fever.

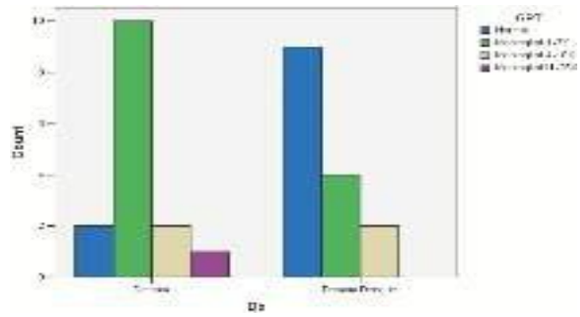

Figure 11. SGPT levels in groups of non-dengue and positive dengue fever.

Based on SGPT level distribution, in non-dengue group the SGPT level increased 1–3 times higher than normal (66.7%, n = 10/15) and in group with positive dengue fever it was still within normal limits (60%, n = 9/15). The results of Chi-Square test showed significant difference with a P value = 0.022 (P < 0.050).

11. TNF-α in groups of non-dengue and positive dengue fever.

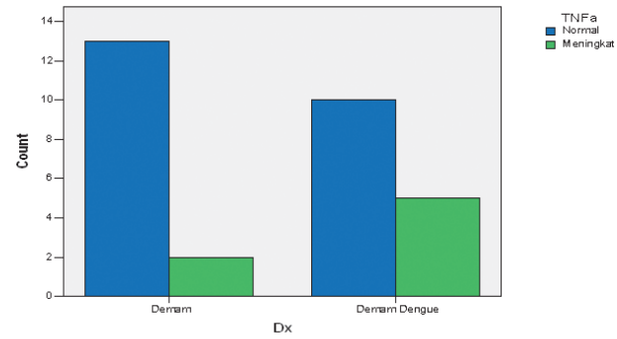

Figure 12. TNF-α in groups of non-dengue and positive dengue fever.

Based on TNF-α distribution, the subjects in both groups were within normal limits. Chi- Square test results showed no significant difference, P = 0.155 (P > 0.050).

Table 1. Laboratory analysis in groups of non-dengue and positive dengue fever on the first day of hospital admission

No.	Variables	Fever N=15	Dengue Fever N = 15	p Value	95% Confidence Interval
1	Sex	Male: 66.7% (10/15)	Male: 66.7% (10/15)	0.300	0.219-4.564
2	Age	15-24 years: 73.3% (11/15)	15-24 years: 40% (6/15)	0.128	-
3	Fever	Day 5-6 53.3% (8/15)	Day 5-6 46.7% (7/15)	0.096	-
4	Grade	Grade II 60% (9/15)	Grade II 86.7% (13/15)	0.090	0.708-26.531
5	Hemoglobin	Normal 73.3% (11/15)	Normal 60% (9/15)	0.248	-
6	Leukocyte	Normal 93.3% (14/15)	Normal 60% (9/15)	0.274	-
7	Thrombocyte	Decrease 93.3% (14/15)	Decrease 86.7% (13/15)	0.388	0.174-26.672
8	Hematocrit	Decrease 53.3% (8/15)	Decrease 73.3% (11/15)	0.161	0.090-1.918
9	SGOT	Increase 1-3x Normal 60% (9/15)	Increase 1-3x Normal 53.3% (8/15)	0.143	-
10	**SGPT**	Increase 1-3x Normal 66.7% (10/15)	Normal 60% (9/15)	**0.022**	-
11	TNF-α	Normal 86.7% (13/15)	Normal 66.7% (10/15)	0.155	0.519-20.370
12	Vomiting	Vomiting > 3x 73.3% (11/15)	Vomiting > 3x 53.3% (8/15)	0.161	0.521-11.104

12. Vomiting incidence in groups of non-dengue and positive dengue fever.

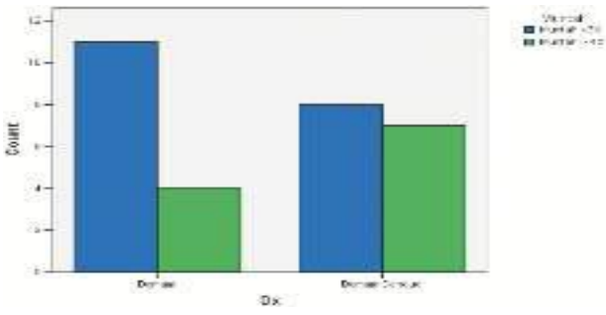

Figure 13. Vomiting incidence in groups of non-dengue and positive dengue fever.

Vomiting incidence in both groups was > 3 times. Chi-Square test results showed no significant differences, with P = 0.161 (P > 0.050).

DISCUSSION

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are diseases caused by dengue virus infection with clinical manifestations of fever, muscle aches and/or joint pain accompanied with leukopenia, rash, lymphadenopathy, thrombocytopenia and hemorrhagic diathesis. Plasma leakage in DHF occurs characterized by hemoconcentration (increased hematocrit) or the accumulation of fluid in the body cavity. Dengue shock syndrome (DSS) is characterized by dengue shock (Balmaseda A, et al 2006, Nasronudin 2007, Soegijanto S., 2006).

NS1 is a glycoprotein with a molecular weight of 50 kDa that is expressed in two forms of membrane-associated (mNS1) and secreted (sNS1) (Paul Y. 2004). NS1 protein has a high immunogenic properties compared with other nonstructural proteins, although its function has not been known. NS3 and NS5 proteins can stimulate humoral immunity, although the effect is very small when compared to the NS1 protein (Earth C, 2006). Upon the entry of dengue virus in the human body, the virus multiplies in the reticuloendothelial cells followed by viremia that lasts for 5-7 days. As a result of this emerging viral infection both humoral immune and cellular responses (antineutralization, antihemagglutination, anticomplementary). The appearing antibodies are generally IgM and IgG. In primary dengue infection, IgM antibodies begin to form, whereas IgG is not yet formed. In secondary infection IgM levels are formed again and IgG antibody levels that already exist will rise (booster effect) (Chen LK, et al, 2003, Soegijanto S. 2006).

SGOT, stands for Serum Glutamic Oxaloacetic Transminase or also called aspartate amino transferase (AST), is an enzyme that is always present in heart and liver cells. AST is released into the blood when the liver or heart is damaged. Very high SGOT levels in blood indicates liver damage due to viral hepatitis or possible occurrence of heart attacks. Some medications can also raise SGOT level.

SGPT, stands for Serum Glutamic Piruvic Transaminase, or also called ALT (alanine amino transferase), is an enzyme found in the liver cells as well as effective for diagnosing hepatocellular destruction. In small quantities this enzyme found in the heart muscle, kidney and skeletal muscle. Generally, when acute liver parenchymal damage is present, ALT level is higher than AST levels, whereas in chronic process the opposite condition is present.

Reference value of SGPT is 0–40 U/L. Conditions that increase SGPT level > 20 times than normal are usually caused by acute viral hepatitis, liver necrosis (due to drug toxicity or chemicals). SGPT increase of 3–10 times more than normal is caused by mononuclear infection, chronic active hepatitis, biliary extra hepatic obstruction, Reye 's syndrome, and myocardial infarction. SGPT increase of 1–3 times more than normal is caused by pancreatitis, fatty liver, cirrhosis Laennec, and biliary cirrhosis.

SGPT is more specific for liver damage. SGPT is an enzyme produced in the liver cells (hepatocytes), so it is more specific for liver disease compared with other enzymes. SGPT increase usually occurs when there is a damage liver cell membrane. Any type of liver inflammation may cause an increase in liver cell membranes. Inflammation of the liver can be caused by viral hepatitis, some medications, alcohol use, and diseases of the bile duct.

SGOT is a mitochondrial enzyme that is also found in heart, kidneys and brain. So this test is less specific for liver disease. In some cases of liver inflammation, an increase in SGPT and SGOT will be similar (Gowda, Desai, Hull, Math, Kulkarni, Vernekar, 2009). The results of a study in India (Itha S, 2005) revealed that SGPT and bilirubin levels rarely elevated in patients with dengue fever. According to Souza LJ (2002) and Kalayanarooj (1997) SGOT levels in dengue infections tend to be higher than SGPT, but also different from the pattern in patients with viral hepatitis, but similar to that in alcoholic hepatitis. The cause of SGOT > SGPT in dengue patients is excessive SGOT release from monocytes damaged during dengue infection. According to Kuo CH (1992), the determination of SGOT levels increasing 1-3 x than normal value and normal SGPT levels can be an early indicator of dengue virus infection. The findings are similar to the results of this study.

The severity of liver dysfunction in dengue infections is associated with the severity of dengue disease. Therefore, a good predictive factor for DHF severity is to identify the extent of liver damage (Kalayanarooj S, 1997). According to Rajoo Singh Chhina (2008) that the extent of liver damage (SGOT levels) was higher in DF and DHF group, not the SGPT levels, whereas in DSS all liver function tests entirely revealed a rise. Similar data have also been reported by Seneviratne et al. (2006) and Souza et al. (2004).

The results of this study showed that whole blood count (hemoglobin level, leukocyte count, platelet count, hematocrit percentage, SGOT levels, and TNF-α levels) and

symptoms (fever days, grade, time vomiting) were the same, except in non-dengue fever group the levels of SGPT was 1-3x higher than normal value, whereas in positive dengue fever the SGPT levels was within normal limits.

CONCLUSION

In patients with fever for 4-5 days , which are grouped into non-dengue fever, SGOT levels increased 1-3 x times and SGPT levels increased 1-3 x from normal value. In positive dengue fever SGOT levels increased of 1-3 x, while SGPT level was still within the normal range (< 40 IU/L), so that SGPT levels can be used as a predictive factor for determining positive dengue fever (DF and DHF).

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Research Report

APPLICATION OF NEURAL NETWORKS ON BLOOD SERUM IMAGE FOR EARLY DETECTION OF TYPHUS

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ABSTRACT

Background: Typhus is a disease caused by Salmonella typhi, Salmonella paratyphi A Salmonella parathypi B, dan Salmonella paratyphi C bacteria that attacks digestive tract and caused infection in small intestine. The common test that performed in the laboratory is widal test. The result reading of the widal test still processed manually with looking the turbidity caused by the agglutination. Aim: The research was made to decrease human error by creating a program based on artificial neural network (ANN) with learning vector quantization (LVQ) method. Method: Input of this program is image of blood serum that has reacted with widal reagen. Image procesing start with grayscaling, filtering, and thresholding. Result: Output of this program is divided into two classes, normal and typhus detected. Conclusion: From this experiment result that using 24 testing data, gives the accuracy of this program 95.833% with 1 error result from 24 testing data.

Key words: *Artificial Neural Network, Learning Vector Quantization, Salmonella, Typhus, Widal*

ABSTRAK

Latar belakang: Penyakit typhus adalah penyakit yang disebabkan oleh bakteri Salmonella typhi, Salmonella paratyphi A Salmonella parathypi B, dan Salmonella paratyphi C yang menyerang bagian saluran pencernaan, sehingga terjadi infeksi saluran pencernaan tepatnya usus halus dan masuk ke aliran darah. Pemeriksaan awal yang umum dilaksanakan di laboratorium adalah dengan melakukan pemeriksaan widal. Pembacaan hasil pemeriksaan widal masih dilakukan secara manual dengan mengandalkan kemampuan manusia memeriksa kekeruhan yang timbul akibat terjadinya aglutinasi. Tujuan: Penelitian ini dibuat untuk mengurangi adanya human error yang terjadi pada pembacaan hasil tes dengan menggunakan program berbasis jaringan saraf tiruan (JST) metode Learning Vector Quantization (LVQ). Metode: Citra yang digunakan adalah citra serum darah yang telah direaksikan dengan reagen widal. Proses pengolahan citra dilakukan dengan teknik grayscaling, filtering dan thresholding. Hasil: Keluaran dari program ini adalah deteksi citra serum darah typhus dan normal. Kesimpulan: Dari hasil penelitian ini dengan menggunakan 24 data uji, memberikan akurasi program sebesar 95,833% dengan 1 kesalahan uji dari 24 data uji.

Kata kunci: *Jaringan Saraf Tiruan, Learning Vector Quantization, Salmonella, Typhus, Widal*

INTRODUCTION

Typhoid fever or commonly referred to as typhus is a disease caused by the bacterium Salmonella typhi, Salmonella paratyphi A Salmonella parathypi B, and Salmonella paratyphi C, which attacks the digestive tract. Acute infectious disease is always there in society (endemic) in Indonesia, ranging in age from toddlers, children and adults. According to WHO (World health organization) in 2003, each year there are approximately 17 million cases with 600,000 cases leading to death in the world. Approximately 2% of patients with typhoid can be a carrier. In Indonesia, there were 900,000 cases with 20,000 deaths case.[1]

A common initial examination was carried out in the laboratory by examining widal. Widal tes is included in the class of serological test, which is done by reacting the blood serum of patients with widal reagents. Widal test is the

examination steps are easy to do and the results are quickly obtained. Widal test involving agglutination reaction that helps detect antibodies in the diagnosis of typhoid fever.[2] Positive Widal test characterized by the appearance of turbidity caused by agglutination arising from the reaction of antibodies in the blood serum of patients with bacterial antigens present in widal reagents. Widal test results can be used as a follow-up diagnosis of typhoid fever. Diagnosis of typhoid disease from widal test must be supported by other examinations, such as checking the physical condition of the patient's own or other laboratory tests such as examination of peripheral blood and blood cultures.[3]

The results of turbidity caused by agglutination widal examination read by relying on human capabilities so that errors can occur due to human error, because each medical staff have different possibility of reading the results of turbidity arising from the blood serum agglutination reagents.[4] Based on the above presentation, to reduce human error and to develop research using soft computing technologies it needs to make a program to solve it is by using a program based on artificial neural networks.

Artificial neural network is an information processing system that has characteristics similar to biological neural networks, which can be applied to one of them in pattern recognition or pattern recognition.[5] Typhus diagnostic research with widal test using artificial neural networks has been studied before with bacpropagation method.[4] The procentage of the result is success detection for 93.75% positive typhoid and negative 90% for typhoid. The study was conducted by using the features in the form of a binary matrix pattern of image processing as input feature propagation. Artificial neural networks have many methods that can be used. Comparative research results using an artificial neural network classification methods bacpropagation and LVQ (Learning Vector Quantization) to obtain the result that the LVQ training process faster and more accurate than backpropagation.[6]

Due to the background, the author will make an application methode of artificial neural network in blood serum image for early detection of disease typhoid. The present study microscopic images of blood serum which has given widal reagents are used as inputs of the software, but the image is processed first using image processing methods. The output of image processing features value then processed using LVQ neural network method and going through the learning phase.

MATERIALS AND METHODS

Sample data collection of blood serum samples is done by taking a blood serum sample data that has been diagnosed from clinical laboratory that consist normal blood serum sample and typhus blood serum samples. Blood serum is then reacted with a reagent widal then conducted observations and image capture using a digital microscope. The whole image is obtained jpeg format, and done cropping on an object the size of 100 x 100 pixels.

Image of the blood serum was observed if there is agglutination in it, if it is exposed to typhus blood serum then there will be agglutination otherwise if there is no agglutination in the serum it is normal serum. Agglutination occurs due to the reaction between serum by cellular antigen or cell body surface. The reaction between the reagent and serum observed under a microscope with magnification 4x10, the results of these observations in the form of images to be processed into the image processing and artificial neural networks.

Broadly speaking, software design schemes undertaken in this study is depicted in Figure 1.

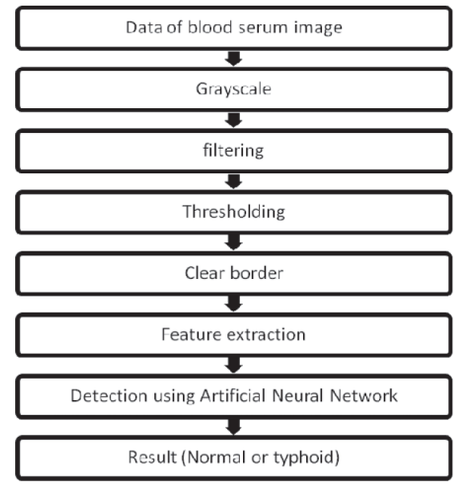


Figure 1. Flow procedure typhus detection program

Blood serum samples processed image using a digital image processing techniques such as grayscale, namely, filtering, thresholding and clear border. First Data of blood serum image was color image that consists of three layer matrix, namely R - layer, G - layer and B - layer converted to grayscale images or images that represent the level of gray. Grayscale process aims to alleviate the computational load while performing data processing. Then the filtering process done to the image that has grayscale form. Filtering is used to improve the quality of the processed image by smoothing noise contained in images of blood serum samples. The research will use median filter as the filter technique. Median filter method serves as a nonlinear filter for the workings of this filter is not included into the category convolution operation. The next process is thresholding, thresholding is a simple and effective techniques for image segmentation of blood serum. This method can be used to extract objects from the background by selecting the threshold value T that separates the background and object and the result of thresholding process will produce a binery image or image with black and white colour. In this case the object needed is agglutination that caused by the reaction of serum with reagents. Agglutination will be represented by the white pixels that result from thresholding

process, so the feature image obtained in the form of the number of pixels that are white. Clear borbeder process after thresholding process is used to eliminate unwanted image on the wall background.

Feature extraction is used to determine the characteristics of the image or pattern of positive and negative blood serum typhus before being put to be processed into the neural network. Results of feature extraction is a number of white pixels, which is where white pixels represent the image in the image agglutination.

Results of feature extraction processing will be used as input to an ANN using LVQ models. The next stage is the determination of the network design. This stage will be determined the definition of the problem, namely the determination of input and output patterns for training and testing the ANN. The next step taken is to initialize the network to be trained or tested. This research was conducted using 64 training sample data consist of 32 normal samples and 32 samples of typhus. The data used for testing amounted to 24 data consists of data from 12 normal and 12 Data typhus. The flow chart of LVQ itself can be shown in Figure 2.

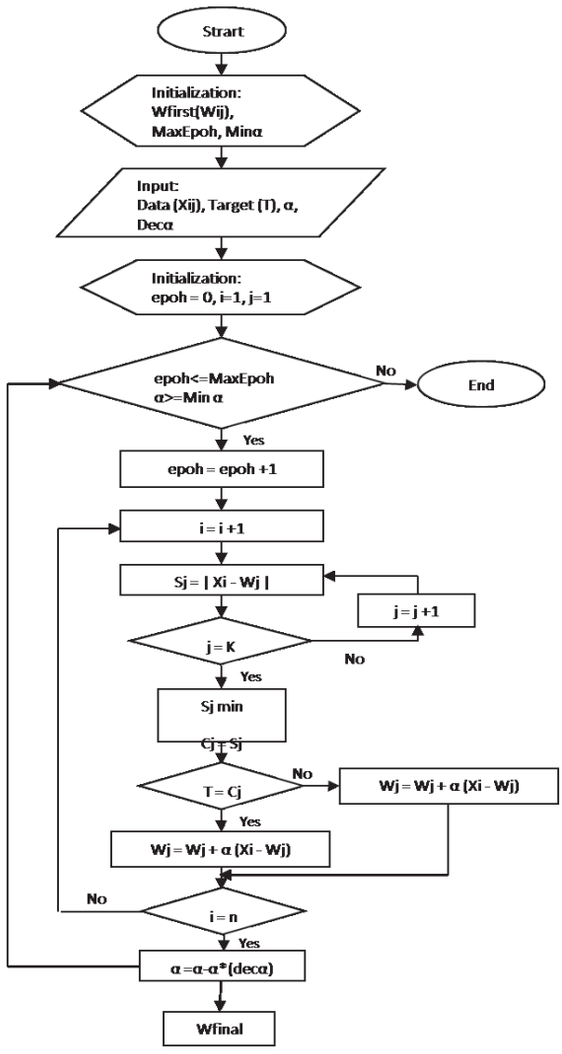


Figure 2. Flowchart LVQ

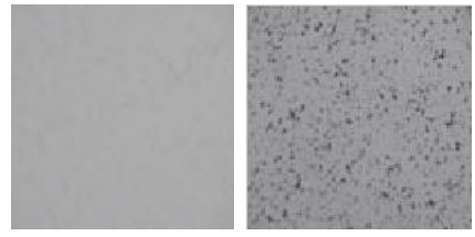
RESULT AND DISCUSSION

This research was conducted using 64 training sample data, where each consisting of 32 normal samples and 32 samples of typhus. The data used for testing amounted to 24 data consists of data from 12 normal and 12 Data typhus. All training sample data processed with image processing and ANN using LVQ methode.

For the first image processing is grayscale, grayscaling process is done for change the image color on the blood serum into gray image to reduce the computational burden. After grayscalling process, image has filtered with median filtering methode to reduce the noise. Grayscalling image processing result is shown in Figure 3.

The next processed is thresholding. Thresholding process in this study is done by taking 115 as the threshold value that obtained by observation the histogram blood serum images were used as training samples. Objects needed in this case is the agglutination that caused by reaction with the reagent serum. Agglutination will be represented by the white pixels caused by the thresholding process. Image processing results for thresholding disaikan in Figure 4.

The last step of image processing that used in this study is clear border. Clearborder process needed for eliminate unneeded image attached or contact with blood serum image to be processed. Image processing results of the process is a clear border binary image like the result of the previous image processing wihich is thresholding. This binary image is used to deteminate the value of feature extraction. At this clearborder process used toolbox matlab syntax "cb = imclearborder (cb)". Results of image processing for clear border is shown in Figure 5.

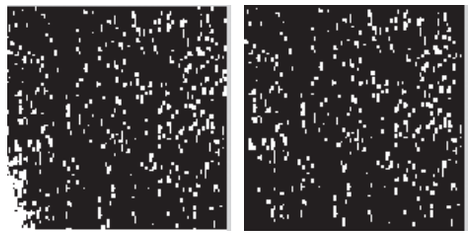


(a) (b)

Figure 3. (a) Grayscale normal serum image (b) Grayscale typhus serum image

(a) (b)

Figure 4. (a) Threshold normal serum image (b) Threshold typhus serum image



(a) (b)

Figure 5. (a) Threshold image typhus serum (b) clearborder image typhus serum

For feature extraction, object that needed in this case is the agglutination that caused by the reaction with the reagent serum. Agglutination will be represented by a white pixel in the resulting binary image. Binary image of blood serum results will be processed for image feature extraction process to count the number of white pixels as features that are used as input for training and testing process in ANN.

Training process of neural network LVQ method for detection of typhus used 64 data of blood serum image, which consists of 32 images of normal blood serum and 32 images of typhoid blood serum. Training process is performed using some variations of Learning Vector Quantization input parameters, as shown in Table 1.

The results of the training process is obtainment of final weight values that saved and used for the testing process. After the data passing through the training process will be performed test matches to the target data which is the result of a doctor's diagnosis. The number of matches data with the target compared to the entire amount of data to get the accuracy rate of the training process. The accuracy obtained from each parameter changes is shown in Table 2.

Table 2 shows the percentage level of accuracy of the results of the training process to some variations of the learning rate (α) and a reduction in the rate of learning (dec α), these variations affect the number of epoch that and the level of accuracy for the training. Parameter variation indicates a accuray changes that does not necessarily(volatile). The most optimal level of accuracy in this study was the learning rate 0.01 with a reduction in the learning rate 0.5 and learning rate 0.001 with the reduction of learning rate 0.1, 0.25, 0.5 by 96.875% accuracy. Display ANN training program is shown in Figure 6.

Table 1. Variation parameter of LVQ

Amount of *training data*	64
Amount of target classification	2
Learning rate (α)	0.1 ; 0.01 ; 0.001
Decrease of learning rate (dec α)	1.01 ; 0.1 ; 0.5 ; 0.25
Minimum learning rate (min α)	0.0000001
Maximum epoch	10000

Table 2. Result accuracy testing value on training data

α	Dec α	Epoch	Akurasi (%)
0.1	0.001	1146	93,75
	0.1	110	93,75
	0.25	41	93,75
	0.5	17	93,75
0.01	0.001	917	93,75
	0.1	88	93,75
	0.25	33	95,3125
	0.5	14	96,875
0.001	0.001	688	93,75
	0.1	66	96.875
	0.25	25	96.875
	0.5	10	96.875

The testing process used 24 data outside of the data that used for training process. 24 data consists of 12 images of normal blood serum, 12 images typhus blood serum. Classification of testing data process performed by finding the minimum distance between features of testing data with the final weight values that obtained from the results of ANN training process. The testing process is done by

Figure 6. Display of training program

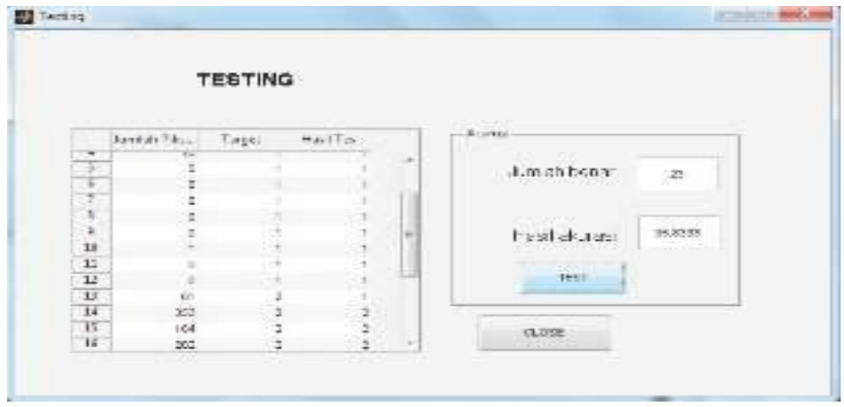


Figure 7. Testing program display

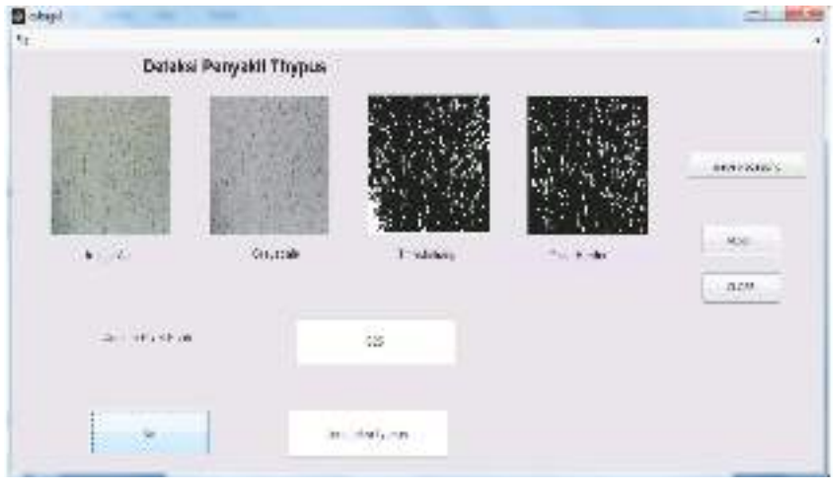


Figure 8. Display of early detection of typhus program

using a variation of the dec alpha and alpha which has the highest value accuracy of training data which is used alpha 0.01 and dec alpha 0.5. Testing process results for testing data using the alpha value of 0.01 and the dec alpha value of 0.5 is obtained the accuracy of 95.833%. From 24 testing data are, there's 1 testing data that does not match the target. Display ANN testing program is shown in Figure 7.

Display program of early detection of typhus which thre are image processing, feature values and the image test results is presented in Figure 8.

In this page display there is a menu bar file, which is used to retrieve data from the directory. Also in this page there are some buttons that have the functions of each. Image processing button, a button that contains the command for perform the process image processing on the data tested. Image processing process shown on this page are the original image, grayscale, binary image from thresholding process and clear border. This button also count the feature extraction value. Test button, a button that contains the commands for perform testing process on the data tested. In this case the results of the test is information whether the data classified in "normal" or classified in "typhosa detected". Reset button, serves to reset or delete the previous data so that the page can be used for perform other testing data. Close button, serves to close the page process the data and return to the home page.

CONCLUSION

Based on the results of the discussion, it concluded that early detection system design on blood serum image based on neural network methode, done by looking for the value of the number of features in the form of white pixels in the images of blood serum which has been processed using image processing. The most optimal parameter values for the typhus early detection design programs is using the value of the learning rate (α) of 0.01 and a reduction of learning rate (dec α) of 0.5 with the accuracy of the program 95.83%.

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Research Report

IMMUNOHISTOCHEMICAL ANALYSIS OF NF-κB (P50/P65) IN PATIENT WITH AGGRESSIVE AND CHRONIC PERIODONTITIS

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ABSTRACT

Background: Nuclear factor-kappaB (NF-κB) is a protein complex that plays a role in transcription factors and in response to inflammation. Periodontitis is a periodontal disorder caused by various bacteria such as A. actinomycetemcomitan and P.gingivalis whose LPS is closely related to NF-κB (p50/p65). Aim: This study observed whether NF-κB (p50/p65) played a role in aggressive and chronic periodontitis. Methods: Data were obtained from periodontal tissue 40 patients with aggressive periodontitis and 40 patients with chronic periodontitis. Samples were derived from periodontal tissue with abnormalities and NF-κB (p50/p65) protein expression test was performed by immunohistochemistry. The statistical test used was the t-test. Results: In NF-κB (p50) the t value was -12 041 and significance 0.000, with α = 5%, showing significant difference in protein expression of NF-κB (p50) between patients with aggressive periodontitis and chronic periodontitis. OR estimation for the value of protein expression of NF-κB (p50) was 0.64 (sign. = 0.000). It shows that if the protein expression of NF-κB (p50) of the respondents is incremented by 1 (one) unit, the risk of chronic periodontitis increases 1.64 times. Box plot diagram shows that the distribution of the protein expression of NF-κB (p50) between patients with aggressive periodontitis and chronic periodontitis patients is significantly different. In NF-κB (p65) the Z value was -7.137 and significance of 0000, with α = 5%, showed significant differences in protein expression of NF-κB (p65) between patients with aggressive periodontitis and chronic periodontitis. OR estimates for protein expression of NF-κB (p65) was 0.66 (sign. = 0.000). This indicates that if the protein expression of NF-κB (p65) respondents is incremented by 1 (one) unit, the risk of chronic periodontitis increases 1.5 times. Box plot diagram shows that the distribution of the protein expression of NF-κB (p65) between patients with aggressive and chronic periodontitis patients is significantly different. Conclusion: The protein expression of NF-κB (p50/p65) has more influence on the incidence of chronic periodontitis patients, so it can be used as a marker for chronic periodontitis.

Key words: *periodontitis, inflammation, NF-κB p50, NF-κB p65, transcription*

ABSTRAK

Latar belakang: Nuclear factor – kappaB (NF-κB) sebagai protein komplek yang berperan pada faktor transkripsi dan berperan pada respons terhadap keradangan. Periodontitis sebagai kelainan periodontal yang disebabkan berbagai kuman seperti A. Actinomycetemcomitan dan P gingivalis dimana LPS kuman ini berkaitan erat dengan NF-κB (p50/p65). Tujuan: Penelitian ini ingin melihat apakah NF-κB (p50/p65) berperan pada periodontitis agresif dan kronis. Metode: Data penelitian didapat dari jaringan periodontal 40 penderita dengan periodontitis agresif dan 40 penderita periodontitis kronis. Sampel berasal dari jaringan yang mengalami kelainan periodontal dan uji ekspresi protein NF-κB (p50/p65) dilakukan secara imunohistokimia. Uji statistik yang digunakan adalah uji–t. Hasil: Pada NF-κB (p50) diperoleh nilai t sebesar -12.041 dan signifikansi 0.000, dengan α = 5% maka terdapat perbedaan bermakna ekspresi protein NF-κB (p50) antara penderita Periodontitis Agresif dan penderita Periodontitis Kronis. Nilai estimasi OR untuk variabel ekspresi protein NF-κB (p50) adalah 0,64 (sign. = 0,000). Artinya, jika ekspresi protein NFκB (p50) responden bertambah 1 (satu) satuan, maka risiko terjadinya Periodontitis kronis menjadi 1,64 kali. Menggunakan Diagram Box plot memperlihatkan sebaran ekspresi protein NF-κB (p50) antara penderita Periodontitis Agresif dan penderita Periodontitis Kronis yang tampak sangat jauh berbeda. Pada NF-κB (p65) diperoleh nilai Z sebesar -7.137 dan signifikansi 0.000, dengan α = 5% maka terdapat perbedaan bermakna ekspresi protein NF-κB (p65) antara penderita Periodontitis Agresif dan penderita Periodontitis Kronis. Nilai estimasi OR untuk variabel ekspresi protein NF-κB (p65) adalah 0,66 (sign. = 0,000). Artinya, jika ekspresi protein NF-κB (p65) responden bertambah 1 (satu) satuan, maka risiko terjadinya Periodontitis kronis menjadi 1,5 kali. Menggunakan Diagram Box plot memperlihatkan sebaran ekspresi

protein NF-κB (p65) antara penderita Periodontitis Agresif dan penderita Periodontitis Kronis yang tampak sangat jauh berbeda. Kesimpulan: Ekspresi protein NF-κB (p50/p65) lebih berpengaruh pada kejadian penderita Periodontitis Kronis, sehingga dapat digunakan sebagai marker untuk periodontitis kronis.

Kata kunci: *periodontitis, keradangan, NF-κB p50 NF-κB p65, transkripsi*

INTRODUCTION

Nuclear factor-kappaB (NF-κB) is a complex protein, a transcription factor that plays an important role in the regulation of immune system in response to inflammation and as regulators of gene expression. NF-κB contributes to the activation of a wide variety of genes, such as proinflammatory cytokines, TNF-α, IL-1 and chemokines. In the inactive state of NF-κB is located in the cytoplasm with the inhibitory protein NF-κB (Jimi et al., 2007). The family NF-κB consists of NF-κB1 (p50/p105), NF-κB2 (p52/p100), RelA (p65), RelB and c-Rel. Its classical form is a heterodimer consisting of p50 and p65 subunits.

NF-κB serves to delay neutrophil apoptosis by altering the levels of Bcl2 protein. When an infection with pathogenic microorganisms, such as that in periodontitis, it activates the transcription factor NF-κB via TLRs stimulation, which is expressed on innate immune system, including macrophages, dendritic cells (DCs) and mucosal epithelial cells (Beinke and Ley, 2004).

NF-κB consists of a heterodimer between Rel polypeptides and protein p50, which acts to control the expression of many adaptive genes, such as MHC proteins and genes important for the regulation of the apoptotic process. NF-κB resides in the cytoplasm in an inactive form along with a regulatory protein I-κB. The interaction between LPS/TLR activates transcription factor nuclear factor-κB (NF-κB) that plays a role in activating the transcription of inflammatory mediators (Ohnishi et al., 2007; Takahashi et al., 2008). NF-κB is activated by LPS in THP-1 cells, the transcription factor NF-kB is associated with metabolic and inflammatory responses, including nuclear receptors, activators of protein (AP-1) and early growth response (EGR). Furthermore, it is suggested that IKK/NF-kB is a target for the treatment of periodontitis disorders (White et al., 2000; Carpenter and O'Neill., 2007, Chen, 2007). As it was believed that the expression of NF-kB (p50/p65) affects the occurrence of aggressive and chronic periodontitis, this study revealed observations of its expression in patients with those disorders.

MATERIALS AND METHODS

This research was an analytical observational studies, case control study design in patients eperienced aggressive and chronic periodontitis. Tissue samples were taken from periodontal tissue affected by periodontitis. The population of the research was patients who came to Periodontics Department in Faculty of Dentistry Airlangga University and had been diagnosed with aggressive or chronic periodontitis.

Informed consent was obtained from all subjects before commencement of the study. A total of 80 gingival tissue (40 aggressive periodontitis and 40 chronic periodontitis) were included in the present study. Gingival tissue were obtained surgically from periodontal tissue under local anesthesia.

The expression of NF-κB (p50/p65) was detected by immunohistochemistry test. NF-κB (p50/p65) was detected with biotin-labeled antibodies were and visualized with DAB-deminobenzidine.

RESULTS

Immunohistochemistry results to observe NF-κB (p50) protein expression for patients with aggressive and chronic periodontitis can be seen in Table 1.

The differences in NF-κB (p50) protein expression in patients with aggressive and chronic periodontitis are seen in Table 1. The mean protein expression in patients with aggressive periodontitis was 9.80 whereas in patients with chronic periodontitis it was 21.33. The mean value indicates that the protein expression of NF-κB (p50) in aggressive periodontitis patients was significantly lower than protein expression in patients with chronic periodontitis. Using independent samples t-test with homogeneous variance data the obtained t value was -12 041 and 0.000 significance, using the $\alpha = 5\%$. Conclusively, there were significant differences in the protein expression of NF-κB (p50) between patients with aggressive periodontitis and chronic periodontitis.

By using simple logistic regression analysis values, we obtained sign. 0,000 , which means that the protein expression of NF-κB (p50) influences the occurrence of aggressive periodontitis and chronic periodontitis. OR estimated values for the protein expression of NF-κB (p50) was 0.64 (p = 0.000). If the protein expression of NF-κB (p50) of the respondents increased by one unit, then the

Table 1. NF-κB protein expression descriptive value (p50), in patients with agressive (PA) and chronic periodontitis (PK)

Periodontitis	N	Mean	Std Deviation	Std Error Mean	t – test
PA	40	9,80	4,040	.639	t = -12,041
PK	40	21,33	4,509	.713	p = 0,000

risk for the occurrence of aggressive periodontitis would be 0.604 times or risk of chronic periodontitis would be 1.64 times higher than that with aggressive periodontitis. Differences in NF-κB (p50) protein expression is described using box plot diagram, which clearly shows the difference in the distribution of data between the NF-κB (p50) protein expression of patients with aggressive periodontitis and chronic periodontitis.

Figure 1 shows that the distribution of NF-κB (p50) protein expression between patients with aggressive periodontitis and chronic periodontitis were very much different.

Immunohistochemical Examination of Periodontal Tissues

In figure 2, NF-κB (p50) protein expression of periodontal tissues samples in aggressive and chronic periodontitis were analyzed by immunohistochemistry method using peroxidase labeled at 400x magnification, suggesting that NF-κB (p50) is expressed and the picture it appears as having brown color (arrow).

Protein expression of NF-κB (p65) patients with aggressive periodontitis and chronic periodontitis patients can be seen in Table 2.

To see the differences in NF-κB (p65) protein expression in patients with aggressive periodontitis and chronic periodontitis patients, we obtained values as seen in Table 2. Median NF-κB (p65) protein expression in patients with aggressive periodontitis of 8.0 whereas in patients with chronic periodontitis it was 22.0. After distribution test using Kolmogorov-Smirnov on the data, statistical test value revealed 1.406 with significance 0.038, which means that the data were not normally distributed. To see the difference we used Wilcoxon Mann Whitney, revealing Z values of -7137 and significance of 0000. If α=5%, it can be concluded there are differences in the protein expression of NF-κB (p65) between patients with aggressive periodontitis and chronic periodontitis.

By using simple logistic regression analysis obtained value sign = 0.000, which means that NF-κB (p65)

Table 2. NF-κB protein expression descriptive value (p65) in patients with aggressive periodontitis (PA) and chronic periodontitis (PK)

Periodontitis	N	Mean Rank	Median	Wilcoxon Mann Whitney
PA	40	22.00	8	Z = - 7,137
PK	40	59	21	p = 0,000

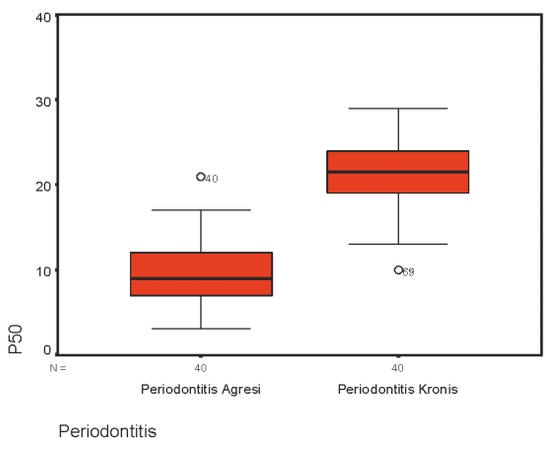

Figure 1. Box plots of NF-κB (p50) protein expression in aggressive and chronic periodontitis

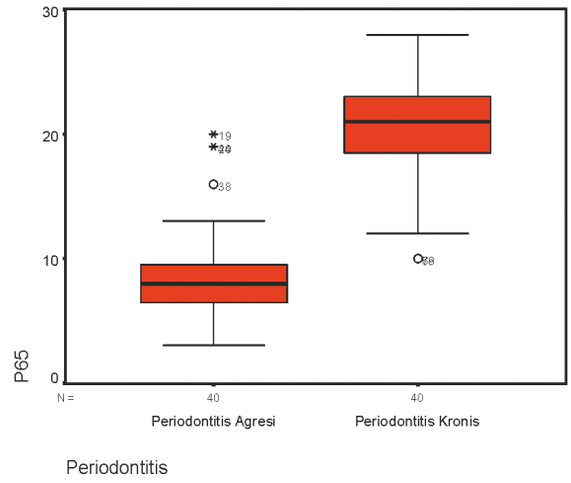

Figure 3. Box plots of NF-κB (p65) protein expression in aggressive and chronic periodontitis patients

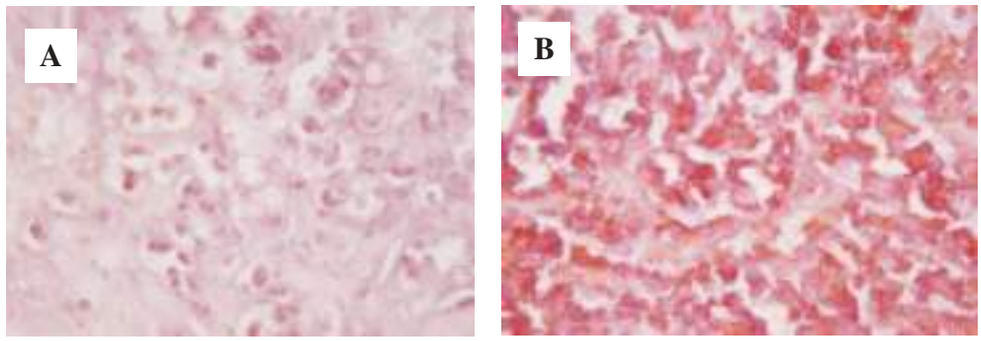

Figure 2. NF-κB (p50) protein expression **(arrow)** of periodontal tissue with DAB peroxidase immunohistochemical staining, magnification 400x. (A) aggressive periodontitis, (B) chronic periodontitis

protein expression influences the occurrence of aggressive periodontitis and chronic periodontitis. OR estimated values of NF-κB (p65) protein expression was 0.659 (p = 0.000). If NF-κB (p65) protein expression of the respondents increases by one unit then the risk for aggressive periodontitis increases 0.66 times or the risk of chronic periodontitis to increased 1.5 times higher than that of aggressive periodontitis. Differences in protein expression of NF-κB (p65) described using Box plot diagram shows clearly data distribution on the difference of NF-κB (p65) protein expression in patients with aggressive and chronic periodontitis.

Figure 3 shows p65 protein expression distribution between patients with aggressive and chronic periodontitis is very much different.

Immunohistochemical Examination of Periodontal Tissues

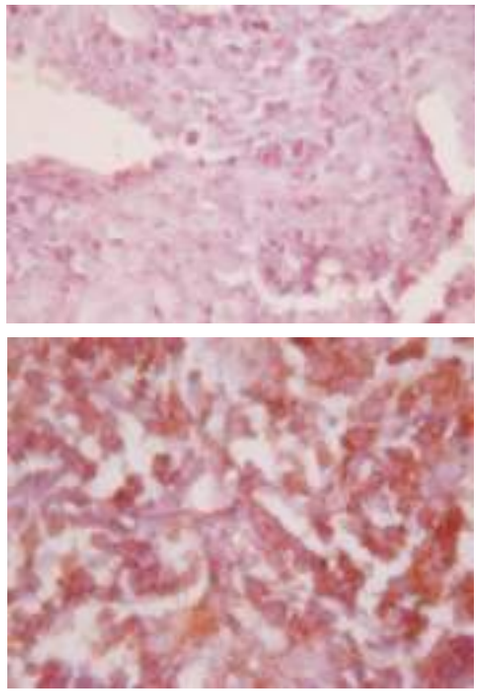

Figure 4. NF-κB (p65) protein expression (arrows) of periodontal tissue with DAB peroxidase immunohistochemical staining, magnification 400x. (A) aggressive periodontitis, (B) Chronic Periodontitis

OR estimates for NF-κB (p65) protein expression was 0.738 (p = 0.03). This indicates that if NF-κB (p65) protein expression of the respondents increases by 1 (one) unit, then the risk of aggressive periodontitis would be 0.738 times or if NF-κB (p65) protein expression of the respondents increases by 1 (one) unit, then the risk of chronic periodontitis will increases to 1,355 times higher.

DISCUSSION

Nuclear Factor-κB (NF-kB) is a transcription factor that bridges the innate immune system with the adaptive immune system, and is essential for the detection of activation of innate immune response because their activation is associated with the TLR2/TLR4 receptors in the innate immune system (Abbas and Lichtman, 2007). NF-κB activity is very dependent on cytoplasm movement to the nucleus and the cell's response to stimuli. NF-κB is a homodimer or heterodimer type, consisting of RelA (p65), c-Rel, RelB, NF-κB1 (p50/p105), NF-κB2 (p52/p100). The family p65, c-Rel and RelB contain C -terminal transcriptional activation domains (TAD) which plays an important role in the stimulation of the target gene expression, whereas p50 and p52 are formed as large proteins but not have the TAD in which p50 and p52 can act as a factor transcription as homodimers.

The research was conducted on a sample of patients with aggressive and chronic periodontitis due to various periodontal pathogens in the oral cavity, so that tolerant endotoxin may occur for the presence of clinical manifestations. In periodontitis bacteria that play a role are the *P. gingivalis* and *A. actinomycetemcomitan* in which LPS activates germ cells using TLR2 pathway and/or TLR4. The interaction between LPS/TLR will activate NF-κB which plays a role in activating inflammatory mediators transcription (Ohnishi et al., 2007; Takahashi et al., 2008). Secretion of cytokines TNF-α, IL-1β and IL-6 will be inhibited only by TLR4 in response to LPS, not by TLR2. Competitive nature between *A. actinomycetemcomitan* and *P. gingivalis* has a role in periodontal tissue damage. When *A. actinomycetemcomitan* is more dominant, there will be aggressive periodontitis. Whereas, when the dominant is *P. gingivalis*, the clinical situation will be chronic periodontitis.

Homodimer complex of NF-κB (p50) and NF-κB (p65) proteins will suppress the expression force of TLR responses, thereby inhibiting the production of proinflammatory cytokines (Sun et al., 2008; Jotwani, et al., 2010). The formation of a heterodimer complex of NF-κB (p50) and NF-κB (p65) is the most common formation in mammalian cells. NF-κB proteins present in human cells cytoplasm and in inactive state and bound to a protein known as the NF-κB. NF-κB signal plays an important role in several aspects of the activity of osteoclasts, osteoblasts and chondroblast (Boyce et al 2010). NF-κB transcription factor plays a role in the expression of various genes that play a role in the regulation of immune response and inflammation, proliferation, tumorigenesis and cell survival. NF-κB acts to regulate genes such as TNF-α and IL-1β and directly enhances the inflammatory response. Activation of NF-κB by the B cell receptor and T cells are also required to stimulate antigen proliferation, cytokine production and survival of B and T cells (Jimi et al., 2007).

Improved cascade pathway of NF-κB kinase results in an increase of NF-κB (p105/p50) transcription factor. This situation is also found in the research by Kaisho and Akira (2006) and Abbas et al (2007). Immunohistochemical examination on NF-κB (p50/p65) in patients with periodontitis appears to increase as compared to that in

healthy samples. NF-κB signaling inhibition can be done through IL-4 inhibition mechanism by osteoclastogenesis process. Most of the existing genes are removed or depreciated due to the presence of inactive IKK/ NF-κB. The involvement of NF-κB in patients with arthritis is apparent in differentiation and osteoclasts activity disorders, where NF-κB as factor that has a major influence on the biological response. (Beinke and Ley, 2004).

The activation of different TLR will also result in the activation of NF-κB and cytokine products through TLR2 and TLR4 in neutralizing antibodies in that it seems that NF-κB (p50) and NF-κB (p65) protein expression is increasing in chronic periodontitis. The results of this study support the research by Jotwani et al., (2010) which states that NF-κB (p50) and NF-κB (p65) were significantly higher in patients with chronic periodontitis.

NF-κB activity is found in NF-κB (p50/p65) heterodimer present in the cytoplasm in an inactive state and binds to NF-κB. NF-κB protein degradation causes the release of p50/p65 heterodimer, and put the protein into the cell nucleus. NF-κB (p50) homodimer is a repressor for transcriptional process and as a mediator in endotoxin tolerans. Increased NF-κB (p50) homodimer in inflammatory cytokines with *P. gingivalis* LPS stimulation will suppress immune response stimulation. Such circumstances is in accordance with the results of this study, which found that NF-κB (p50) differed significantly between patients with aggressive periodontitis and chronic periodontitis. Patients with chronic periodontitis showed a higher value. This situation shows that *P. gingivalis* LPS, which is the cause of chronic periodontitis, activates NF-κB (p50).

NF-κB (p50) is stimulated through TLR2 pathway, so what happens is a clinical state of chronic periodontitis in which NF-κB (p50) serves as an important protein involved in the regulation of different transcription process, while NF-κB (p65) regulates the activation of NF-κB. NF-κB (p50) and NF-κB (p65) are respectively in the form of homodimer, while the frequently found common form is in the form of heterodimer to become NF-κB (p50/p65) (Jimi et al. 2007; Espinosa et al., 2008).

Statistical analysis showed that NF-κB (p50/p65) has effect on the occurrence of chronic periodontitis. This is consistent with the logistic regression analysis on NF-κB (p50) and NF-κB (p65) in this study, in which the risk of towards chronic periodontitis was 1.6 times and 1.5 times. According to research conducted by Jotwani et al, (2010), NF-κB (p50/p65) increased after stimulation with *P. gingivalis* LPS compared with stimulation with E coli. *A. actinomycetemcomitan* has the same potential with E Coli. E. coli LPS stimulates inflammation through TLR4, whereas *P. gingivalis* LPS via TLR2 and/or TLR4 to activate the cell. The secretion of inflammatory cytokines TNF-α, IL-1β and IL-6 would be inhibited by TLR4 antibody in response to *P. gingivalis* stimulation. Based on the classification, *P. gingivalis* is a periodontal pathogenic bacteria, which are weak in stimulating the activity of the immune response. NF-κB (p50) has immunosuppressive ability, so that when there is a change, it would affect the NF-κB (p50) homodimer complex that is expressed in tissues from patients with chronic periodontitis. Activation of dendritic cells by *P. gingivalis* LPS will increase p50/p65 ratio in patients with chronic periodontitis. This study showed that increased p50/p65 occured in chronic periodontitis respondents. In samples of periodontitis patients, it was known that periodontal pathogenic bacteria are varied, so it can be concluded that patients with chronic periodontitis were affected by *P. gingivalis* so that NF-κB (p50/p65) was activated through TLR4. Good response to treatment was detected in NF-κB activated through (p65) protein phosphorylation (Espinosa et al., 2008).

CONCLUSION

Based on this study, it appears that patients with chronic periodontitis show increased NF-κB (p50/p65) protein expression compared with protein expression in patients with aggressive periodontitis. Through NF-κB pathway, chronic periodontitis is more likely caused by *P. gingivalis* bacteria.

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