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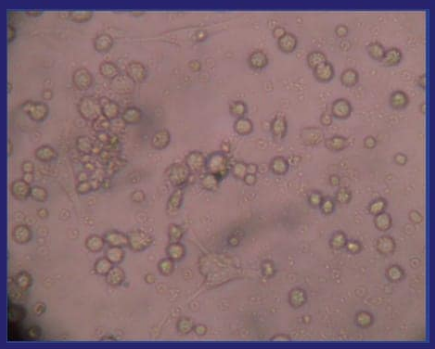

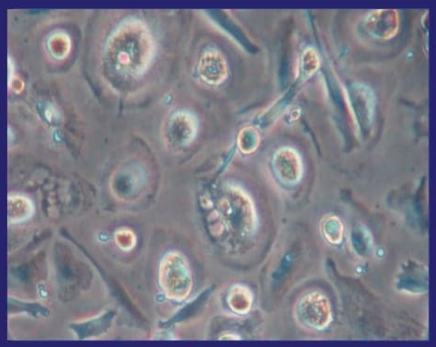

Evaluation of Salmonella sp Contamination and its Antibiotics Resistance Patterns Isolated from Broiler Meat Sold at Wet Market in Center of Surabaya

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Research Report

EVALUATION OF *Salmonella* sp CONTAMINATION AND ITS ANTIBIOTICS RESISTANCE PATTERNS ISOLATED FROM BROILER MEAT SOLD AT WET MARKET IN CENTER OF SURABAYA

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ABSTRACT

Antibiotic resistance now days become a main issue to the medical researches as found many positive result of antibiotic resistance test. One of the causes of antibiotic resistance is using antibiotic as a feed additive to animal. Bacteria that are resistant to antibiotics can be a danger to humans, in this case the resistant bacteria as a result of treatment errors animals, especially chickens that uses low-dose antibiotics as growth promoters. This study aimed to determine the contamination of Salmonella sp and its antibiotics resistance patterns of Salmonella sp isolated from broiler meat sold at wet market in the Center of Surabaya: (a) Pasar Kembang, (b) Pasar Kupang, (c) Pasar Dukuh Kupang, (d) Pasar Kedungsari, (e) Pasar Kedungdoro and (f) Pasar Keputran. The method that used in this study was bacteriological isolation and identification method. The method started with pre-enrichment using Buffered Pepton Water, selective enrichment using Tetrathionate Broth and Selenite Cysteine broth, selective media using Salmonella-Shigella Agar, Biochemical test using Triple Sugar Iron Agar, Simon Citrate, Methyl Red – Voges Proskauer, and Sulfide Indol Motility, and followed with susceptibility test according to Kirby-Bauer method using Mueller-Hinton Agar. The antibiotics that used in susceptibility test were: (a) Meropenem, (b) Ampicillin Sulbactam, (c) Amikacin, (d) Ofloxacin and (e) Nalidixic Acid. The results of this study were found 90% or 27 of 30 samples positive contaminated with Salmonella sp. The results of antibiotics resistance from 27 isolates 0% were resistant to Meropenem, 0% were resistant to Amikacin; 3.7% were resistant to Ampicillin-Sulbactam; 11.1% were resistant to Ofloxacin and 44.4% were resistant to Nalidixic Acid.

Key words: *Salmonella sp, wet market, broiler meat, antibiotic resistance, Center of Surabaya*

ABSTRAK

Resistansi antibiotik sekarang menjadi isu utama pada penelitian medis seiring ditemukannya banyak hasil positif pada uji resistansi antibiotik. Satu dari penyebab resistansi antibiotik adalah penggunaan antibiotik sebagai makanan aditif pada hewan. Bakteri yang resistan terhadap antibiotik dapat membahayakan manusia, pada kasus ini resistansi bacteria merupakan hasil dari kesalahan perlakuan pada hewan, terutama pada ayam yang menggunakan antibiotik dosis rendah sebagai pemicu pertumbuhan. Penelitian ini membahas mengenai cemaran dan resistensi terhadap antibiotika dari bakteri Salmonella sp yang diisolasi dari daging ayam broiler di Pasar Tradisional Surabaya Pusat (Pasar Kembang, Pasar Kupang, Pasar Dukuh Kupang, Pasar Kedungsari, Pasar Kedungdoro dan Pasar Keputran). Penelitian ini menggunakan metode isolasi dan identifikasi bakteri yang dilanjutkan dengan uji sensitivitas antibiotika menggunakan metode difusi dari Kirby Bauer. Antibiotika uji yang digunakan pada uji sensitivitas adalah: (a) Meropenem, (b) Ampicillin Sulbactam, (c) Amikacin, (d) Ofloxacin dan (e) Nalidixic Acid. Hasil dari penelitian ini adalah ditemukan

27 dari 30 sampel positif terkontaminasi bakteri Salmonella sp. Hasil uji sensitivitas terhadap antibiotika, 0% resisten terhadap antibiotik meropenem dan amikacin; 3,7% resisten terhadap antibiotika ampicillin sulbactam; 11,1% resisten terhadap ofloxacin dan 44,4% resisten terhadap nalidixic acid.

Kata kunci: *Salmonella sp, pasar basah, daging ayam broiler, resistensi antibiotika, Surabaya Pusat*

INTRODUCTION

The poultry product consumption especially broiler meat is predicted will climb up as increaseas the number of Indonesian population, lifestyle changes and the high awareness of the importance of protein consumed. On 2008, broiler meat consumption got up to 3,8 kg/capita/year. The total of broiler meat consumption reached at 84.07% from total consumption of the other livestock.[1] Broiler meat is a product that easy contaminates with pathogenic or non-pathogenic microorganism.[2] One of the microorganisms that often contaminate broiler meat is *Salmonella* sp, a bacteria caused Salmonellosis and recorded as the main cause of food borne disease.[3] There are 21.6 million cases of Salmonellosis in the world with 216.000 victim dies, and more than 90% happened in Asia.[4] Directorate General of Medical Services, Indonesian Department of Health in 2008 reported typhoid fever was on second rank of the top ten main diseases of inpatients in Indonesia's hospitals with 81.116 cases (proportion 3,15%), the first rank was occupied by diarrhea with the amount of 193.856 cases (proportion 7,52%).[5]

As the high level of demand for broiler meat, many farmers choose a shortcut way to increase the chicken's perform with giving feed additive, such as antibiotic to fast the growth of the chicken. Monitoring and surveillance in 2004 at Padang and Palembang reported that there were chicken, meat, and egg contained antibiotic residues. In Padang, from 98 specimens were found 3% contained tetracycline residues and 2% contained aminoglycoside residues. In Pekanbaru, from 22 specimens were found 4,8% contained penicillin residues.[6] 317 *Salmonella* sp isolated from Immanuel Hospital in Bandung were testedthe resistance of antibiotic and found that resistant to trimetoprim-sulfametisazol (7,89%), trimetoprim (6,95%), ciprofloxacin (4,11%), chloramphenicol (0,95%), and amoxicillin (0,62%).[7]

Seeing the potential incidence of salmonellosis and broiler meat as the media vulnerable to contamination by bacteria and the phenomenon of antibiotics as a feed additive for maintenance broilers, the researchers wanted to know the existence of contamination of *Salmonella* sp in broiler chicken meat sold in wet markets in the center of Surabaya and its antibiotic resistance against Salmonella sp.

MATERIAL AND METHODS

A total of 30 specimens (*musc. Pectoralis*) were collected randomly from 7 wet markets at Center of Surabaya between November–December, 2014. The list of wet market presented in Table 1.

Bacterial test including isolation, identification and susceptibility test were done at Gastroenteritis and Salmonellosis Laboratory, Institute of Tropical Disease, Airlangga University.

The bacteriological test started with pre-enrichment, 25 gram specimen put into an Erlenmeyer with 225 ml Buffered Peptone Water sterile (OXOID®) and incubates for 24 hours at 37°C.[8] The next day, inoculate 1ml isolate from pre-enrichment media to selective enrichment media using 10 ml Tetrathionate Broth (BD) and 10 ml Selenite Cystine Broth (BD), incubate for 24 hours at 37°C.[8]

The culture from each selective enrichment media were inoculated on selective media: Salmonella Shigella Agar (OXOID®) sterile with streaking using sterile loop on the surface of the plate, incubate all media for 24 hours at 37°C.[8] Biochemical tests were started with colony selection. Colonies that showed suspect of *Salmonella* sp were the colonies with black spot. Take five colonies and inoculate each to biochemical media: Triple Sugar Iron Agar (OXOID®), Simons Citrate Agar (OXOID®), Sulfide Indol Motility (BD), and Methyl-Red Voges-Proskauer (OXOID®), incubate for 24-48 hours at 37°C, then confirmation the positive *Salmonella* sp isolates. Purify the positive *Salmonella* sp using Nutrient Agar (Merck®).

Table 1. List of Wet Market in Center of Surabaya and Total Specimens

No	Wet Market	Spesimens
1	Pasar Kembang	5 specimens
2	Pasar Keputran	5 specimens
3	Pasar Dukuh Kupang	4 specimens
4	Pasar Kupang	4 specimens
5	Pasar Pandegiling	4 specimens
6	Pasar Kedungsari	4 specimens
7	Pasar Kedungdoro	4 specimens
	Total	30 specimens

Each of purified positive *Salmonella* sp from Nutrient Agar were sub-cultured to PZ sterile and the turbidity of the isolates equivalent to 0.5 McFarland. Susceptibility of isolates to selected antibiotics was carried out using the Kirby Bauer's disk diffusion method on Mueller-Hinton Agar (BD).[9] Susceptibility to the following antibiotics was determined: Ampicillin-Sulbactam 10 µg (OXOID®), Amikacin 30 µg (OXOID®), Meropenem 10 µg (OXOID®), Ofloxacin 1 µg (OXOID®), Nalidixic Acid 30 µg (OXOID®).

RESULT AND DISCUSSION

Any colonies that grow on Salmonella Shigella order taken five colonies were selected for the best. Then conducted to biochemical tests on Triple Sugar Iron Agar, Simon Citrate, Indol Motility Sulfide and Methyl Red-Voges Proskauer. Out of a total of 30 specimens examined, 27 (90%) were positive for *Salmonella* sp (Table 2).

Contamination could happen when processing on Poultry Slaughter House until the meats were consumed. The contaminants are soil contamination, dirt, water, processing equipment, air, human.[10]

Samples with positive results and then tested again to see the level of sensitivity to antibiotics. This sensitivity test using Kirby-Bauer method and the antibiotics that used are a class of β-Lactam antibiotics (ampicillin sulbactam), a sub-class of carbapenems (meropenem), aminoglycosides (amikacin), fluoroquinolones (ofloxacin), and quinolones (nalidixic acid).

High percentages of theisolates were susceptible to Meropenem and Amikacin (100%), Ampicillin Sulbactamand Ofloxacin (88.9%). However, 44,4% isolates were resistant to Nalidixic Acid.

Mechanism of antibiotic resistance could be transferred via plasmid (R factor), a genetic mutation of bacteria that could change the location of binding sites of antibiotics, bacterial metabolic change so that is not affected by antibiotics, or the change of bacteria cell membrane permeability and its difficult to be penetrated by antibiotics.[11,12]

Table 2. *Salmonella* sp Contamination on Broiler Meat at Wet Market in Center of Surabaya

No	Wet Market	Number of Specimen	Positive Salmonella sp	
			Total	Proportion
1	Ps. Kembang	5	3	60%
2	Ps. Kupang	4	3	75%
3	Ps. Dukuh Kupang	4	4	100%
4	Ps.Pandegiling	4	4	100%
5	Ps. Kedungsari	4	4	100%
6	Ps. Kedungdoro	4	4	100%
7	Ps. Keputran	5	5	100%
Total		30	27	90%

Table 3. Antibiotic Susceptibility pattern of *Salmonella* isolated from Broiler Meat at Wet Market in Center of Surabaya

No	Antibiotics	S[a]	%	I[b]	%	R[c]	%
1	Ampicillin Sulbactam	24	88,9%	2	7,4%	1	3,7%
2	Meropenem	27	100%	0	0%	0	0%
3	Amikacin	27	100%	0	0%	0	0%
4	Ofloxacin	24	88,9%	0	0%	3	11,1%
5	Nalidixic Acid	13	48,2%	2	7,4%	12	44,4%

[a] Sensitive, [b] Intermediate, [c] Resistant

Meropenem has a good result, 27 samples was susceptible to *Salmonella* sp. Meropenem is antibiotic that could be the final choice for treating the Gram-negative bacteria infection. From Center of Disease Control and Prevention (CDC) report on Antibiotics Resistance Threat in the United States 2013, Antibiotic resistance of carbapenems sub-category could be found on Gram-negative bacteria, included Pseudomonas and *Acinetobacter* spp. After the bacteria became resistant to carbapenems, the bacteria normally resistant to all β-laktam antibiotics. Amikacin resistance occured due to the expression of the gene encoding β-lactamase. This gene encodes the enzyme β-lactamase that inactivates β-lactam ring of Amikacin, therefore becoming resistant to Amikacin.[13]

Amikacin is a good antibiotic for *Salmonella* sp, 100% samples were positive sensitive to this antibiotic. Amikacin is one of semi synthetic aminoglycoside antibiotic that is highly resistant to enzymes modification. Resistance may occur because of three things, The decline retrieval; the absence of oxygen-dependent transport system for aminoglycosides, Lack of receptor; 30s ribosomal sub-unit has a low affinity for aminoglycosides, Modification of the enzyme; plasmids that carry R.factor which encodes an enzyme formation (example: acetyl transferase, nucleotidyl transferase and phosphotransferase) change and inactivation of aminoglycosides antibiotics.[14]

Antibiotic ampicillin-sulbactam has only one positive isolates resistant. Two samples including intermediates to antibiotics and the rest of 88.9% is still sensitive samples. The occurrence of resistance to ampicillin-sulbactam due to the expression of the gene, i.e. the gene encoding β-lactamase is located on Gram-negative bacteria chromosome. This gene encodes the enzyme β-lactamase that inactivates β-lactam ring of ampicillin by means of hydrolyzing β-lactam ring, thereby becoming resistant to ampicillin.[15]

Antibiotic sensitivity of ofloxacin is 88.9% of isolates of *Salmonella* sp still sensitive to these antibiotics. Three isolates were resistant or 11.1%, so it can be said that

Salmonella sp begin resistant to ofloxacin. Ofloxacin is an antibiotic that belongs to the class of fluoroquinolones. The mechanism of resistance to fluoroquinolones is this antibiotic bound to the β subunit of the bacterial enzyme DNA gyrase and block the activity of enzymes that are essential in maintaining DNA supercoiling and important in the process of DNA replication. Mutations in encoding gene of the DNA gyrase could produce active enzyme but could not be bound by fluoroquinolones.[14]

Bacteria *Salmonella* sp has the highest resistance to the antibiotic level Nalidixic acid as many as 12 samples or 44.4% resistant and two samples or 7.4% intermediates. Nalidixic acid is active against Gram-negative bacteria coliform. These antibiotics work by inhibiting the enzyme activity of bacterial DNA gyrase that disrupts DNA supercoiling.[16] Nalidixic acid resistance to antibiotics is not transferred via plasmids (R factor), but by other mechanisms. The mechanism is a genetic mutation of bacteria that can change the location of the protein and binding sites of antibiotics, bacterial metabolic change so it is not affected by antibiotics, or bacteria alter the permeability of the cell membrane so difficult to be penetrated by antibiotics. This resistance has led to clinical problems, bacteria normally resistant to Nalidixic acid is Pseudomonas spp.[11, 12]

CONCLUSION

From the result of this study, Broiler meat sold in wet markets Surabaya center 90% positive contaminated with *Salmonella* sp (27 of 30 samples). *Salmonella* sp isolated from Broiler meat sold in wet markets Surabaya center were 0% resistant to Meropenem and Amikacin, Ampicillin Sulbactam (3.7%), Ofloxacin (11.1%), and Nalidixic Acid (44.4%).

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Research Report

DIFFERENCES OF UNIVERSAL AND MULTIPLEX PRIMER FOR DETECTION OF DENGUE VIRUS FROM PATIENTS SUSPECTED DENGUE HEMORRHAGIC FEVER (DHF) IN SURABAYA

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ABSTRACT

Dengue Hemorrhagic Fever (DHF) is a global health problem in tropical and subtropical regions, as well as endemic in110 countries around the world. Indonesia is one of the largest countries in the region of endemic dengue. In Indonesia, dengue virus infection has become a contagious disease that was very important and was reported in 1968. Many molecular epidemiological approaches have been developed to look for factor that has been assumed as the cause of the increase of prevalence dengue virus infection in the world. The aim of this study is for the detection and determination of serotype of dengue virus in Surabaya. The method used was the technique of Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and Polymerase Chain Reaction (PCR) with specific primers for dengue virus. Samples suspected DHF patients were obtained from various health center and hospital in Surabaya. Results of this research detected negative result for dengue virus in all samples of patients suspected DHF. Negative results caused by dengue virus titers in serum samples of patients who had been dropped due to long storage time and taken after the third day of fever in early period.

Key words: *Dengue Hemorrhagic Fever, PCR, RT-PCR, Dengue Virus*

ABSTRAK

Demam Berdarah Dengue (DBD) merupakan permasalahan kesehatan global di daerah tropis dan subtropis, serta penyakit endemik di 110 negara di dunia. Indonesia adalah salah satu negara terbesar di wilayah endemik DBD. Di Indonesia, infeksi virus dengue telah menjadi penyakit menular yang sangat penting dan dilaporkan pada tahun 1968. Banyak pendekatan epidemiologi molekuler telah dikembangkan untuk mencari faktor-faktor yang telah dianggap sebagai penyebab meningkatnya infeksi virus dengue prevalensi di dunia. Penelitian ini bertujuan untuk deteksi dan penentuan serotipe virus dengue di Kota Surabaya. Metode yang digunakan adalah teknik Reverse Transcription - Polymerase Chain Reaction (RT-PCR) dan Polymerase Chain Reaction (PCR) dengan primer spesifik untuk virus dengue. Sampel pasien diduga DHF didapatkan dari berbagai puskesmas dan rumah sakit di Kota Surabaya. Hasil penelitian terdeteksi negatif virus dengue pada semua sampel pasien yang diduga DHF. Hasil negatif yang disebabkan oleh titer virus dengue dalam sampel serum dari pasien yang telah turun karena waktu penyimpanan yang lama dan diambil setelah hari ketiga demam pada periode awal.

Kata kunci: *Dengue Hemorrhagic Fever, PCR, RT-PCR, Surabaya, Virus Dengue*

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is a global health problem in tropical and subtropical regions, also endemic diseases in 110 countries around the world.[1, 2, 3, 4] Indonesia is one of the largest country in dengue endemic regions, with a population reaching 251 million people.[5] DHF is caused by the dengue virus and transmitted through

mosquito vector *Aedes aegypti* and *Aedes albopictus*.[6] In 2010, Indonesia became the first ranked country in ASEAN by the highest number of cases in DHF and hyperendemic predicate.[7]

The dengue virus is the Flaviviridae family members and genus flavivirus. Consists of four dengue virus serotypes (DENV-1, DENV-2, DENV-3 and DENV-4).[8,9,10] Dengue virus serotype DENV-1 was first discovered in Hawaii in 1944 and DENV-2 in Papua New Guinea in the same year. Dengue virus serotype DENV-3 and DENV-4 was found at Philippines in 1956.[11] Dengue virus is transmitted to humans by the mosquito *Aedes aegypti* and *Aedes albopticus*.[12] In Indonesia, dengue virus infection has become a contagious disease that was very important and was reported in 1968.[13,14] Many molecular epidemiological approach have been developed to look for factors that has been assumed as the cause of the increase of prevalence dengue virus infection in the world. That ranges of strains in serotypes classified in different groups can genetically sequencing revealed the dengue virus. Differences of nucleotide caused biological diversity in nature and their antigenicity.[15,16]

The aim of this study is for detection and determination of serotype of dengue virus in Surabaya. Dengue virus serotypes that have been known can be compared with previous studies, so the movement of dengue virus serotypes could be discovered.

MATERIAL AND METHODS

Samples

Serum samplesof patients suspected of dengue put in eppendorf tubes and storedina refrigerator at a temperature -80ºC in Dengue Laboratory, Institute of Tropical Disease Airlangga Univesity.

Extraction of dengue virus Ribonucleic acid(RNA)

Ribonucleic acid or RNA of dengue virus extracted from serum samples with extraction device QIAamp Viral RNA Mini Kit (QIAGEN), following a step works: put 560 µL of buffer AVL and carrier RNA in a 1.5 mL eppendorf tube, put1 40 µL of serum samples and vortex for 15 seconds, incubation at room temperature for 10 minutes and then centrifuge, added 560 µL of ethanol 96-100% then vortex for 15seconds and centrifuged, put 630µL of solution to the QIAamp mini coloumn, centrifuged at 8000 rpm for 1 minute, the remaining solution was included to QIAamp mini coloumn, centrifuged at 8000 rpm for 1 minute, put 500 µL of buffer AW1, centrifuged at 8000 rpm for 1 minute, put 500 µL of buffer AW2, centrifuged at 8000 rpm for 1min, transferred to a 1.5 mL microtube, added 60 µL of buffer AVE, incubated at room temperature for 1 minute, centrifuged at 8000 rpm for 1 minute and showed RNA.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and Polymerase Chain Reaction (PCR) for the detection and determination of dengue virus serotype

Molecular examination using RT-PCR and PCR for detecting nucleic acid of dengue virus sample and also for testing dengue virus serotypes is known as serotyping. The function of RT-PCR is to transcript the RNA into cDNA, then thecDNAwas amplified byPCR. Primers used for serotyping is D1 (forward), TS1, TS2, TS3 and TS4 (reverse).

In the process of RT-PCR was performed with 3 stages: the first stage component of reagents consists of 1 µL of primer, 5 µL of RNA, 1 µL of dNTP, and 7 µL of water; the second stage components of reagents consists of 4µLof 5x FS buffer, 1 µL of DTT, 0.5 µL of water, 0.5 µLof RNAse out, and 0.5 µLof superscript; the third stage was added 0.5 µL RNAseH. The first stage of the RT-PCR reaction carried out at a temperature of 65 °C for 5 minutes, the second stage carried out at a temperature of 55 °C for 50 minutes and 85 °C for 5 minutes, and the third stageincubated at a temperature of 37 °C for 20 minutes. In the process of PCR, component of reagents consists of 5 µLof cDNA, 2 µL of 10x PCR buffer, 2 µLof dNTP, 2 µL of primer, 0.1 µL of rTaq, and 9 µL of water. PCR reactions were performed as many as 30 to 40 cycles of PCR, the temperature was 94 °C for 4 minutes (pre-denaturation), 94 °C for 1 minute (denaturation), 50 °C for 1 minute (annealing), 72 °C for 12 minutes (extension) and 4 ºC. Temperature 94 °C for 5 minutes in a PCR reaction aiming to denature double-stranded DNA, so DNA into single strands. PCR products were analyzed by electrophoresis on a 1.5% agarose gel and staining is done with ethidium bromide. Marker used is a 100 bp ladder. On the implementation of the detection

Table 1. Primer oligonucleotides used for amplification and determination dengue virus serotypes

Primer	Sequences
D1	5'-TCAATATGCTGAAACGCGCGAGAAACCG-3'
TS1	5'-CGTCTCAGTGATCCGGGGG-3'
TS2	5'-CGCCACAAGGGCCATGAACAG-3'
TS3	5'-TAACATCATCATGAGACAGAGC-3'
TS4	5'-CTCTGTTGTCTTAAACAAGAGA-3'

According Lanciotti *et al*. (1992) [17] and Harris *et al*. (1998) [9], dengue virus serotype determination was based on the size of DNA band formed after visualization on agarose gel electrophoresis, 482 bp for DENV-1, 119 bp for DENV-2, 290 bp for DENV-3, and 389 bp for DENV-4.

Table 2. Primer oligonucleotides used for detection of flavivirus group

Primer	Sequences
cFD2	5'-GTGTCCCAGCCGGCGGTGTCATCAGC-3'
MAMD	5'-AACATGATGGGRAARAGRGARAA-3'
FS778	5'-AARGGHAGYMCDGCHATHTGGT-3'

reaction and serotyping, always included a positive comparison (positive control dengue virus).

Additionally, in this studyuseda specific primer for flavi virus, which cFD2, MAMD, and FS778. The process of RT-PCR with specific primers for flavi virus was done in 3 stages: the first stage component of reagent sconsists of 1 µL of cFD2 primer, 5 µL of RNA, 1 µL of dNTP, and 7 µL ofwater; the second stage components of reagent sconsists of 4 µL of 5 xFS buffer, 1 µL of DTT, 0.5 µL of water, 0.5 µL of RNAse out, and 0.5 µL of superscript; the third stage was added 0.5 µL of RNAseH. The first stage of the RT-PCR reaction carried out at a temperature of 65°C for 5 minutes, the second stage carried out ata temperature of 55°C for 50 minutes and 85°C for 5 minutes, and the third stage incubated at a temperature of 37°C for 20 minutes.

In the process of PCR, component of reagents consists of 5 µL of cDNA, 2 µL of 10x PCR buffer, 2 µL of dNTP, 2 µL of primer (1 µL of cFD2 and 1 µLof MAMD), 0.1 µL rTaq, and 9 µL of water. PCR reactions were performed by 25 cycles of PCR, the temperature was 94°C for 4 minutes (pre-denaturation), 94 °C for 1 minute (denaturation), 54°C for 1 minute (annealing), 72°C for 1 minute (extension), and 72ºC for 10 minutes (extension). Next process was heminested-PCR, component of reagents consists of 5 µL of DNA,2 µL PCR buffer 10x, 2 µL of dNTP, 2 µL primer (1 µL of cFD2 and 1 µL of FS778), 0.1 µLof rTaq, and 9 µL of water.

Heminested-PCR was performed by 25 cycles of PCR, 94°C for 2 minutes (pre-denaturation), 94°C for 30 seconds (denaturation), 55°C for 30 seconds (annealing), 60°C for 4 minutes (extension), and 60°C for 10 minutes (extension). Heminested-PCR products were analyzed by electrophoresis method on a 1.5% agarose gel and staining is done with ethidium bromide. Marker used is a 100 bp ladder.

RESULT AND DISCUSSION

Reverse transcription-polymerase chain reaction or RT-PCR and polymerase chain reaction or PCR for detection of dengue virus performed on serum samples of patients suspected of DHF were taken from Medokan Ayu Health Center, Manukan Kulon Health Center, Pacar Keling Health Center, Tenggilis Health Center, Krembangan Selatan Health Center,and Soerya Hospital Child and Maternity. This method, obtained negative results for all

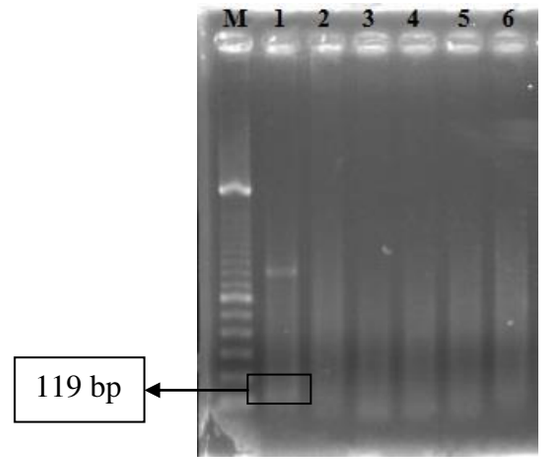

Figure 1. Results of electrophoresis of PCR samples obtained from Soerya Hospital Child and Maternity with primer D1, TS1, TS2, TS3, TS4. M: Marker; 1: Positive Control DENV-2; 2-6: Samples.

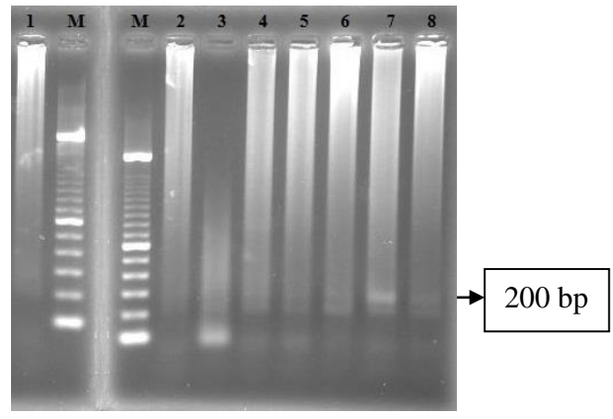

Figure 2. Results of electrophoresis of PCR samples with primer cFD2, MAMD, and FS778. M: Marker; 1: Medokan Ayu Health Center; 2: Manukan Health Center; 3: Pacar Keling Health Center; 4: Tenggilis Health Center; 5: Krembangan Selatan Health Center; 6: Manukan Health Center; 7: Positive Control; 8: Positive Control.

samples. Negative results caused by dengue virus titers in serum samples of patients who had been dropped due to long storage time and taken after the third day of feverin early period.

In Figure 1 was a serum samples obtained from Soerya Hospital Child and Maternity. The results obtained through out the sample is negative, there is no dengue virus DNA bands appearing. However, the positive control dengue virus DNA bands appear. Positive control was used the dengue virus serotype DENV-2 (119 bp).

In Figure 2 was a serum sample obtained from Medokan Ayu Health Center, Manukan Kulon Health Center, Pacar Keling Health Center, Tenggilis Health Center, and Krembangan Selatan Health Center. The negative results of the samples based on the condition that there is no DNA

band of dengue virus appearing. Negative results caused by dengue virus titers in serum samples of patients who had been dropped due to long storage time and taken after the third day of fever in early period. However, the positive control of dengue virus DNA bands appear at about 200 bp position. Primers used were primer for flavi virus, which are cFD2, MAMD, and FS778. Primer of flavi virus used to detect a group of viruses belonging to the genus *Flavivirus*.

In addition, dengue virus consists of four serotypes (DENV-1, DENV-2, DENV-3 and DENV-4).[8,9,10] In Indonesia gained dominance serotype DENV-2, followed DENV-3 in 2003 to 2005.[18] In Indonesia, the four serotypes of dengue virus have been discovered but DENV-3 is often associated with severe dengue cases.[19,20] In Surabaya in 2005 was dominated serotype DENV-2, followed by DENV-3 and DENV-1. In Surabaya in 2008-2009 was also dominated by DENV-2.[21] According Aryati et. al. (2012),[22] in Surabaya dominated by DENV-1, followed by DENV-2, DENV-4, and DENV-3.

Detection of dengue virus serotypes is very important because secondary infection with a different serotype may impact more severe. Likewise, an infection caused by two serotypes or more in a single individual (double infection) can contribute to the severity of the infection. Then serotyping very important in the management of patients with dengue virus infection.[23,24]

Virus serotype can be demonstrated by molecular techniques such as PCRandRT-PCR. This is very important because it changes serotypes causing an indication of the threat of dengue fever in this population.[25] Unavailability of vaccines or antiviral drugs for the prevention of dengue virus infection is the cause of the development of research based surveillance system is needed in early warning (early warning) DBD. Such information can be used as apreventive measure and alert in preparation for an outbreak of dengue. Early warning is given each year prior to the extraordinary incident in dengue. Societies can play an active role in efforts to combat the vector which is an important factor for breaking the chain of transmission and prevention of dengue disease that reappeared in the future.

CONCLUSION

In this study, all samples of patients suspected of Dengue Hemorrhagic Fever (DHF) which is obtained from various health center and hospital in Surabaya detected negative and dengue virus serotype can't be known.

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Research Report

AN APPROPRIATE DIAGNOSIS OF DENGUE VIRUS INFECTION IN SOME CASES WHO HAD AND WERE BEING TREATED IN SOERYA HOSPITAL SEPANJANG – INDONESIA

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ABSTRACT

Since January 2014, Soerya Hospital has found many cases with positive result of NS_1 or IgM and IgG Dengue. The clinical manifestations mostly were high fever with headache, vomiting and also malaise convulsion and unconsciousness. Aim of the study is to find out an appropriate diagnosis of Dengue Virus Infection. Observasional study had been done since January–April 2014 with 50 cases of dengue Virus Infection. The diagnostic procedure was made based on the WHO 2011 criteria. Result Many cases had come with fever within couple days, some of them showed convulsions. Therefore, it should be made a differential diagnosis with other disease, such as acute tonsilopharingitis, etc. The patient also had to be tested with NS_1 if the patient come in the first, second and third day of fever and followed by IgM/IgG dengue on the fourth, fifth or sixth days of fever. The diagnosis of Dengue Virus Infection was made based on the WHO criteria 2011. This study showed that not all cases showed positive result of NS_1 or IgM/IgG dengue on the first or second test. For the negative result, we should not think that the case is not a case of Dengue Virus Infection, especially if it happens at Aedes aegypti breeding season, the patient should be observed and performed the test again to get a proper diagnosis for Dengue Virus Infection. Monitoring clinical manifestation should always be done, to predict the appropriate diagnosis of Dengue Virus Infection.

Key words: *Dengue Virus, Diagnosis Dengue Virus NS_1, IgM test, IgG test, WHO criteria*

ABSTRAK

Sejak Januari 2014, Rumah Sakit Soerya telah menemukan banyak kasus dengan hasil positif dari NS1 atau IgM dan IgG Dengue. Manifestasi klinis sebagian besar adalah demam tinggi dengan sakit kepala, muntah dan juga kejang, lemas. Tujuan penelitian untuk mengetahui diagnosis yang tepat Dengue Virus Infeksi. Materi dan metode penelitian Observasional telah dilakukan sejak Januari–April 2014 dengan 50 kasus Infeksi Virus dengue. Prosedur diagnostik dibuat berdasarkan kriteria WHO 2011. Hasil yang ditemukan diantaranya demam dalam beberapa hari, beberapa dari mereka menunjukkan kejang. Oleh karena itu, harus dibuat diagnosis diferensial dengan penyakit lain, seperti tonsilopharingitis akut, dll. Pasien juga harus diuji dengan NS1 jika pasien datang pertama, kedua dan ketiga hari demam dan diikuti oleh IgM/IgG dengue pada hari keempat, kelima atau keenam demam. Diagnosis Virus Dengue Infeksi dibuat berdasarkan kriteria WHO 2011. Studi ini menunjukkan bahwa tidak semua kasus menunjukkan hasil positif dari test NS1 atau IgM/IgG dengue pada pertama atau kedua menunjukkan hasil negatif, kita tidak harus berpikir bahwa kasus ini bukan kasus Dengue Virus Infeksi, terutama jika hal itu terjadi pada musim nyamuk Aedes aegypti, pasien harus diamati dan dilakukan tes lagi untuk mendapatkan diagnosa yang tepat untuk Dengue Infection. Kesimpulannya Pemantauan virus berdasarkan manifestasi klinis harus selalu dilakukan, untuk memprediksi diagnosis yang tepat Dengue Virus Infeksi.

Kata kunci: *Dengue Virus, Diagnosis Virus Dengue NS1, tes IgM, tes IgG, kriteria WHO*

INTRODUCTION

Dengue fever and severe dengue infection an important causes of morbidity in tropical and sub tropical region. Most half world population live in area at risk infection.[1,2] One step Dengue NS1 antigen test is a highly conserved glycoprotein that seems to be essential for virus viability, but has no established biological activity, This NS1 antigen is present, at high concentration in the sera of dengue virus infection patients during the early clinical phase of the disease so it could be used as a suitable marker of dengue virus infection.[3,4,5]

Since January 2014, Soerya Hospital has found many cases with positive result of NS_1 or IgM and IgG Dengue. The clinical manifestations mostly were high fever with headache, vomiting and also malaise convulsion and unconsciousness.

Pathogeneis of DHF and DSS is still controversial. Two theories, which are not mutual exlusive-were frequently invited to explain the pathogenetic changes; secondary infection or immune enchacement hypothesis, viral virulence theory. Both theory is supported by epidemiologic and laboratory evidence, are most probably valid.

Risk factor reported for DHF; virus strain, pre-existing anti-dengue antibody: previous infection, maternal antibodies in infant, host genetics, age, Higher risk in secondary infections, higher risk in locations with two or more serotypes circulating simultaneously at high levels (hyperendemic transmission).

Diagnosis dengue NSI Ag as Rapid test is an in vitro immunochromatographic, one step assay designed to detect Dengue virus NS1 antigen human serum, plasma or whole blood.[11,12,13] Diagnosis early acute dengue infection to detect NSI antigen. Dengue NSI Ag can be detected from day 1 after on set of fever.[14] Sensitivity-92.8%, Spesificity-98.4%. The Speciment: Serum, plasma an wholeblood (100 µl).[9,10] Test result: 15–20 minutes. The introduction of few device model: fully covered device.

Diagnosis Dengue IgG/IgM test is a solid phase in vitro immunochromatographic test for the qualitative and differential detection of IgG and IgM antibodies to dengue virus serotype DEN-1,2,3 and 4. Differential detection of IgG and IgM antibodies. Serum, plasma, and Whole blood. Test result: 3-lines (IgG, IgM, control). Highest accuracy in low titer specimen.[15]

Presumptive differentiation between primary and secondary dengue infection have good correlation with Haemagglutination-Inhibition (HI) test.[11]

MATERIAL AND METHODS

To make a diagnosis a cases, the doctor showed ask to family who brought the patient to the hospital for getting history why the patient suffer for illness. What is the reason?

Based on his answer or her history: the doctor in charge should make a plan the laboratory examination with can support the diagnosis. What kind laboratory should be done?

Laboratary examination was done based on clinical manifestation that had been found. For doing laboratory examination the doctor in charge should know the clinical manifestation of cases based on answer of the question. Therefore the doctor incharge had found a sign an symptoms of Dengue Virus Infection (DVI).

Observasional study had been done since January–April 2014. There were 50 cases of Dengue Virus Infection had been studied. The diagnostic procedure was made based on the WHO 2011 criteria.

Sample collection and diagnosis of Dengue. The patient came early had to be tested with NS_1 if the patient come in the first, second and third day of fever and followed by IgM/IgG dengue on the fourth, fifth or sixth days of fever. The diagnosis of Dengue Virus Infection was made based on the WHO 2011 criteria. The patient came late (4, 5, 6 dengue of fiver) should be test IgM IgG dengue.

RESULT AND DISCUSSION

10 cases who came early (1, 2, 3 dengue of fiver) showed positip NS1 test and the other 7 cases also came early but showed negatif NS1 test. See table 1.

And then 7 cases who had a negatif result NS1, were followed IgM IgG test on the fourth until sixth day fever.

Table 1. NS1 Test as Early Diagnosis in Suspected DVI who had come earlier in Soerya Hospital Sepanjang

Day of Fever	Ns1 Test examination		Total Cases
	+	–	
First day		1	1
Second day	5	3	8
Third day	5	3	8
Total Cases	10	7	17

Table 2. IgM/IgG/IgM & IgG rapid test Followed negatif test Ns1 that had been done in earlier cases Dengue Virus Infection

Day of Fever	Dengue test examination		Total Cases
	IgM (+)	IgM & IgG(+)	
Fourth Day	1	2	3
Fifth Day	3		3
Sixth Day	1		1
Total Cases	5	2	7

Table 3. IgM/IgG/IgM & IgG rapid test for Identification Dengue Virus Infection in who had came late to the Hospital

Day of Fever	Dengue test examination			Total
	IgM	IgG	IgM & IgG	
Fourth	8	2	6	16
Fifth	6		3	9
Sixth	2		2	4
Seven	3		1	4
Total Cases	9	2	12	33

The result there were 5 cases showed an IgM (+), 2 cases showed IgM IgG positip. See table 2.

There were 33 cases suspected Dengue Virus Infection came late: all of them had been identified IgM IgG Dengue test. The result 9 cases should IgM (+), 2 cases IgG (+), 12 cases IgM IgG Dengue (+). See table 3.

Buy doing faster test of IgM IgG an all suspected dengue cases with day of fever 4, 5, 6,7 could be identified as a true cases of dengue virus infection.

Dengue virus NS1 antigen was detected in 199 of 213 acute serum samples from patients with laboratory confirmation of acute dengue virus infection. The dengue NS1 antigen – capture ELISA Sensitivity of 93.4%, Specificity of 100%. The sensitivity was significant higher in acute primary dengue (97.3%) than in acute secondary dengue (70.0%). The positive predictive value the dengue NS1 antigen –capture ELISA 100%. Negative predictive value was 97.3%. Virus isolation gave on overall positive isolation rate 68.1% with a. Positive isolation rate 73.9 for acute primary dengue and 31.0% acute secondary dengue. Molecular detection of dengue RNA by RT-PCR gave on overall positive detection rate 66.7%. Detection rate of 65.2 for acute primary dengue and 75.9% for acute secondary dengue.[14]

We have found that NS1 serotype-specific immunoglobulin G (IgG) enzyme linked immunosorbent assay (ELISA) can be used differentiate primary and secondary dengue virus infection.[4,6] This is due to the fact that the NS1 specific IgG antibody cannot be detected before day 6 of illness for primary infection. So the NS1-specific IgG antibody measure in acute phase serum some of them as previous infection. Comparison of NS1 specific IgG ELISA with envelope-and membrane-specific capture IgM and IgG elisa in the differentiation of primary and secondary dengue virus infection showed correlation (95,90% agreement). Most important we have found that the serotype of the dengue virus from the majority of patients with primary infection could be correctly identifitied when convalescent-phase or postinfection sera were analyzed by NS1 serotype-specific IgG ELISA. These findings suggested the NS1 serotype-specific IgG ELISA could be reliably applied for serodiagnosis and seroepidemiological study of dengue virus infection.[7,8,15]

50 cases of suspected Dengue Virus Infection who had been admitted in Soerya Hospital Sepanjang Sidoarjo and had been collected since January 1–April 30, 2014 had been studied. They had come with clinical manifestation of fever, vomitting, convulsion, head ache and gastric pain. And than two groups of cases suspected Dengue Virus Infection had been made, as: 1. first) who had come on the first, second and third of fever and 2. second) who had come on the fourth, fifth and sixth of fever. NS1 test had been done in the first group cases of Dengue Virus Infection and the result showed on table 1.

There were five cases who had shown a clinical manifestation on the second days of fever and had been identified as a positive result of NS1 test. These event were also found on the following five cases that had a clinical manifestation on the third days of fever. The result showed that there were 10 cases who came early had shown as a positive result of NS 1 test but the others 7 cases who came early had shown as a negative result of NS 1 test. It mean that all cases of suspected Dengue Virus Infection who had early come in hospital had been test by NS 1, not always shown totally had a positive result but only 58,8% showed positive result. These negative result cases should be observed and followed on the next day for getting IgM, IgG and IgM & IgG test, the result had been showed on table 2. All of them were positive. This experience give an idea that: if we found some cases which have been identified as the true a suspected clinical manifestation of Dengue Virus Infection, we should try to follow the clinical manifestation and try to do the other test Dengue related with occurring antibody. For some cases who came late more than 3 days of fever we should test IgM & IgG dengue. The result were showed on table 3.

There were 19 cases positive only IgM, it mean that all cases has been suffered from primary infection of dengue virus. All of them showed a mild clinical manifestation and didn't show plasma leakage and shock. 2 cases showed a positive IgG and 12 cases showed a positive IgM & IgG; it mean that all cases had been suffered from secondary infection dengue; these cases showed severity of clinical manifestation of Dengue Hemorrhage Fever. It was due to enhancement Ag Ab reaction that promoting increasing plasma leakage and shock. In some cases this event occurred and showed a clinical manifestation of plasma leakage and promoting shock and need a special treatment.

CONCLUSION

Monitoring clinical manifestation should always be done, to predict the appropriate diagnosis of Dengue Virus Infection for making a good management of DF or DHF and DSS.

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Research Report

COMPARATIVE STUDY OF FILARIAL DETECTION BY MICROSCOPIC EXAMINATION AND SEROLOGICAL ASSAY UTILIZING BMR1 AND BMXSP RECOMBINANT ANTIGENS FOR EVALUATION OF FILARIASIS ELIMINATION PROGRAM AT KAMPUNG SAWAH AND PAMULANG, SOUTH TANGERANG DISTRICT, BANTEN, INDONESIA

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ABSTRACT

South Tangerang district is one of the endemic areas for filariasis; and based on an evaluation study in 2008-2009 which covered several subdistricts, the prevalence of microfilaria was between 1–2.4%. Nevertheless, the evaluation by serological assay has never been reported. A cross-sectional study was conducted to detect the microfilaremia and anti-filarial IgG4 antibody status in Kp Sawah and Pamulang subdistricts. Cluster sampling was performed in Kp Sawah by collecting finger-prick blood (FPB) and venous blood samples from inhabitants who lived with and nearby the four elephantiasis subjects in the area. The FPB were only collected in Pamulang area by consecutive sampling method. The detection method included microscopic evaluation of FPB and serological detection using recombinant antigens BmR1 and BmSXP by ELISA and lateral flow rapid tests. Symptomatic patients who had 2nd and 3rd degree of elephantiasis were clinically determined in 10% (4/40) subjects. Among those with elephantiasis, 2 were positive serologically but their microscopic results were all negative (40/40). Meanwhile, the microscopic result for 107 subjects from Pamulang were all negative. The results of the rapid tests showed that 15% (6/40) of the positive cases were detected by Brugia Rapid and 27.5% (11/40) by PanLF. Meanwhile, the ELISA showed that 20% (8/40) of the cases were positive with BmSXP, whereas only 2.5% or 1/40 sample was found to be positive with BmR1. Even though the sensitivity of the Rapid test was lower when compared to microscopic examination for these samples, the assay showed good specificity ranging from 72.5 to 97.5%. The optical density (OD) values of ELISA has ranged between 0.3–3.045.

Key words: *Microfilaremia, BmR1, BmSXP, Brugia rapid test, PanLF*

ABSTRAK

Kabupaten Tangerang Selatan merupakan salah satu wilayah endemik filariasis; dan berdasarkan studi evaluasi tahun 2008-2009 yang mencakup beberapa kecamatan dengan prevalensi antara 1–2.4%. Namun demikian, belum ada laporan tentang hasil evaluasi secara serologi. Studi potong lintang dilakukan untuk mendeteksi status mikrofilaremi dan keberadaan antibodi anti-filaria IgG4 di kecamatan Kp sawah dan Pamulang. Pengambilan sampel dilakukan secara Cluster sampling dengan sampel darah jari (SDJ) dan sampel darah vena dari penduduk yang tinggal di sekitar empat penderita elefantiasis di wilayah Kp Sawah. Sedangkan di wilayah Pamulang hanya dilakukan pengambilan darah jari dengan metode consecutive sampling. Metode deteksi dilakukan secara mikroskopis terhadap SDJ dan secara serologi dengan menggunakan rekombinan antigen BmR1 dan BmSXP dengan cara ELISA dan tes cepat Brugia Rapid. Penderita simptomatik yang terdeteksi elefantiasis berjumlah 10% (4/40) diketahui dengan status limfedema ekstremitas derajat 2 dan 3. Diantara penderita elefantiasis tersebut, 2 orang terdeteksi positif secara serologis, namun hasil mikroskopisnya negatif (40/40). Sementara itu, hasil mikroskopis dari 107 SDJ di wilayah Pamulang seluruhnya negatif. Hasil tes cepat menunjukkan 15% (6/40) positif terhadap Brugia Rapid dan 27.5% (11/40) positif terhadap PanLF. Hasil ELISA pada sampel penelitian ini menunjukkan

20% (8/40) positif terhadap BmSXP, namun hanya 2.5% (1/40) yang positif terhadap BmR1. Meskipun nilai sensitifitas tes cepat lebih rendah dibandingkan mikroskopis pada sampel penelitian ini, namun nilai spesifisitasnya tinggi yang berkisar antara 72.5 to 97.5%. Nilai optical density (OD) dari hasil ELISA berkisar antara 0.3–3.045.

Kata kunci: *Microfilaremia, BmR1, BmSXP, Brugia rapid test, PanLF*

INTRODUCTION

Lymphatic filariasis is targeted for the Global Elimination Program initiated by WHO and the program is expected to be successful by 2020. An epidemiological data maps out that until 2008, there are 316 regencies/ municipalities out of 471 regencies/municipalities in Indonesia which have been declared as the endemic areas of filariasis.[1] The South Tangerang regency is one of endemic area for filariasis with a prevalence of microfilaria ranges between 1–2.4% covering several subdistricts as mentioned by an evaluation in 2008.[2]

The Health Department of South Tangerang district in the same period found that the prevalence of filariasis in Ciputat subdistrict has reached 1.6% with 8 patients has clinically suffered from lymphedema or elephantiasis in 2002; while in other subdistricts including Pondok Aren, Setu and Pamulang, the prevalence are 1.8%, 1%, and 2.4%, respectively. An area is defined to be endemic for filariasis when the microfilaria rate has 1% of prevalence.[2] A previous study to evaluate microfilaremia and antigenemia status, which was conducted in Kp Sawah, Ciputat, South Tangerang district in 2012, showed that 5% subjects were positive for microfilaria and 27.5% subjects had positive results for IgG4 antifilarial antibody using rapid test.[2]

There are some factors that may affect the success of filariasis elimination program, i.e. accurate diagnosis and evaluation on the success of continued diagnostic work-up and treatment.[3] Mass Drug Administration for filariasis in South Tangerang district, has been performed annually and been evaluated microfilaremia by using finger-prick blood (FPB) since 2002. Nevertheless, an evaluation by serological assay to detect antigenemia in blood vein has never been reported.

The present cross-sectional study was conducted to identify the microfilaremia and antibody anti-filarial IgG4 status in Kp Sawah (Ciputat) and Pamulang areas. The method included microscopic evaluation for FPB and serological detection using recombinant antigens BmR1 and BmSXP1 for blood vein samples of inhabitants living in Kp Sawah area. Diagnostic tests were also performed to identify the sensitivity and specificity of both antigens in detecting the presence of antibody anti-filarial IgG4 in the blood.

MATERIAL AND METHODS

A cross-sectional study was designed to conduct filariasis evaluation by observational, questionairy, and laboratory methods. Diagnostic tests were performed to detect the presence of microfilaria by microscopic and IgG4 antibody antifilarial by rapid test and ELISA. Samples were collected using cluster sampling technique in Kp Sawah by obtaining samples from some inhabitants who lived nearby the 4 patients who had been diagnosed with elephantiasis in the area. finger-prick blood (FPB) was also collected in West Pamulang area by consecutive sampling; however, the local Health Department advised that the blood vein samples should not be collected at the time. Samples were collected at night (10 pm – 2 am.) as the microfilaria activity in peripheral blood reaches its peak in those hours.

Microscopic examinations were performed at the Parasitology Laboratory Faculty of Medicine and Health Sciences, Syarif Hidayatullah State Islamic University on 40 samples obtained from Kp Sawah and 107 samples obtained from West Pamulang. The FPB were prepared into thick-blood smear slide and subsequently stained using Giemsa staining (Merck©) before they were examined under microscope. The volume of blood for microscopic was 1-2 drop(s) of peripheral blood. The calculation of microfilaria found in FPB was performed using the following formulation:

$$\text{Mf density (mfd)} = \frac{\text{Total number of microfilariae found in the sample}}{\text{Total number of slides with positive Mf}} \times 50^{*}$$

* 50 is the correctional factor for blood volume of 20 µl; while for different blood volume, the correctional factor is also different.[4]

Subsequently, the vein blood was examined using recombinant antigens BmR1 and BmSXP to detect antibody anti-filarial of IgG4 in blood circulation. The serological examination was performed at the laboratory of Institute for Moleculer Medicine (INFORMM) in University Science Malaysia (USM) using both rapid test and ELISA.

The measurement of rapid test was done using recombinant antigens (BmR1 and BmSXP) and the results were characterized by the development of 2-3 strips (bands) indicating positive results or the presence antibody anti-filarial of IgG4 in sample serum. The instrument used for detecting the presence of *Brugia sp* infection is *Brugia Rapid test*; while for detecting *W. Bancrofti,* the ICT

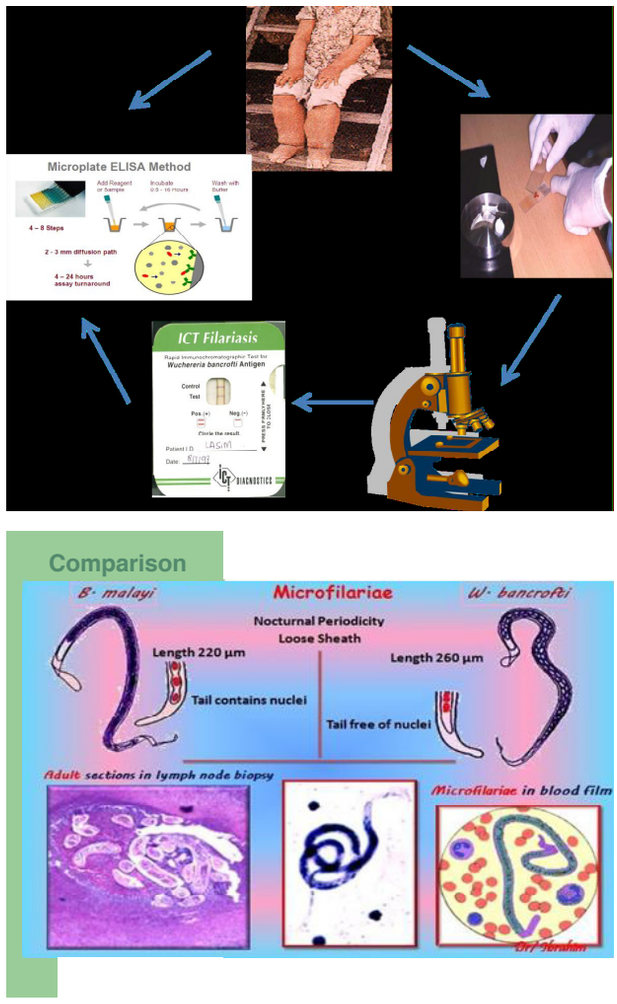

Figure 1. Method of diagnostic stages to detect Lymphatic Filariasis

bancrofti or Pan LF was utilized. (Reszon Diagnostik International. Bhd, Malaysia).

The measurement of IgG4 antibody antifilarial level using ELISA technique was also performed according to the standard procedure at the laboratory of INFORMM-USM (Penang, Malaysia) as mentioned by Rahmah et al (2001a). Each well of the ELISA plate was coated with 100 µL of recombinant antigen BmR1/BmSXP in 20 µg/mL $NaHCO_3$ buffer (pH 9.6).

Conjugates containing monoclonal anti-human IgG4-HRP (Horseredish Peroxidase-CLB Netherland) were inserted in each well of the plate as much as 1: 4500 in PBS. Additional substrate of ABTS (Boehringer Mannheim, Germany) was given for each well after washing and the plate was subsequently covered with aluminium foil and incubated for ½ hour.[5]

The result of reaction was read using ELISA spectrophotometer (Dynatech, USA) at 410 nm wavelength. The measurement results were presented in Optical Density (OD) with a cut-off point of 0.300.[5] Serum sample with OD ≥ 0.300 was categorized as sample with positive IgG4 and those with OD < 0.300 was considered negative.

RESULT AND DISCUSSION

Evaluation on the success of filariasis elimination program was supported by instruments of assay, which had high sensitivity and specificity in detecting the presence of specific infection of filarial species in filariasis endemic area.[4]

The use of recombinant antigens of *BmR1* and *BmSXP* by utilizing rapid test and ELISA had higher sensitivity and specificity compared to using microscopic examination.

Table 1. Results of Diagnostic Test for Samples from Kp. Sawah and Pamulang

Index	Detail from Kp Sawah	Total result Kp Sawah	Result from Pamulang
Microscopic Examination:			Amicrofilaremic: 100% (107/107)
➢ Microfilaremic	0		
➢ Amicrofilaremic	100% (40/40)		
Rapid test	27.5% (11/40)	Rapid test:	No serological test was performed
➢ Positive *PanLF*	15% (6/40)	➢ Positive: 27.5%	
➢ Positive *Brugia Rapid*	15% (6/40)	➢ Negative 72.5%	
➢ Positive *Brugia Rapid & PanLF*	60% (24/40)		
➢ Negative			
ELISA:		ELISA:	
➢ Positive *anti BmXSP1* (OD: 0.3–3.045 or strong positive)	20% (8/40)		
➢ Positive *anti BmR1* (OD: 0.645 or strong positive)	2.5% (1/40)	➢ Positive: 20%	
➢ Positive *W. bancrofti* and *Brugia s*	2.5% (1/40)	➢ Negative: 80%	
➢ Negative	77.5% (31/40)		

It can detect and differentiate filarial infection caused by *Wuchereria bancrofti* and *Brugia sp*, both for individuals with amicrofilaremia and those with microfilaremia. The recombinant antigen is to confirm microscopic result, which have some limitations, i.e. the sensitivity depends on blood volume and parasite periodicity; therefore, it is less sensitive, particularly for individual with symptomatic amicrofilaremia.[6]

Several study report by using microscopic in some endemic filariasis area in South Tangerang and Banten province has found negative result in all slides of FPB. However, serological examination has never been reported for these areas.

An evaluation on filariasis elimination program in the last 5th-annual period in 2013 at Ciputat and Pamulang area reveal the following results:

Symptomatic subjects who had 2nd and 3rd degree of elephantiasis at Kp Sawah in the present study were 4/40 subjects or 10% of all subjects in the study. Among the respondents who had elephantiasis, there were only 2 subjects who had positive serological results for *W. Bancrofti* filariasis, but their microscopic results were all negative; therefore, the inhabitants in the study site could be categorized into:

a. Asymptomatic microfilaremic patient: 0
b. Asymptomatic amicrofilaremic patients: 90% (36/40)
c. Symptomatic microfilaremic patients: 0
d. Symptomatic amicrofilaremic patients: 10% (4/40)

Meanwhile, the microscopic result for 107 subjects from Pamulang were all negative, and there was no blood vein collection nor serological test was performed at the time. Total for microscopic result of 147 samples from the two areas are negative.

The results of the rapid tests showed that 15% (6/40) of the positive cases were detected by Brugia Rapid and 27.5% (11/40) by PanLF. This is not surprising since both recombinants antigens can detect both kinds of filariasis, however BmSXP has greater diagnostic sensitive for bancroftian filariasis while BmR1 is more sensitive in detecting brugian filariasis. Noordin, 2003 has reported that BmSXP antigen showed 91% sensitivity using serum of *W. bancrofti*- infected individuals and 39% sensitivity using serum from brugian filariasis patients.

Meanwhile, the ELISA showed that 20% (8/40) of the cases were positive with BmSXP, whereas only 2.5% or 1/40 sample was found to be positive with BmR1. These results indicated that the study site is endemic for bancroftian filariasis and this idea is supported by the clinical manifestations. The optical density (OD) values ranged between 0.3–3.045. Even though the sensitivity of the ELISA test was lower when compared to microscopic examination, the assay showed good specificity ranging from 72.5 to 97.5%.

The serological diagnostic test can also detect and differentiate infection specifically between *Wuchereria bancrofti* and *Brugia sp* since there is a recombinant filarial antigen of BmR1 and BmSXP1 coated on the rapid test as well as for the ELISA. The results of serological test, which were mostly positive with recombinant antigen of *BmSXP,* indicates that the study site was endemic for Bancrofti and this idea is supported by the clinical manifestations, which revealed the presence of 2nd and 3rd degree of elephantiasis. However, positive result of *Brugia* found by ELISA in one single sample of asymptomatic subject and in 6 samples of PanLF rapid test has indication of possibility for potential transmission of Brugria filariasis in the area.

Appropriate results may become a reference point for evaluation of filariasis program, whether the program is successful or not in the endemic area. It will affect the future policy of filariasis program that should be taken into consideration by the local health department, i.e. whether they will continue the filariasis program or whether it should be stopped. The process of stopping MDA for filariasis is illustrated in the figure below.

CONCLUSION

Serological detection using antigen BmR1 and BmSXP for inhabitants in Kp sawah and Pamulang area shows that infection of filaria *W. bancrofti* and *Brugia sp* is remained endemic. Eventhough sensitivity of the ELISA test was lower when compared to microscopic examination, the assay showed good specificity ranging from 72.5 to 97.5% for the presence of *W. bancrofti* and *Brugia* filaria with titer of IgG4 antifilarial antibody ranging between 0.3–3.045.

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I would like to express my honor and gratitude for all the contribution and coordination to:

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c. Head of Health Department of South Tangerang District and staff P2M Subdit Filariasis
d. Head of UPT Puskesmas Kp Sawah and staff
e. Head of District Health Laboratory (Labkesda) South Tangerang
f. All Participants from Kp Sawah, Ciputat and Pamulang Barat for their samples contribution

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PROFILE OF HEMATOCRIT LEVEL CAPTURED BY DIGITAL HEMATOCRIT TEST

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ABSTRACT

The dengue fever is a disease caused by Dengue virus which is transmitted via Aedes aegypti and Aedes albopictus vector. This Dengue Haemorrhagic Fever (DHF) case in Indonesia tend to rise from year to year caused by delayed detection and inadequate handling. The laboratory parameter of hematocrite had regularly been performed using invasive method by taking the blood from the patient. This method is still not been able to monitor patients with DHF by repetitive and accurate measurament. This research project aims is to perform a digital hematocrit test (DHT) with non-invasive accurate sensors. Digital Hematocrit Test (DHT) is needed to presenting fast, exact, economical and accurate detection methods of hematocrit level. Measureable magnitude by the instrumentation is non-absorb intensity electromagnetic waves 560 nm emitted by transmitter captured by receiver. Signal captured by the receiver then converted into electrical signal. The electrical signal from receiver was the levels of hemoglobin. Levels of hemoglobin then converted to hematocrit. Hematokrit is three times the level of hemoglobin. Technology of hematocrit monitoring is aimed to control DHF patient clinical symptoms continuously and acquisitively.

Key words: *Hematocrit (HCt), Hemoglobin (Hb), Wave 540–900 nm, Non invasive, Lambert Beer Law*

ABSTRAK

Demam berdarah merupakan penyakit yang disebabkan oleh virus dengue yang ditransmisikanmelalui Aedes aegypti dan Aedes albopictus. Kasus demam berdarah di Indonesia cenderung meningkat dari tahun ke tahun dikarenakan terlambat dideteksi serta penanganan yang kurang memadai. Parameter laboratorium dari hematokrit telah dilakukan menggunakan metode invasive denngam mengambil darah pasien. Metode ini masih belum bisa memonitor pasien dengan DBD melalui pengukuran berulang dan akurat. Penelitian ini bertujuan untuk menunjukkan tes hematokrit digital (THD) dengan sensor akurat yang non-invasif. Tes Hematokrit Digital (THD) diperlukan untuk menunjukkan deteksi yang cepat, tepat, ekonomis dan akurat dari kadar hematokrit. Besaran yang dapat diukur oleh instrumentasi ini adalah gelombang elektromagnetik 560 nm yang diemisikan oleh transmitter dan ditangkap oleh receiver. Sinyal yang ditangkap oleh receiver dikonversi menjadi sinyal elektrik. Sinyal elektrik dari receiver menggambarkan kadar hemoglobin. Kadar hemoglobin kemudian dikonversi menjadi hematokrit. Hematokrit merupakan tiga kali kadar hemoglobin. Teknologi memonitor kadar hematokrit bertujuan untuk mengontrol pasien dengan gejala klinis DBD secera berkelanjutan.

Kata kunci: *Hematokrit (HCt), Hemoglobin (Hb), Gelombang 540–900 nm, non invasive, Lambert beer Law*

INTRODUCTION

The dengue fever Dengue Hemorrhagic Fever (DHF) is disease caused by dengue virus transmitted through Aedes aegypti and Aedes albopictus mosquito. Both types of these mosquito are to be found almost in the whole parts of Indonesia, with the exception of a height more than 1000 meters above the sea level. Dengue fever disease often misdiagnosed by other diseases such flu or thypus. This is because an infection dengue virus that causes of dengue fever can be asimptomatik or obscure the symptoms. Based on child data of Cipto Mangunkusumo Hospital, dengue patients often showing symptoms such cough cold, vomiting, nausea and diarrhea. The problem might increase because the virus could enter at the same time with other disease such influenza or thypus. The understanding of disease infection by Dengue virus, pathogenesis, and clinical observation discernment. Using good and complete clinical examination supported by adequate laboratory examination then DHF diagnosis could be set up especially when symptoms are not enough.

The first time dengue fever in Indonesia was discovered in 1968, in Surabaya it happened in 1972. Since then, the disease spread across the area, until 1980 to every province in Indonesia. There were, for the first time show an increase of the number of cases in the area of or infected by or in a sporadic extraordinary occurance always happening every year. The biggest extraordinary occurance of DHF happened in 1998, with incidence rate (IR) = 35,19 per 100,000 population and CFR = 2%. In 1999, a sharp declination from 10,17%, but the next year is likely to increase from 15,99 (in 2000); 21,66 (in 2001); 19,24 (in 2002); 23,87 (in 2003).

The high prevalency of DHF could be caused by many factors. Delayed diagnosis, incautiousness of patients's family to monitor the physical symptoms adn inadequate laboratory examination. The dengue diagnosis according of WHO criteria are thrombositopenia: < 100.000 mg/dl and hemoconsentration: Pack Cell Volume increase > 20%.[1] Hemoconcentration mean there was plasma leakage and it is main indicator to determine whether the patient already fall into Dengue Shock syndrome or not. There are several cause of inadequate detection of hematocrite by laboratory examination which could direct to false result such as first blood capillary contain interstitial liquid, sometimes blood specimen was not directly examine therefore could increase hematocrite level result, examination specimen was not mixed well until homogen, blood specimen could not contain clot.[2,3]

Based on it above, test hematokrit digital (THD) is needed to presenting detection methods levels a hematocrit fast, exactly, economical and accurate. Magnitude measured by instrumentation system is non-absorb intensity of electromagnetic waves emitted by transmitter captured by receiver as the result the remaining non-absorb waves of 560 nm. Signal captured by receiver then converted into electrical signal. The electrical signal of receiver is showed levels of hemoglobin in the veins. Levels of hemoglobin then converted to hematocrit level. Through the technology of this hematocrit level as an indicator plasma leakage could be monitor as often as possible continuously to prevent Dengue Shock Syndrome.

MATERIAL AND METHOD

Material

SpO_2 Oximetri Nellcor, LCD Graphic, arduino uno R3, Shield Arduino, Mini-LCD Probe, Baterai Li-Po 2200 mAh.

Method

SpO_2 Hardware accuracy

SpO_2 data accuracy by took the normal data patient to check accuracy and calibration. By connecting hardware shield with SpO_2 on arduino to ensure Red and IR in the right process.

Software Process and Filtering

Output SpO_2 data resulting from infra red (IR) and LED RED managed to find value intesity wide light absorb (R) on a finger/parts of patient bodies.[4] Absorbs the intensity (R) may be known by measuring value AC_{red} and DC_{red} divided by value AC_{ir} and DC_{ir}, results either absorption managed to get a saturation oxygen (SaO_2) by dividing value HbO_2 with the result the number HbO_2 + Hb and multiplied 100%.Value Hb obtained by inserting value results saturation oxygen (SaO_2) by reduction constant value (110-25 x either absorption (R)) and multiplied by constants absorption Hb of 13.7.Value HCt is value 0.33 of the value Hb.

RESULT AND DISCUSSION

In this study, the average oxygen saturation measurement results of male samples with a DHT is 97.26% and in accordance with the normal range is 95–100% SpO_2 levels. Average Hb values of all the male data is 13,328 g/dl approaching the normal range of Hb values which is 13.5–18 g/dl for male.[5] While the average male HCt value of all male samples is 39,976% approaching the normal range of HCt in male which are 40–54%.[5]

In this study also found the average measurement of the oxygen saturation results of female with a DHT is 97.5% which is in accordance with the normal range of female SpO_2 levels which is 95-100%. The average value of 13.326 g Hb/dl were in the normal range of hemoglobin values is 12-16 g/dl for female.[5] While the average value of 40.1% of female HCt in appropriate range of normal values HCt 37–47% of female.[5]

Hematocrit (HCt) is an indicator of the determination of the most indicative of the symptoms of dengue fever.[6]

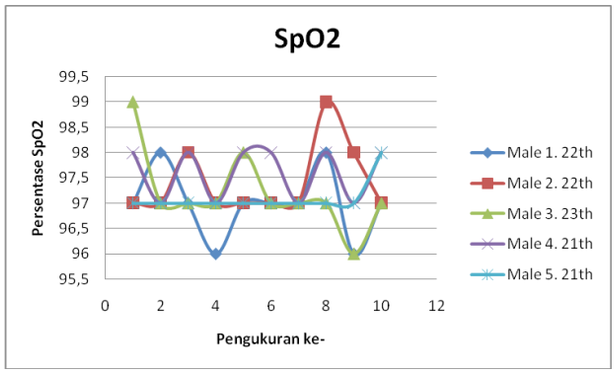

Figure 1. Percentage SpO2 of male

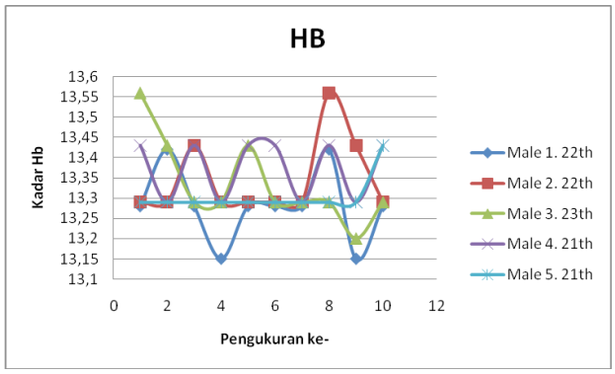

Figure 2. Hb level of male

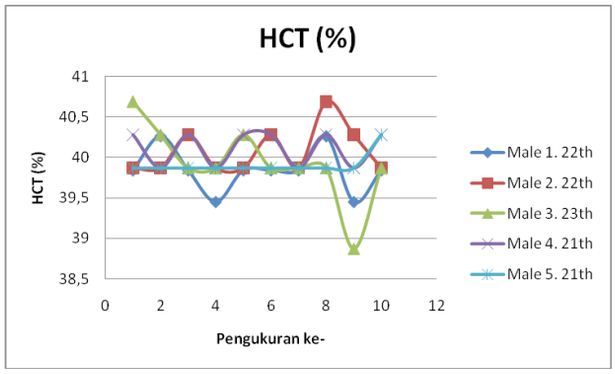

Figure 3. HCT of male

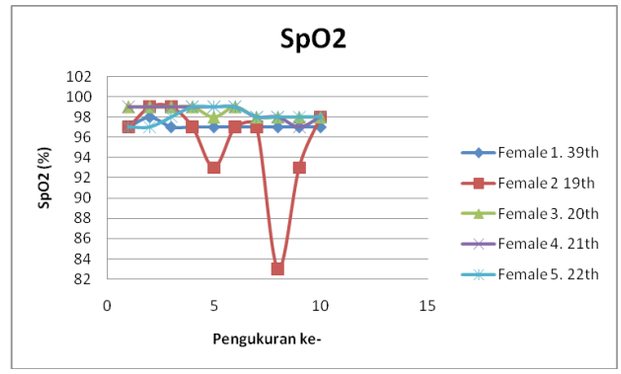

Figure 4. Percentage SpO2 of female

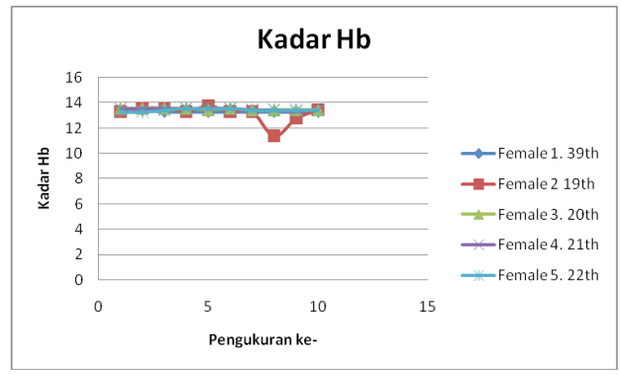

Figure 5. Hb level of female

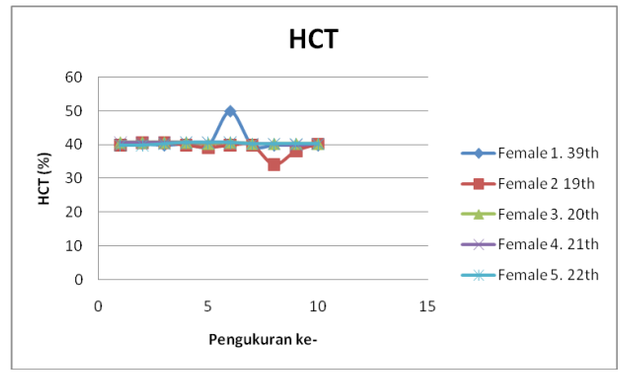

Figure 6. HCT of female

Initial hematocrit levels related to the degree of clinical DHF according to WHO criteria. Not only to assess the factual condition of the patient, but also to estimate or act as predictors the worst risk facing the patient, so it can be taken countermeasures and early prevention. As mentioned previously, plasma leakage is a causal factor that sparked the beginning of hypovolemia shock in dengue cases. And it has been proven that plasma leakage has occurred since the beginning of fever before the seizure.[7,8,9]

Digital Hematocrit Test l (DHT) utilizing RED value at a wavelength of 540–900 nm and IR. HbO_2 and Hb values are the result of constituent values of saturation (SaO_2). To find the value of the voltage absorption wavelength generated by HbO_2 and Hb should be based on the amount of voltage that is absorbed in the SpO_2. The division of red voltage value with IR voltage value generating absorption voltage values (R) which were used to search Hb value patient. Saturation value minus the result of filtering constantas (110-25 x absorption voltage (R)). hemoglobin (Hb) is obtained by multiplying the value of the saturation (SaO_2) to hemoglobin absorption constant value of 13.7. Percentage of HCt values obtained from 1/3 hemoglobin value.[10]

Average male HCt value 39,976% of all male data approaching the normal value range HCt for male which are 40 to 54%. The average value of female HCt 40.1% were

in the range of normal values female HCt 37–47%.[5] Based on the results of measurements on male and female normal samples with 10 times showed that the measurement results are in accordance with the normal range of hematocrit values of male and female.

CONCLUSION

From the research it can be concluded that, a large percentage of the value of SpO_2 values can be used to find the value of Hb and HCt. The data obtained from the normal samples showed that SpO_2, Hb, and HCt value is still in the normal range.

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