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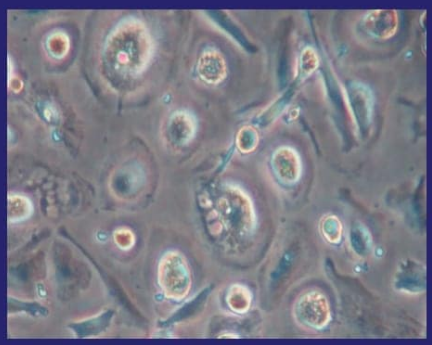

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Research Report

RELATIONSHIP BETWEEN CLINICAL MANIFESTATIONS AND ANTIBODY SERUM IN OUTBREAKS ANTHRAX

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ABSTRACT

Introduction: *Anthrax is a zoonotic disease that often affects the grass-eating animals, which occurs due to the entry of spores into the bodies of animals and can be transmitted to humans. This disease often appear in certain seasons and occurs in endemic areas, including Indonesia. Cutaneous anthrax is the clinical manifestations that often arise on outstanding events in the area. This study aims to determine how the relationship between the clinical manifestations of the serum antibodies in people who are exposed to anthrax.* ***Material and methods:*** *This study is an observational cross sectional analytic approach, in people exposed to anthrax to assess the clinical manifestations and antibody serum Anthrax.* ***Results:*** *Obtained in this study respondents were 101 people with a history of contact with animals suffering from anthrax. The number of respondents with the highest age distribution was 31 to 40 years by 42%, and most were female gender, which is 57.7%, the highest level of education is 74% finished elementary school. Forty-four percent of working as a housewife. Risk factors are the most direct contact with and consume the flesh of animals as much as 34.6%. Results of Ig G antibody serum showed 50% negative, 15.4 borderline and 34.6% positive. Clinical manifestations that occur in the skin as much as 13.5%, that is the eschar on all respondents and 92.8% showed positive Ig G. While 86.5% did not show any clinical signs of anthrax, of that number 25.5% with Ig G positive, 16.6% and 57.7% showed borderline negative with p 0.02.* ***Conclusion:*** *There was a significant association between the clinical manifestation with antibody serum anthrax. But also found a positive Ig G without the appearance of clinical signs in the skin.*

Key words: *clinical, manifestation, anthrax, serum antibody ELISA, eschar*

ABSTRAK

Pendahuluan. *Antraks adalah salah satu penyakit zoonosis yang sering menyerang pada hewan pemakan rumput, yang terjadi karena masuknya spora ke dalam tubuh hewan dan dapat ditularkan ke manusia. Penyakit ini sering muncul pada musim tertentu dan terjadi di daerah endemi, termasuk Indonesia. Cutaneous Anthrax merupakan manifestasi klinis yang sering timbul pada kejadian luar biasa di suatu daerah. Penelitian ini bertujuan untuk mengetahui bagaimana hubungan antara manifestasi klinis terhadap serum antibodi pada orang yang terpapar antraks.* ***Bahan dan Metode.*** *Penelitian ini merupakan observasional analitik dengan pendekatan Cross Sectional, pada orang yang terpapar antraks dengan menilai manifestasi klinis dan serum antibodi Antraks.* ***Hasil.*** *Pada penelitian ini didapatkan responden sebanyak 101 orang dengan riwayat kontak dengan hewan yang menderita antraks. Jumlah responden dengan sebaran umur tertinggi adalah pada 31 sampai 40 tahun sebanyak 42 %, dan jenis kelamin terbanyak adalah perempuan, yaitu 57,7 %, tingkat pendidikan terbanyak adalah lulus SD 74 %. Empat puluh empat persen bekerja sebagai ibu rumah tangga. Faktor risiko terbanyak adalah kontak langsung dan mengkomsumsi daging hewan sebanyak 34,6%. Hasil pemeriksaan Ig G antibodi serum menunjukkan 50% negatif, 15,4 borderline dan 34,6% positif. Manifestasi klinis yang terjadi pada kulit sebanyak 13,5 % , yaitu adanya eschar pada semua responden dan 92,8% menunjukkan Ig G positif. Sedangkan 86,5% tidak menunjukkan adanya tanda klinis antraks, dari jumlah tersebut 25,5% dengan Ig G positif, 16,6% menunjukkan borderline dan 57,7% negatif dengan p 0,02.* ***Simpulan.*** *Ada*

hubungan yang bermakna antara manifestasi klinis dengan hasil serum antibodi Antraks. Namun juga didapatkan adanya antibodi Ig G positif tanpa disertai munculnya tanda klinis di kulit. Sehingga perlu dilakukan deteksi dini pada orang yang terpapar antraks.

Kata kunci: *manifestasi, klinis, antraks, antibodi serum ELISA, eschar*

INTRODUCTION

Anthrax is one of the types of zoonotic diseases, which can be transmitted to humans, animals suffering from anthrax. The disease is caused by bacillus anthracis. Anthrax commonly often attacked livestock such as cattle, sheep, goats and camels. Transmission to humans occurs when there is direct contact of animals or animal products that suffer from anthrax, can be skin, blood and flesh.[1,2]

Anthrax incident in Indonesia in the last ten years has occurred five times the plague that is 1996 to 2000 in West Java.[3,4]

Since the outbreak of anthrax 15 years ago in Indonesia, the patient sample should be sent abroad (USA) for the diagnosis of anthrax investigation. Based on these events, the Moewardi hospital cooperate with Integrated Biomedical Laboratory of the Faculty of Medicine, University Sebelas Maret has been trying to develop anthrax test-based immunoassay using enzyme-linked immunosorbent assay (ELISA) for the detection of proteins PA by using the Anthrax Protective Antigen Calbiotech (PA) IgG ELISA Kit, as catcher agents that are sensitive to the ELISA was able to detect PA (≥ 1 ng / ml PA) in the serum of patients with suspected anthrax.[4,5]

This Problem is how is anthrax protective antigen serum antibodies (PA) Ig G ELISA in people who are exposed to anthrax and its relationship with clinical manifestations in outbreak area ?

Diagnosis approach

Anthrax Diagnosis is made through history, clinical examination, laboratory and serology :

1. History: Early diagnosis of anthrax is difficult to know because it does not show the typical signs and symptoms, usually preceded by the appearance of reddish nodule with pain and swelling. It needs to be asked is whether previously had contact with an animal that died of anthrax, either direct contact or eating meat or contact with animals or their products (skin, bone), how the employment status (farmers fields, ranchers, RPH, tanners) and whether residence in endemic areas of anthrax.[2,6]
2. Clinical manifestations: There are 3 clinical manifestations that may arise in people is cutaneous anthrax, gastrointestinal and inhalation:
   a. Cutaneous anthrax
      Most cases (95%) anthrax is happening in the world is cutaneous anthrax. Patients usually have a history of contact with animals or their products, the anthrax bacteria or spores enter through the skin through a lesion on the skin, for example, when doing the slaughtering process (cutting, skinning or divide meat) cattle infected with anthrax. Then came a low germination rate at the location where the entry of spores and cause lesions on the skin that itch, then papuler lesions arise and develop into vesicles accompanied by edema and pain. These lesions became necrotic eschar formation and accompanied local soft tissue edema. Germination occurs within 1-3 hours after inoculation, but germination can not cause infection of the skin intact. Endospores will undergo phagocytosis by macrophages and then be taken to the regional lymph nodes, causing lymphadenopathy and lymphangitis. Hematogenous dissemination can occur, but with the provision of adequate spread of systemic antibiotics is quite rare. Several case reports of infections caused by insect bites suspected of being infected (eating carcasses containing anthrax bacillus).[4,5]
      Common location is on the face, extremities or neck. Endospores enter through skin abrasions or wounds. One to seven days after entry endospores, formed the primary skin lesions that are not painful and itchy papules. Twenty-four to 36 hours later lesions forming vesicles containing clear fluid or serosanguineus, and contains many Gram-positive bacteria. Vesicles then undergo central necrosis, dry out and cause eschar (necrotic ulcers) blackish typical vesicles surrounded by edema and purple. Edema usually occurs more severe on the head or neck than the body or limbs. Lymphangitis and lymphadenopathy that pain can be found following systemic symptoms occur. Although cutaneous anthrax can heal itself, but still need to be given antibiotics (to reduce systemic symptoms). In 80-90% of cases the lesions healed completely without complications or scarring. Malignant edema are rare, characterized by severe edema, induration, multiple bullae, and shock. Malignant edema can occur in the neck and chest area that causes difficulty breathing, requiring corticosteroids or intubation.[5,6]
   b. Gastrointestinal Anthrax
      Gastrointestinal anthrax, although it can be fatal, has not been reported in the US. Symptoms usually appear 2-5 days after eating raw or undercooked meat that is contaminated with germs. Some cases may occur in the home. On pathological examination under a microscope can be found in

the mucosa and submucosa basil lymphoid tissue and mesenteric lymphadenitis. Ulceration almost always be found. A large number of Gram-positive bacteria can be found in the peritoneal fluid. Mediastinal widening can also occur.

Clinical symptoms can include fever, diffuse abdominal pain, constipation or diarrhea. If there is ulceration of the bowel becomes blackish. Ascites can occur with clear liquids until purulent.[1,6]

c. Anthrax inhalation

Inhalation anthrax spores began with the entry into the alveolar cavity, then macrophages will fagocyt spores and some of the spores will lysis and broken. Spores that survive will spread to the lymph nodes and mediastinal nodes. The process of change in vegetative forms occur approximately 60 days later. The slow process of change in shape is not known with certainty, but well-documented cases of anthrax in Sverdlovsk that inhalation occurs between day 2 to day 43 after exposure. Once germination has occurred, the disease will arise quickly and replication of bacteria causing bleeding, edema and necrosis. The term anthrax pneumonia is not used because it turned out after pathological examination abnormalities were obtained mainly in the form of thoracic lymphadenitis and mediastinitis hemorhagis without typical bronchopneumonia. However, in the event of inhalation anthrax in Sverdlovsk, 25% of fatal cases was found bleeding focal necrosis and pulmonary lesions.[1,2]

Anthrax meningitis

A complication of cutaneous anthrax, inhalation and gastroitestinal, but is most common in inhalation anthrax (> 50%). Often addressing bleeding and meningoencepalitis. Anthrax death rate is over 90%.[6,8]

3. Investigations : In the diagnosis of anthrax needed routine blood tests, culture swab the wound or blood (on the skin), sputum (on inhalation) chest X-ray (on inhalation), electrolyte (gastrointestinal) and serology using ELISA (Enzyme linked immunosorbent Assay) and PCR (Polymerasi Chain Reaction). Samples were taken for laboratory examination of the above is the blood serum, rub the injured area, sputum and land near the cage or a dead animal.[9,10]

Criteria of Diagnosis

The criteria used in the diagnosis of anthrax consists of three types, namely suspected (suspect), Probable (possibility) and Confirmed (Confirmation).[10,11]

Suspected, is clinically shown one form of anthrax and there is epidemiological evidence that exposure to anthrax environment, but there is no definitive laboratory evidence. Probable, is in clinical symptoms of anthrax but do not meet the definition of confirmation, but shows one of the following : (1) In Epidemiology, there are environmental exposures. (2) B. anthracis DNA evidence collected from the lesion, usually sterile (such as blood or CSF) or lesion of other affected tissue (skin, lung or digestive) (3) Positive serology IgG ELISA Anthrax Lethal Factor (LF) in the examination of the positive Spectrometry. Confirmed, is in clinical symptoms of anthrax with one of the following : (1) B. anthracis culture positive (2) Demonstrate B. anthracis antigens of the network by immunohistochemical staining using the cell wall and capsule monoclonal antibody B. anthracis (3) Proven 4x increase in antibody titer during the acute period and fixes the quantitative examination of anti-PA IgG ELISA testing (4) The presence of environmental exposure to anthrax and PCR test positive.[10,11]

METHOD

This study is an observational analytic with cross sectional approach, by screening immunoassay based on people exposed to anthrax outbreak in the area in 2011. The location of sampling is an area of outbreak anthrax, Boyolali and Sragen, Central Java. All the people who are exposed to dead animals suffering anthrax, a blood sample for examination IgG antibodies in serum by ELISA.[3,4] Intepretation ELISA will get three categories: positive, borderline and negative, with inclusion criteria such as direct contact with infected animals anthrax, do not suffer from a disease that causes a decrease in the body's immunity, there are currently using Immune suppressant drugs, there are pregnant or breastfeeding and not in hormone therapy. There were exclusion citeria such as not willing to follow research and medium serious illness, such as sepsis.

Operational definitions of variables

Dependent variable: clinical manifestations Is the result of the interview and physical examination in people who are exposed to anthrax animals.

The independent variables: levels of Ig G Anthrax Serology examination to assess the titer of anthrax protective antigen (PA) Ig G ELISA in order to confirm the presence of infection with Bacillus antracis in human blood. Interpretation of test results :

<0.9 : No detectable IgG antibodies against PA protein in ELISA.

0.9 - 1.1 : Borderline

> 1.1 : Detected the presence of IgG antibodies to the protein PA, indicated patients were infected or infected with a never Bacillus anthracis.

Scale: nominal

RESULT

In this study, 101 people with a history of contact with animals that died of anthrax. Respondents in the youngest age is 6 years old (1%) and the oldest 80 years (1%). The distribution of the highest age at 21 to 40 years as much as 39.6%, and most are women sex, ie 57.4%. The education level of respondents at most 74.3% graduated from elementary school. Subsistence farmers as much as 21.8%. The basic characteristics of the study subjects are shown in Table 1.

Table 1. Baseline Characteristics of research subjects (n = 101)

Variable	N	%
Gender		
• Male	43	42,6
• Female	58	57,4
Age		
• 0 - 20 year	2	1,9
• 21 – 40 year	40	39,6
• 41 – 60 year	37	36,6
• 61 – 80 year	22	21,7
Pendidikan		
• Elementary	75	74,3
• Junior high school	15	14,9
• Senior high school	5	5
• University	6	6
Profession		
• No work	48	47,6
• Farmer	22	21,8
• Civil	5	5,0
• Private	26	38,8

Of the total sample, showed serum Ig G antibodies showed negative 50.5%, borderline 15.8% and 33.7% positive. ELISA serology results can be seen in Table 2.

Table 2. Results of ELISA

Variable	N	%
• Positive	34	33,7
• Borderline	16	15,8
• Negative	51	50,5

In cross-table analysis results between risk factors contact with ELISA serology results obtained at the same respondents who cook and eat the highest risk on positive serologic results 20.8%. Serology results Elisa Risks associated with the contact can be seen in Table 3.

Table 3. Results of serology Elisa Risks associated with contact

Risk Factors	Elisa		
	Positif	Border line	Negatif
• Wash the meat	1	0	1
• Eating	10	5	16
• Wash and eat	3	2	0
• Cooking and eating	11	6	19
• Slaughtering and eating	9	3	13
• Located near the cage	0	0	0

Overall there are 11.9% of respondents who showed clinical signs of the appearance of the skin in the form of vesicles, accompanied by fever and ulcers which ended with eschar formation. The skin manifestations can be seen in Table 4.

Table 4. Distribution of clinical manifestation

Clinical manifestation	N	%
• Eschar	12	11,9
• No Eschar	89	88,1

At respondents with 10.9% positive serology results indicate a skin manifestation of the emergence of eschar, while only 1.0% with borderline serology showing the skin manifestations. There are as many as 22.8% with positive ELISA results, but does not cause any skin manifestation in the form of eschar or other clinical signs (fever, myalgia, stone, spasms, nausea and vomiting). Result of serology associated with skin manifestations such as eschar can be seen in Table 5.

Table 5. Results of serology Elisha associated with skin manifestations in the form of eschar

Eschar	ELISA		
	Positive	Borderline	Negative
• Yes	11 (11%)	1 (1,0 %)	0 (0,0 %)
• No	23 (23%)	15 (14,8 %)	51 (51%)

P 0.02

Contact risk factor for the emergence of manifestations in the skin, especially on the respondents were slaughtered at once ate beef (6.0%), followed by the washing and eating meat, which is 3.0%, whereas only 1.0% wash and meating. The relationship between contact with the manifestation of the emergence of the eschar can be seen in Table 6.

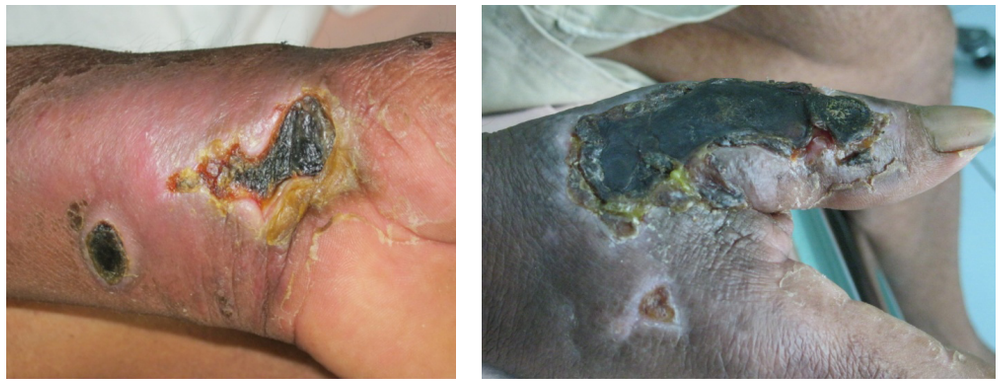

Figure 1. Anthrax manifestations in the skin with the advent of eschar

Table 6. Relationship between contact with the manifestation of eschar

Variable	Eschar	
	Yes	No
• Wash the meat	1 (1,0 %)	1 (1,0 %)
• Eating	2 (2,0 %)	29 (28,7 %)
• Wash and eat	3 (3,0 %)	2 (2,0 %)
• Cooking and eating	0 (0,0 %)	36 (35,6 %)
• Slaughtering and eating	6 (6,0 %)	19 (18,9 %)
• Located near the cage	0 (0,0 %)	2 (2,0 %)

DISCUSSION

During the 2011 outbreak of anthrax in Central Java. Initially obtained a cow belonging to one of the people who had collapsed and accompanied by seizures. The owner decided to slaughter cattle meat and sold to residents of 40 packs. Beef meat and blood samples examined in laboratory of Central Java Province and tested positive for anthrax. Seven days later, seven people were complaining there are small bumps and itching, swelling and lesions accompanied wet in the area under the eyes, hands, legs or feet, then taken to the health center and declared suspected anthrax. Then in May 2011 in Sragen also occur the same thing and be some people who show symptoms of anthrax skin contact.

Clinical manifestations in the form of eschar present in 11.9% with cutaneous anthrax. Respondents were taken from both locations, obtained 101 samples were then examined serological Anthrax serum Ig G antibodies. Of these 50.5% negative and 33.7% positive, while 15.8% borderline. Clinical manifestations in the form of a skin disorder that begins their edema or injury which led to edema and ends with eschar present in 10.9% of the respondents who Ig G antibody positive and 1.0% of respondents with Ig G borderline results. This is due to the emergence of antibodies against anthrax bacteria on respondents who had clinical manifestations in the skin, but that can not be explained is the result obtained antibodies also borderline clinical manifestations (see Figure 1).

Twenty-two percent of respondents with a positive serum Ig G antibody, did not lead to clinical manifestations. It might be due to the durability of the respondents, bacterial virulence factors and the amount of exposure that occurs may not be too much. But this can not be explained further, because of the endurance factor all pretty much the same condition, which probably is due to the virulence of the bacteria and germs that enter the number.

The risk of direct contact, ie cooking and eating meat of infected animals showed 30.5% Ig G positive results, but does not cause clinical manifestations with the advent of eschar (0%). This may be due to immune factors of patients as well as virulence B antrhacis that enters the body. Risk factors eating only 32% of respondents showed positive results. While the risk factors slaughter and eat 24% manifested by the appearance of skin eschar.

CONCLUSIONS

The conclusion of this study is the increase in serum antibody titer Ig G anthrax does not occur in all of the respondents were exposed to the anthrax outbreak area, all respondents were obtained eschar followed by an increase in Ig G antibody titers.

Researchers suggest screening anthrax using anthrax Ig G antibodies can be done in areas that are outbreaks of anthrax and can proceed with dealing with how the eschar and its effect on Ig G antibody titer anthrax.

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Literature Review

MICRONUTRIENT THERAPY FOR SEPSIS

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ABSTRACT

Micronutrients are nutrients which are needed by the body to perform the function of body. The amounts is less than 100% µg per day and consist of vitamins and minerals. It cannot be synthesized in the body. Research in the US mentioned that the rate prevalence of sepsis is tended to be increased 8.7 % annually. In sepsis, nutrition is one of the important component which could drive the success treatment. Micronutrient, especially a vitamin which is soluble in fats, it would be toxic if the number exceed the capability of body to receive it. Although there are guidance and mutual agreement about sepsis using, it still need to concern on micronutrient which potentially giving bad effect. In sepsis case, micronutrients also determine the success of treatment due to redistribution of vitamin and trace element from circulation to the tissue which involved in the proteins formation and immune system. The conclusions of the latest 7 experiments and 4 random controlled studies of multi-centre support the micronutrients supplementation because it can decrease mortality rate. However, it still need to be aware to the toxicity of fat soluble micronutrient if the doses are excessive.

Key word: *Micronutrients, sepsis, nutrition, mineral, vitamin*

ABSTRAK

Mikronutrien adalah zat gizi (nutrien) yang diperlukan oleh tubuh manusia dalam jumlah kecil yaitu kurang dari 100% µg per hari untuk melaksanakan fungsi fisiologis. Mikronutrien terdiri dari vitamin dan minera dan tidak dapat disintesis oleh tubuh. Penelitian di AS menyebutkan angka kejadian sepsis cenderung meningkat 8,7% setiap tahunnya. Pada sepsis, nutrisi adalah salah satu dari komponen utama yang menentukan keberhasilan pengobatan. Mikronutrien, terutama vitamin larut lemak, membawa risiko toksisitas pada pemberian melebihi dosis standard. Meskipun ada petunjuk dan kesepakatan bersama tentang sepsis, masih diperlukan perhatian pada mikronutien yang berpotensi memberikan dampak buruk. Pada kasus sepsis, mikronutrien juga menentukan keberhasilan pengobatan karena redistribusi vitamin dan diikuti unsur-unsur dari peredaran darah ke jaringan yang terlibat dalam pembentukan protein dan sistem imun. Kesimpulan terhadap 7 percobaan terbaru dan 4 penelitian acak terkontrol multi-centre mendukung suplementasi mikronutrien pada sepsis karena dapat menurunkan angka kematian. Namun demikian, tetap perlu diwaspadai kemungkinan terjadinya toksisitas mikronutrien yang larut dalam lemak jika melebihi dosis.

Kata Kunci: *Mikronutrien, sepsis, nutrisi, mineral, vitamin*

INTRODUCTION

Micronutrient is nutrient substance (nutrients) required by human body in small quantities to give physiological functions. Micronutrient consists of vitamins and minerals/ trace elements which could not be synthesized in the body. Micronutrient is important for body continuously in small quantities, less than 100 µg each day. Different to macronutrient, carb, protein and fat are important for body in large quantities. [1]

Vitamin is an organic substance which could not be synthesized in the body. Trace elements has a role as co-factor for some enzymes such as mineral which has a little amount in the body. The amount of trace elements is about 0.01 % of body mass and the total amount is less than 1 %.[2]

Systemic Inflammatory Response Syndrome (SIRS) occurs redistribution of vitamins and trace elements from circulation to tissues involved in protein synthesis and the immune system. Concentration of iron, selenium, zinc and protein carrier in circulation are decrease while copper and manganese are increase.[1]

In sepsis, nutrition is one of the main component to determine the success of the treatment. Micronutrient is still important beside the optimal carbohydrate, lipid and protein combination. Supplementation micronutrient research on sepsis focused on 5 selenium micronutrient, namely zinc, copper, clusters of vitamin B, vitamin C and E. [1]

Research in the US stated that sepsis occurrence rate is tended to increase 8.7% annually.[3] Since systematic review by Heyland *et al*. at 2005, some experiments was done (including experiment multi-centre greater) so it is increase the micronutrient using.[4] The latest 7 experiments and 4 random controlled studies of multi-centre support the previous findings that showed micronutrient supplementation sepsis is associated with the reduction of mortality rate especially in 28 days.[1]

Although there are guidance and consensus about sepsis using, it still need to concern on micronutrient which potentially giving bad effect. Micronutrient, especially fat soluble vitamin carries toxocity risk if the dose given is exceed the standard. [1]

SEPSIS

According to Surviving Sepsis Campaign 2010 consensus, SIRS defined as 2 or more criteria from a compilation of clinical manifestationand laboratory examination such as temperature more than 38,3°C (101°F) or less than 36,0°C (96,8°F), tachycardia (more than 90bpm), tachypnea (more than 20 times per minute), PCO2 less than 4,3 kPa (32 mmHg), hyperglycemic (glucose of blood >7,77 mmol/L [120 mg/dL]) without record of DM, mental status changed in acute, leucosyte more than 12109/L (12.000/microliter) or less than 4109/L (4000/microlitre) or WBC normal with granulocytes >10%

Metabolic response begin with rising energy of *Resting Energy Expenditure* (REE) catabolism proteins and fats, balance nitrogen negative hyperglycemia and increasing glucose liver production, *Cuthbertson* clearly described the three phases of response when sepsis are, ebb phase when initial shock response decrease metabolism of the body, flow phase (catabolic phase), convalesen phase (anabolic phase) when a body beginning to synthesize ramifications.[4]

On sepsis occurs metabolic changes which called stress metabolism because the influence of inflammatory mediator. In ebb phase and ebb phase, changes that happened could be considered as the basic guidance to give therapeutic action depend on its starting time, composition and also the provision period.[4]

In the early time phases, ebb phase is found and occurs energy conversion at that time. Flow phase which started soon after ebb phase, as distinguished further into two stages: acute flow phase and adaptive flow phase. In acute flow phase, energy needs is increasing even sometimes flashy.[4]

On adaptive flow phase, the healing process started. For optimum recovery, proper indispensable nutrients are needed along the three stages. In acute flow phase where cardiac output and blood pressure are increased, reserve fat and muscle are having a catabolism process and generated energy. Protein in muscle tissue produce an amino acid for liver gluconeogenesis, hormone stress (glucagon, catecholamine and glucocorticoid) is quickly produced and increasing the glucose level. As the parsing of muscle tissue, potassium, phosphorus and sulfur are lost proportionately. Acute flow phase is generally lasting for 3 or 4 days and will end in 7 to 10 days if there is no complication happened. Hyperglicemia often occurs in this phase.[4]

Disorder motility often occurs in sepsis. In a retrospective study, patients with sepsis are having a greater time of gastric emptying (GE) postponement compared with patients with heart disease and failed breath. There are multifactor but mostly are still unclear. Shock, a cytokine, electrolyte disorder, hyperglicemia, underlying disease and treatment. Catecholamine decrease the motility through ß-adrenergic stimulation. Dopamine decrease the acetylcholine and hindered the antrum contraction and prolong the intestine transit time.[5]

Motility of the gastrointestinal normal tract is controlled by the central nervous system (CNS), autonomous nerve, enteric nervous system (ENS) and triggered by peptide located within the walls of the alimentary canal .ENS Neuron system divides into 2 plexus namely plexus mienteric and submucosal. Mienteric plexus located between longitudinal and circular layers that serves as motility agent. Acetylcholine and P-substance transmitter are the main excitatory motoric neurons while nitric oxide (NO), vaso-active and ATP are the main transmitter of inhibitory neurons.[5]

Response neuroendocrine will arise due to sepsis as increased catabolic hormone such as cortisol, glucagon and catecholamine which accompanied by resistance to insulin so that substrate non essential can be turned into energy accelerated healing wounds. There are conclusion process the response of neuroendocrine such as Gluconeogenesis, glucose/glutamin/fatty acid mobilization, proteolysis jaringan peripheral tissue and balance negative nitrogen, increasing REE, retention of water, insulin and GH resistance.[4]

DEFINITION OF MICRONUTRIENT

Micronutrient is an important element which are needed by body in small amount that only 100 mg/day or less than

1 % weight. Micronutriens consists of micromineral and vitamins. Micromineral has iron, cobalt, chromium, bronze iodine, manganese, selenium, zinc and molybdenum as its subtance. Although mineral needs can only 5 % obtained from food, but it is very useful for body organs.[6]

FUNCTION OF MICRONUTRIENT

The role of micronutrients in metabolism process is to maintain the function of body tissue. Hypermetabolism led to an increasing production of Reactive Oxygen Species (ROS) as an impact of increasing oxidative metabolism which can damage cell, mainly on unsaturated fatty acids which are found in the cell membrane and nucleus.[6]

Zinc, iron and selenium are absorbed in duodenum and jejunum while chromium and copper are absorbed in ileum. Micronutrient take part on helping the body to neutralize the negative effects of free radicals. Deficiency micronutrient usually accompanied by more than one drawbacks except zinc, iron and vitamin A. Some interactions could happen in provision of micronutrient. Zinc decrease the absorption of copper while iron decrease the absorption Cu and zinc.[7]

Zinc (Zn)

Several enzymes that play role in setting of oxidant defense including SOD, catalase, and glutathione reductase depend on normal condition of zinc. It is suitable because in sepsis occurs declining capacity of ROS detoxification.[8]

Zinc has a very important role for immune system, oxidative stress response, wound healing process and protecting homeostasis. It is difficult to distinguish between the symptoms of zinc deficiency and sepsis.[9]

Zinc is a co-factor of more than 200 enzyme that play roles in the immune system. It is very important for wound healing process, regenerating new cells and balancing of acid base.[10]

The normal concentration of zinc in the body is between 70-150 mcg/dl. Low concentration of alkaline phosphatase (ALP) represent that the body has a low concentration of zinc. Granting zinc 150 mg/day twice a week for 6 weeks can be lowered the response of lymphocytes stimulation towards fitohemaglutinin as well as it can decreased chemotaxis and bacteria phagocytosis through PMN. Granting 15 mg of zinc can improve response of the body against infection. Zinc become a toxic if it is given in 50 mg/day dose.[9]

Zinc supplementation triggers the decreasing of copper concentration through the competitive interaction in the intestines absorption process. Zinc stimulates enterocyte metallothionein. Metallothionein breaks copper up faster than zinc does so thatas copper can not be transferred and absorbed by the intestines. The excessive provision of zinc is causing anemia deficiency as well as netropenia and lecopenia. Symptoms that appear when indigestion occurs are pain at epigastrium, nauseous, and vomit. Zinc intoxication happens if a dose of 30 mg / day is causing the occurrence of acute phase response and increase IL-6.[11]

IRON (Fe)

On sepsis, the declining iron concentration occurs due to the improving permeability so that transferin move from intravascular into interstitial liquid. Increased production of ferritin in the liver caused by induction IL-6 so that more Fe stored in liver. On sepsis, hepsidin production is increasing and it will inhibit the Fe transporting.[6]

Neutrophils and macrophages need Fe for phagocytosis and intermediate oxygen formation which is toxic in killing bacteria. Reduction of nitroblue tetrazolium and hydrogen peroxide on neutrophils and macrophages are decreasing if lack of iron substance. Iron also taking part in Crebs cycle as a source of essential energy. Several enzymes such as glutathione, peroxidase, catalase and dehidrogenase need the iron as a free radical antidote.[7,12]

The improvement of veins permeability causing a leak, transferin to interstitial fluid. Iron triggers bacterial growth because of its role as an essential nutrients for the growth of bacteria.[6]

Polymorphonuclear (PMN) release laktoferin along inflammatory process and bend the iron than it will be processed by macrophages. Netrofil and macrophages need the iron for phagocytosis and killing bacteria. Otherwise, excessive iron can decrease the ability of macrophages to do phagocytosis. It happens because of the production of free radicals are increasing and damage the peroxydases fat located in phagosom membrane.[12]

Iron is also a growth factor for some bacteria and increase proliferation thoroughly in vivo. Granting iron in parenteral way can inhibit the migration of neutrophi and weaken the host defensibility. Oxidative stress controlled by iron free can trigger inflammatory through some stages, for example is factor-kappa ß(NF-jB). Once it is been activated will cause a mediator inflammatory production like TNF-α, cytokine, etc.[13]

Selenium

In a state of sepsis, the issuance of selenoprotein-P in intravascular is increasing. Selenoprotein-P is a form of

Table 1. Recommendation for Trace Elements in Critical Illness

Trace Element	RDA	Standard Dose		Additional Supplementation
		PN Formula	EN Formula	
Zinc	15 mg	2.5 - 5 mg	11 - 19 mg/L	10 - 30 mg/day
Selenium	50 - 100 mcg	20 - 60 mcg	20 - 70 mcg/L	100 - 400 mcg/day
Iron	0 – 15 mg	0	12 - 20 mg/L	-
Copper	900 mcg	300 - 500 mcg	-	-

Source: S Afr J Clin Nutr 2010;23(1) Supplement: S60[14]

selenium which are rich of protein. One of the uniqueness of selenium is having a dual function as pro-oxidant and as anti-oxidant. Sodium selenite as a pro-xidant while atoms of selenium as an anti-oxidant. Selenium translocation to interstitial cause the improvement of NF kappa B activityso that raises protein during acute phase.[6]

In sepsis there has been a decrease levels of selenium as 40%. Selenoenzymes including glutathione peroxidase (GPx) plays an important role in controlling the process of inflammatory including protection against species reactive oxygen (ROS). The provision of high doses selenium for 9 consecutive days in sepsis and reduce the dose for every 3 days can decrease the number of deaths, score of the APACHE III and serum creatinin.[6,15,16]

Maximum doses of selenium is 400 µg (5µg/kg BB/day) but even granting 800 µg is considered has no side effect report. In some research ,granting 750-1000 µg selenite combined with 800 µg selenium per day in iv and enteral is safer in terms of the dosage provision.[1,15]

According to Kuklinsky, *et al*., granting selenium as a bolus is more effective than as a drip. To reduce the binding of NF-κB with DNA and trigger the apoptosis, it needs high concentration of selenium in plasma that can only be achieved if it granting as a bolus.[15]

Angstwurm *et al*., experimented with selenium by giving high doses for 9 days and got lowered every day. It can fix APACHE III score, decrease creatinin serum and decrease the mortality rate because of sepsis.[16]

Copper

Sepsis is often accompanied by acidosis and release cupric ion from seruloplasmin and another proteins. As the needs of oxygen is increasing which not accompanied by oxygen availability is causing ischemia and acidosis in early sepsis and release cupric ion.[17]

Copper is a essential component of several enzymes like superoxide dismutase (SOD), cytochrome oxidase and some coenzymes. It is needed for free radical detoxification, hem formation, antioxidant effect, immune function and synthesis of collagen. Copper consumption is restricted for liver failure and colestasis patients because it is excrete through the gall bladder and will cause toxicity if it heaped.[11]

Some previous studies show that copper inhibits the activity of activated anticoagulant and protein. In addition, copper trigger the epinephrine oxidation to be inactive and adenokrom which is toxic for the heart.[17]

Copper is important for wound healing process, hematopoiesis and as an anti-oxidant. Besides, it is also as co-factor of metalloenzymes and norepinephrine synthesis. Gastrointestinal absorption is not optimal in this process and having low daily requirement. Copper deficiency can cause neuropathy and pancytopenia. Recommended Dietary Allowance (RDA) for copper in adults is 900 mcg and parenteral dosage is 300 - mcg 500 per day.[7]

Vitamin

In sepsis, the ascorbic levels in plasma and cerebrospinal liquid are decreasing. Vitamin C may decrease the expression of ascorbic iNOS and can overcome radical which produced by immune system. Ascorbic also decrease or prevents endotoxin translocation from intestines and directly bactericydal, also it can increase the GSH concentration in liver circulation. Ascorbic also prevents the enzymes reduction in liver and responsible for any endotoxin cleaning.[18]

The 19 RCT meta-analysis results shows that high doses of vitamin E increase the risk of all causes of mortality and it depends on the doses which begin in 150 IU / days.[1] A research conducted by Crimi *et al*., granting high dose of vitamin C and vitamin E can decrease death rate until 67,5 % to 45,7 %.[6]

Vitamins B1 (thiamine) is co-factor of pyruvic dehydrogenase, an enzyme that responsible for pyruphate conversion into asetil-coenzim A. Lack of thiamine can cause pyruvic failure entering enter the tricarboxylic acid cycle so anaerobic metabolism will happen. Thiamine deficiency is correlated to acidosis lactate tacking.[19]

SUMMARY

Micronutrients are nutrient substance (nutrients) required by human body to perform a physiological function in amounts of less than 100% µg per day and consist of vitamins and trace elements. In sepsis case, micronutrients also determine the success of treatment due to redistribution of vitamin and trace element from circulation to the tissue which involved in the proteins formation and immune system.

The conclusions of the latest 7 experiments and 4 random controlled study of multi-centre support the argument that micronutrients supplementation in sepsis may decrease mortality rate. However, it still need to be particularly aware of the possibility of micronutrients toxicity in fat soluble if the doses is excessive.

Zinc is co-factor of more than 200 enzymes that have a role in the immune system, regeneration new cells and balancing acid bases. Normal level is between 70-150 mcg /dl. The low alkaline phosphatase (ALP) can be used as a parameter that the body has low level of zinc.

In sepsis occurs the improvement production of hepsidin so can block Fe transportation. Neutrofil and macrophages need the iron to do phagocytosis and killing bacteria.

Copper is an essential component of several enzymes e.g. superoxide dismutase (SOD). Copper inhibits the activity of anticoagulant and protein C which has been activated. Recommended Dietary Allowance (RDA) for copper in adults is 900 mcg and parenteral dosage is 300 - 500 mcg per day

Selenium uniqueness is having a dual function as pro-oxidant and anti-oxidant. Granting high doses selenium

in sepsis for 9 consecutive days and decrease the dose every 3 days may lower mortality rates, decrease the score of APACHE III and creatinin serum. Maximum dose of selenium 400 µg (5µg/kg BB/day) but even granting 800 µg is considered has no side effects report.

Vitamin C can lowered the iNOS expression.Vitamin B1 (thiamin) is co-factor of pyruphate dehidrogenase, the enzyme which is responsible for pyruphate conversion into asetil-coenzim A.

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Research Report

THE PREVALENCE OF HUMAN IMMUNODEFIENCY VIRUS-1 (HIV-1) SUBTYPES AND TRANSMISSION METHOD AMONG HIV/AIDS INFECTION PATIENT IN TULUNGAGUNG, EAST JAVA INDONESIA

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ABSTRACT

The rapid epidemic growth of HIV is continuing in Indonesia. There are some factors which have influenced the spreading of this epidemic in Indonesia, such as the poor awareness to avoid unsafe free sex attitude and the sharing of needles and syringes among intravenous drug users (IDUs). The sexual transmission of HIV has also apparently increased in Tulungagung. Commercial sex workers play a significant role in the spread of HIV in Tulungagung. People in Tulungagung have worked at other countries as Indonesian migrants. This condition can cause the increase number of HIV-1 case and the possibility of genetic variation (subtype) HIV-1 in Tulungagung. This research is aimed to analyze the subtype and to determine estimation of transmission mode on infected patient of HIV-1 and AIDS who came to Seruni clinic Dr. Iskak hospital in Tulungagung. 40 HIV?AIDSpatients were interviewed to determine the subtype and the transmission mode. The results showed that 14 of 40 plasma samples (35%) were successfully to amplified and sequenced. OverallCRF01-AE wereidentified as predominant subtype among HIV/AIDS patients in Tulungagung. Based on individual information, 31 of 40 subjects (77%) were heterosexual transmission.

Key words: *prevalence, subtype, transmission mode, HIV-1, Tulungagung*

ABSTRAK

Pertumbuhan epidemi HIV di Indonesia berkembang sangat pesat. Ada beberapa faktor yang mempengaruhi penyebaran epidemi di Indonesia, diantaranya kesadaran masyarakat dalam melakukan hubungan seksual secara aman dan berbagi jarum suntik di kalangan pengguna narkoba suntikan (penasun). Pola penyebaran HIV melalui hubungan seksual meningkat di daerah Tulungagung. Pekerja seks komersial memiliki peranan penting dalam penyebaran HIV di Tulungagung. Banyak warga di Tulungagung yang bekerja di luar negeri sebagai imigran Indonesia. Kondisi ini dapat mempengaruhi jumlah kasus HIV/AIDS dan kemungkinan munculnya variasi genetik (subtipe) HIV-1 baru di Tulungagung. Penelitian ini bertujuan untuk menganalisis subtipe dan menentukan estimasi transmisi pada pasien yang terinfeksi HIV-1/AIDS yang datang ke klinik Seruni RS Dr Iskak Tulungagung. Dalam penelitian ini, sebanyak40 pasien HIV/AIDS yang diwawancarai untuk mengetahui subtipe dan polapenyebaran HIV/AIDS. Hasil menunjukkan bahwa dari 40 sampel plasma terdeteksi 14 (35%)sampel positif secara kualitatif. Hasil analisa RIP dan pohon filogentik menunjukkan bahwa 14 sampel tersebut termasuk dalam kelompok rekombinan CRF01-AE. Berdasarkan informasi pasien, pola penyebaran HIV/AIDS melalui heteroseksual sebanyak 31sampel(77%).

Kata kunci: *prevalensi, subtipe, pola transmisi, HIV-1, Tulungagung*

INTRODUCTION

Humanimmunodeficiency virus/aquiredimmune deficiency syndrome (HIV/AIDS) causes a serious health problem and has a big impact on Indonesian economics. In addition, rapidly growing epidemic of HIV-1 is a serious public health problem in Indonesia.

In Indonesia, the number of people living with HIV was estimated to be 591,823 in 2012 and 735,256 in 2015. Whereas newly infected with HIV were estimated to be 71,879 in 2012 and 85,523 in2015[1].

Several factors affect the rate and magnitude of growth of HIV prevalence, but two of the most important are injecting drug use and the commercial sex workers; transmission between men who have sex with men usually has a secondary role [2,3].

In Indonesia, CRF01_AE continually dominates HIV epidemic, although HIV subtype B is responsible for a large amount of infection sexually transmitted. Furthermore, Circulation Recombinant Form (CRF) can occur from one subtype to another one and nowadays it has been found 43 CRF. It is stated that different HIV subtype can have different effect on its transmission, causing drug resistance and disease progressivity[4].

The growth and finding of HIV and AIDS cases in Tulungagung are increasing rapidly and its transmission is getting wider. Supposedly, it is triggered by hidden prostitution activities in two ex-brothels which have been closed down since 2012[5,6]. This research is intended to identify subtype HIV-1 and to know the method of HIV-1 infection transmission in Dr. Iskak General Hospital, Tulungagung.

By doing so, it is very essential to get data analysis on subtype HIV-1 and its infection transmission of HIV-1 at the newest and more representative hospital, Dr. Iskak General Hospital.

METHODS

Study Population and Data Collection

A total of 40 peripheral blood samples from HIV/AIDS patients in Dr. Iskak General Hospital, Tulungagung were collected. A cross-sectional survey, the study population were interviewed in Dr. Iskak General Hospital, Tulungagung using a questionnaire that collected information on socio-demographic characteristics, and transmission.

5-8 ml whole blood samples werecollected from 40 HIV/AIDS patients by using spuit 5 cc. Whole blood sample were added into a EDTA tube. Plasma was then

isolated from peripheral blood samples by centrifugation for 10 min at 2,000 rpm.Plasma were analyzed at HIV/AIDS laboratory, Institute of Tropical Disease (ITD), Universitas Airlangga, Surabaya.

The Examination of Polymerase Chain Reaction (PCR) HIV

The examination of Polymerase Chain Reaction (PCR) HIV was done to identify cDNA, as the following steps: RNA HIV was extracted from plasma and synthesis of cDNA HIV, amplification reaction with PCR, gel electrophoresis, and photo development using gel electrophoresis.

RNA virus was changed into cDNA using Super Script III First-Stand Synthesis Kit (Invitrogen, Carlsbad, CA, USA) with reverse primer, K-env- RI, 5'-CCAATCAGG-GAAGAAGCCTGG-3'.

Fragment HIV-1 gene pol 33 base pair encoding fragment partial integrase was amplified with rested PCR using Ex Taq (Takara Bio, Shiga, Japan) and primary set as follows:

For amplification of fragment gene pol virus, UNIPOL5; 5' –TGGGTACCAGCACACAAAGGAATAGGAG GAAA -3' (nt 4152 to 4183) and UNIPOL6; 5' –CCA CAGCTGATCTCTGCCTTCTCTGTAATSGACC-3' (nt 4934 to 4901) is used for *first PCR*. UNIPOL1; 5' –AGTGGATTCATAGAAGCAGAAGT-3' (nt 4470 to 4492) and UNIPOL2; 5' –CCCCTATTCCTCCCCTTCTT TTAAAA-3' (nt 4806 to 4781) is used for *nested PCR*.

The condition of first PCR: 94ºC for 3 minutes for denaturation (one cycle), 94ºC for 1 minute for denaturation (35 cycles), 72ºC for one minute for extension (35 cycles) and 72ºC for 5 minutes for the last extension.

The condition of second PCR: 94ºC for 3 minutes (1 cycle), 94ºC for 1 minute (35 cycles), 50ºC for 1 minute (35 cycles), 72ºC for 1 minute (35 cycles), 72ºC for 5 minutes.PCR products amplified at the end-point dilution of DNA templates were subjected to sequencing analysisto examine the genomic fragment of the major viral population in a sample.

The Analysis of Sequencing Results

Sequencing analysis on fragment genomic HIV-1 which has been amplified is done by using sequencing cycle kit BigDye Terminator V1.1 with ABI PRISM310 genetic analyzer, and data is analyzed by Genetyx ver10 software. HIV-1 subtyping was carried out using the Recombinant Identification Program (RIP) available on the HIV sequence database website (http://www.hiv.lanl.gov/). In addition, neighbor-joining (NJ) trees with the Kimura two-parameter model were constructed using MEGA6.2 software[7,8].

RESULTS

Demographic and behavioural characteristics

We interviewed 40 HIV/AIDS infected patients who came to Seruni Clinic, Dr. Iskak General Hospital, Tulungagung on June 2014.

Table 1. Sample Characteristic based on Age and Sex

Age (in years)	Gender		Total
	Male	Female	
21-29	7	7	13
30-39	4	9	13
>40	8	6	14
Total	19	21	40
	(47.5%)	(52.5%)	(100%)

Based on questionnaires, it indicates that the number of female patients is 21 (52.5%), age ranges from 30-39 while there are 19 male patients (47.5%). Most of them were over 40.

Table 2. Sample Characteristic based on Age and Transmission Methods

Age (in years)	Transmission way					Total
	Heterosexual	Homosexual	IDUs	Transfusion	Vertical	
21-29	7	4	2	-	-	13
30-39	13	-	-	-	-	13
>40	11	1	1	1	-	14
Total	31	5	3	1	-	40
	(77.5%)	(12.5%)	(7.5%)	(2.5%)	-	(100%)

There were 31 (77.5%) heterosexual patients infected by HIV-1, their ages are various, 30-39 and only 5 persons (12.5%) homosexual patients got infected by HIV-1 as well. They were 20-29 years of age.

Table 3. Sample Characteristic based on Age and Marital Status

Age (in years)	Marital Status				Total
	Married	Unmarried	Widower	Widow	
21-29	9	4	-	-	13
30-39	9	2	1	1	13
>40	12	1	1	-	14
Total	30	7	2	1	40
	(75%)	(17.5%)	(5%)	(2.5%)	(100%)

The data gives us information that there were30 persons (75%), ranges of age are mostly over 40 and 7 persons (17.5%) of 20-29 are not married yet.

Table 4. Sample Characteristic based on Age and The Length of ART Usage

Age (in years)	ART Duration				Total
	Haven't taken ART	< 6 months	6 months – 2 years	< 2 years	
21-29	2	7	2	2	13
30-39	3	1	3	6	13
>40	1	3	4	6	14
Total	6	1	9	14	40
	(15%)	(27.5%)	(22.5%)	(35%)	(100%)

Data obtained from ART indicate that patients who have got ART treatment for over 2 years are 14 (35%). They are mostly 30-39 years old, and over 40 years old. In addition, those 6 persons (15%) of 30-39 did not receive ART treatment.

HIV-1 Subtyping

HIV subtyping has been an important molecular tool for monitoring geographic changes in the worldwide AIDS epidemic[9].Of the 40 samples derived from HIV patients, 12 *pol* genes were succsessefully sequenced. Based on the results of RIP and phylogenetic tree analyses for *pol* genes, overallCRF01_AE has been identified as the predominant HIV-1 subtype, similar to the epidemics in Malaysia, Thailand, and Taiwan[10,11]andno other unique recombinant forms. Our results were consistent with previous findings.

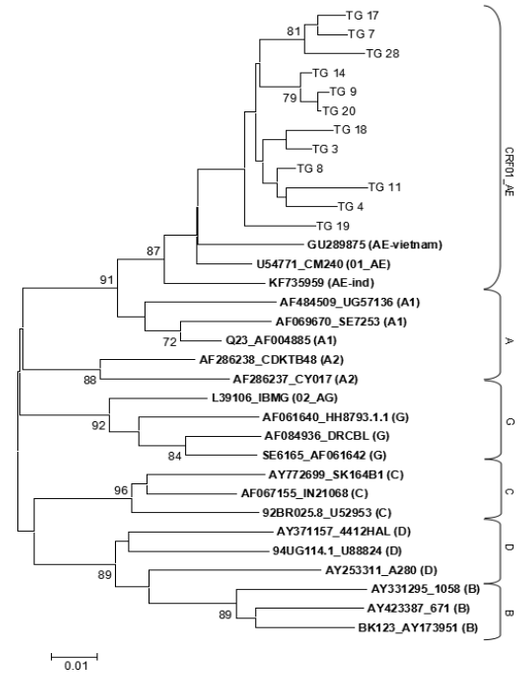

Figure 2. Phylogenetic Tree (Dendogram) Subtype HIV-1

Phylogenetic analysis is done to determine gene sequence pol HIV-1 with strain reference virus gen HIV-1 which shows the plasma sample. Those samples are: CRF01_AE (01_AE). *Accession Number* GU289975 (AE-vietnam) from Vietnam, KF735959 (AE-ind) from Indonesia, AF484509 (A1) from Uganda, AF069670 (A1) from Somalia, AF286238 (A2) from Republic of Congo, AF286237 (A2) from Cyprus, AY423387 (BY) from Netherlands, AY331295 (B) from USA, AY772699 (C) from South Africa, AF067155 (C) from India, AY371157 (D) from Cameroon, AY253311 (D) from Tanzania, AF084936 (G) from Republic of Congo, AF061640 (G) from Finland, L39106 (02_AG) from Nigeria, U54771 (01_AE) from Thailand.

A molecular analysis is done toward nucleotide as a DNA HIV sequence result taken from patient's plasma sample and compared with nucleotide sequences from HIV subtype which has been published before. In later analysis, from HIV-1 pol gene, the all gained sample are CRF, especially one branch with HIV CRF01_AE which comes from Asia. Those are Thailand, Japan, Malaysia, China, and Hongkong.

DISCUSSION

The characteristic of research sample based on age and gender is taken that women are the greatest number who suffer from HIV-1 (52.5%) age ranges from 30-39. This case may happen because the women can easily infected by HIV-1. The greatest number of spreading way is sexual activity with men who are infected. Biologically, the women are easier to be infected with HIV because the flatten areas for HIV are mostly in women (vagina, cervix, and uterus) than in the men (gland penis and urethra). The women are also greater in number who roll out with ejaculate than the men (vagina liquid contains less HIV than semen). The perimenopausal and postmenopausal women who undergo increasing of mucosa genital secondary fragility toward the changing of hormones on its cycle so that it increases the spreading risk of HIV[12].

The characteristic of research sample based on age and assumption of transmission way is reported that heterosexual is the greatest number of transmission way (77.5%) in age between 30-39 years old. Heterosexual is the transmission way which has greatest factor to infect HIV-1 in the world. It becomes worse because of risky sex behavior, for example, having sex with someone with HIV-1 infected risk (a prostitute) or without using condoms.

The second way of transmission is probably homosexual (12.5%) age ranges from 20-mostly 29. It shows that homosexual is risky behavior which can infect HIV-1. There is a trauma in rectum causing wound which can be an easier way of HIV to human body. Rectum consists of lymphoid tissue which can make HIV-1 get into fragile lymphosit cell easily[13].

The characteristic of research sample based on age and marital status is reported that the biggest number is marital status (75%) age ranges from 20-29. The second biggest sample is 17.5% unmarried people with age ranging from 20-29. It has been stated that the biggest sample is married people more than 40 years old when at these age most people have been married and had children. In the previous research, 76% from HIV sufferers are married women. This fact supports the dynamics of HIV-1 infection where in many cases, the first found HIV-1 infections are men who infected their wives.

The characteristic of research sample based on age and assumption of ART duration is reported that the longest ART duration is more than 2 years (35%) ranging from 30-39 years old and more than 40 years old. Most respondent state that they have been diagnosed to be infected by HIV-1 since 2 years ago and at that time, the CD4 examination was done and they continue to take ARV regularly. Medicine drinking obedience is always informed by the medical officer and also by ODHA fellows to others which take the medicine regularly in SeruniClinin Dr. Iskak General Hospital in Tulungagung. The role of counselor, KPA and every element in society has helped medicine drinking obedience and routine control for ODHA community in Tulungagung. The meeting of ODHA community is filled by informing from medical officers (doctors) and also giving the moral supports from ODH fellows to enrich the quality of their lives.

This research explains that the result of blood sample PCR of HIV-infected patients is that the percentage of positive RNA HIV-1 (35%) is lesser from the percentage of negative RNA HIV-1 (65%). The negative PCR HIV-1 result which is quite much is possibly caused by the research subjects who have been given ARV therapy. The research subjects which have not been given ARV therapy are reported to have no many significant differences in the PCR result between negative RNA HIV and positive RNA HIV. On the negative RNA HIV samples in the research, there is possibly a changing or mutation of the nucleotide sequences in the annealing primer. So that the primer cannot attach then it causes the PCR result to be negative.

HIV-1 is marked by the large heterogeneity and divided into 4 groups: M (*major*), O (*outlying*), N (*new or non-M, non-O*) and P *(pending*). The viruses in group M, which are responsible for the HIV pandemic in the world, are further classified into many subtypes and *circulating recombinant forms (CRFs)*. HIV-1 subtype B is the most apparent subtype in USA, Europe, and Australia whereas the subtype non-B is the subtype which causes an epidemic in Africa and Asia. Lately, new CRFs, CRF33_01B and CFRF34_01B, have been isolated in Indonesia[14,15]. In the previous research, it has been found the genome virus fragment CRF01_AE, a CRF is which is dominant in Southeast Asia. That CRF is predominantly found in HIV-positive prostitutes in Surabaya, Indonesia and still a dominant virus strain[16]. In order to know the unidentified subtype,

we need to examine the virus subtype in this research. The genomic fragment of virus CRF01_AE which was found in this research is still the most apparent HIV-1 subtype in Tulungagung, East Java, Indonesia.

CONCLUSION

Based on the research result, it can be concluded that the most found subtype in HIV-1-and-AIDS-infected patients in Dr. Iskak General Hospital, Tulungagung is CREF01_AE. It is relevant with the subtype which develops in Southeast Asia. The greatest number of transmission way in HIV-1-and-AIDS-infected patients in Dr. Iskak General Hospital, Tulungagung is heterosexual.

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Literature Review

MANAGEMENT PATIENT OF SWINE INFLUENZA

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ABSTRACT

Influenza is an acute respiratory diseases caused by various influenza virus which infect the upper and lower respiratory tract and often accompanied by systemic symptoms such as fever, headache and muscle pain. Influenza spreads through the air. Swine influenza comes from swine and can cause an outbreaks in pig flocks. Even this is a kind of a rare case but the swine influenza could be transmitted to human by direct contact with infected swine or through environment that already being contaminated by swine influenza virus. There are 3 types of swine influenza virus namely H1N1, H3N2 and H1N2. Type H1N1 swine-virus had been known since 1918. Avian influenza virus infection is transmitted from one person to another through secret containing virus. Virus is binded into the mucous cells of respiratory tract before it is finally infecting the cells itself. Management patients with H1N1 influenza is based on the complications and the risk. Besides, it is also need to consider the clinical criteria of the patient. Therapy medicamentosa is applied to the patients by giving an antiviral, antibiotics and symptomatic therapy. Prevention can be done by avoid contact with infected animal or environment, having antiviral prophylaxis and vaccination.

Key words: *influenza, swine, infection, H1N1, management patient*

ABSTRAK

Influenza atau flu merupakan penyakit pernafasan akut yang disebabkan oleh bermacam virus influenza yang menginfeksi saluran pernafasan bagian atas maupun saluran pernafasan bagian bawah serta seringkali disertai gejala sistemik seperti demam, sakit kepala dan nyeri otot. Flu dapat menular melalui udara. Flu babi adalah jenis flu yang berasal dari babi dan dapat mewabah pada kawanan ternak babi. Flu babi dapat menular ke manusia melalui kontak langsung dengan hewan babi atau lingkungan yang telah terinfeksi virus flu babi, meskipun hal ini terbilang jarang terjadi. Terdapat 3 jenis virus flu babi yaitu H1N1,H2N3 dan H1N2. Virus flu babi tipe H1N1 telah diketahui sejak tahun 1918. Infeksi virus avian influenza ditransmisikan dari satu orang ke orang lain melalui pembawa virus tertentu. Virus melekat pada sel mukosa pada saluran respirasi sebelum akhirnya menginfeksi sel tersebut. Penatalaksanaan pasien dengan H1N1 didasarkan pada komplikasi dan risiko yang mungkin terjadi. Selain itu, perlu juga ada pertimbangan mengeneai criteria klinis yang dimiliki pasien. Terapi medikamentosa diterapkan kepada pasien dengan memberikan antivirus, antibiotik dan terapi sistomatis. Tindakan pencegahan dilakukan dengan cara menghindari kontak langsung dengan hewan maupun lingkungan yang terinfeksi virus, serta melakukan profilaksis antivirus dan vaksinasi.

Kata kunci: *flu, babi, infeksi, H1N1, penatalaksanaan pasien*

INTRODUCTION

Influenza is an acute respiratory diseases caused by various influenza virus that infect the upper and lower respiratory tract and often accompanied by systemic symptoms such as fever, headache and muscle pain. Influenza spreads through the air. Several epidemical evidence of influenza cases showed high morbidity rate especially for patient with higher risk caused by pulmonary complication.[1,2]

Swine influenza comes from pig and could cause an outbreaks in pig flocks. Clinical signs of this disease are fever, appetite loss, weight loss, sluggishness, cough, sneezes, cold and asphyxiate. Pig incubation usually given

in 1 to 3 days. It has low mortality rate (1% to 3%) and the infected animals are usually getting better in 5 until 7 days. Some pigs are showed a severe pneumonia symptoms as a major cause of death. Secondary bacteria infection could occurs. The process of viral internalization could be happened within 24 hours after infection and become disease after 7 to 10 days.[3,4,5]

Animal to human swine influenza virus transmission is rarely happen. But it can be transmitted through a direct contact between infected pig and human, also by living in environment that already being contaminated with swine influenza virus under special circumstances. Once human are infected, they could transmitted the virus to one and another with the same way of influenza virus works through cough or sneeze.[3]

There are 3 type of swine influenza virus namely H1N1, H3N2 and H1N2. Swine virus type H1N1 has been known since 1918, causing pandemic and death of 40-50 million people. This virus was dissapear until in year 1957 Influenza A (H2N2) pandemic was infected 250.000 people in Hongkong within 3 weeks before it abated. In 1968, another influenza pandemic caused by Influenza A (H3N2) was named Hongkong Influenza. In 1977, epidemic swine influenza (H1N1) was re-emerge in Fort Dix, New Jersey and infected more than 200 people, some of them were severe and one was dead.[6,7]

In March 2009, Influenza A (H1N1) first emerged in Mexico, then it was reported in United States and spread in some parts of other countries, including Indonesia. On April 2009, WHO was announced the occurrence of the pandemic phase 5. Until October 2009, 195 countries were reported their Influenza A (H1N1) cases.[5,8]

INFLUENZA A VIRUS

Influenza virus A is the RNA virus which is the member of the Orthomyxoviridae family. There are 3 type of influenza virus which are Influenza virus A, B and C. RNA nucleus consist eight segments of genee and surrounded by 10 layers of protein (influenza A) or 11 layers of protein (influenza B).[2]

Influenza virus A is divided into subtype based on two protein on the surface, Hemaglutinin (H) and Neuraminidase (N). There are 16 kind hemagglutinin subtype and 9 neuraminidase subtype.Virus type is based on these proteins, for example Influenza A subtype H3N2 which express Hemaglutinin 3 and Neuraminidase 2. Influenza A has 16 kind of H subtype and 9 kind of N subtype. Geneome Influenza A and Influenza B have eight segments single stranded RNA which coding the structural and non structural protein.[3]

Influenza A is more pathogeneic than Influenza B. Influenza A is zoonosis infection and usually infect the most of birds, pigs, horse, dogs and humans. Reasort genees segment can occur when influenza virus infects a cell simultaneously.[2]

H1N1 virus called as swine influenza because the laboratory test showed that several genees in this virus was equal to influenza virus that infect the swine in North America but further studies show that these two viruses are different. HINI virus has 2 genees of the common influenza virus which usually possessed by European swine called gene NA segment, gene HA segment, gene NP segment and gene NS segment from the classical swine and triple reasortment swine. PB2 and PA derived by triple reasortment swine which come from avian species and PB1 derived by swine triple reasortment from human.[9]

PATHOPHYSIOLOGY

Avian influenza virus infection is transmitted from one person to another through secret containing virus. Virus is binded into the mucous cells of respiratory tract before it is finally infecting the cells itself. Influenza infection is occured after secret transfer in the exhalation from one to another. If it is not neutralized by antibodies, virus would strike the respiratory tract and the cells. In host cell, after cellular dysfunction and degeneeration happens, along with replication of the virus and release of viral replication. Almost all cells in the respiratory could support replication of the virus. After the virus has led to respiratory tract infections, the virus infects many cell replication and damage epithelial cilia through direct cytopatic effect or apoptosis. The amount of virus in the respiratory tract is correlated with the severity of the disease and the degree of inflammatory cytokine pro-kemokin. Increased levels of cytokines pro inflammatory such as interferon α, interleukin 6, tumor necrosis factor occur in the blood and respiratory secretions. And may contribute to systemic symptoms and fever. Duration of viral replication depend on the age and the invulnerabILIty status. Shelding usually lasting during three to five days in the adult, often reaches the second week in children, and could survive for weeks on a host immunocompromised.[1]

CLINICAL SYMPTOMS

After the incubation period is complete 1- 5 days, the onset of disease is tend to increase rapidly were actually causing symptoms. Clinical symptoms divided into specific symptoms and non specific, based on CDC (2009) stricken with symptoms reported is: cough (98 %), febris (96%), weak (89 per cent), headache (82 %), cold (80 %), muscle pain (80 %), nausea (55 %), abdominal pain (50 %), diarrhea (48 %), shortness of (48 %), joint pains (46 %). The symptoms above are known as ILI-fever (Influenza Like Illnes) which has temperature more than 39,8^{o}C accompanied by one or more symptoms of cough ingest, pain on swallowing, without any causes other the influenza.[10]

There are mild criteria (outpatient with surveillance) such as without a symptom or symptoms at least, without fever tightness, no pneumonia, no comorbid and young age. Middle criteria (isolation room) such as comorbid factor, asfixia, pneumonia, old age, pregnancy, diarrhea and vomiting. Severe criteria (ICU) such as pneumonia broad, breath failure, sepsis, shock, declining consciousness, Artery Respiratory Distress Syndrome (ARDS) and Multiple Organ Dysfunction Syndrome (MODS).[8]

CASE IDENTIFICATION

Definitin of cases

A. Cases of alleged (suspect): someone with symptoms ILI accompanied by history:
   - Contact with the confirmation influenza virus H1N1 2009, 7 days before sick.
   - Visit where areas there is one or more cases of influenza virus with new confirmation H1N1 2009, 7 days before ILI
   - Reside in areas where there is one or more cases confirmation.

B. Probable case: someone with symptoms alleged (suspect positive laboratory result of influenza but cannot detect the subtype, with the ILI in accordance with clinical symptoms who died because of failed cause of breath which could not be explained and related with epidemology with a case or confirm probable.

C. Must case (confirmation examination): someone from the laboratory is infected by a new influenza virus H1N1 2009 through one or more examination such as Real-Time (RT) PCR, virus culture and increasing 4 times in antibiotic specific influenza a H1N1 viruses with new tests in the neutralization.[8]

CLINICAL FINDINGS

Patients with must case (confirmed) H1N1 virus 2009 with symptoms of the ILI to pneumonia based on the degrees:

1. People with mild state (confirmed): must be the case of the symptoms of ILI fever > 38^0c (cough without pain ingest) with ILI symptom.
2. Heavy state: someone with a must case be obtained (confirmed) clinical syndrome which is ILI accompanied by respiratory infection is a low breath and pneumonia, failed a breath, dehydration and until death. Occurring severe condition previously to be a chronic disease.

Weight degree criteria : fever > 38^o C, Rr> - 24 x/ minutes, hipoxia (SO2 < 90 %), disfungsi organ hipotensi (<90/60), declining conscious, tachicardia, hiperglikemia, photo thoraxabnormal.[11]

Complication ILI :

1. Severe primary viral infection by fail of breath: happens when lung infection viruses infect with clinical symptoms, is not good in the thorax to infiltrate intersisial, and there's a sign ards with severe hypoxia.
2. Pneumonia bacteria, patients often complained of a fever and respiratory symptoms arise who had already died down.Bacterial pathogenes to infect *Staphylocuccus aureus* and are often *Steptococcus pneumonia* and *Hemofilus influenza*.
3. A bacterial virus pneumonia and pneumonia, often occurs in patients who had the disease; a chronic previous (PPOK asthma, DM, GGK, heart failure etc)

Patients with ILI having a high risk occurring complication such as children under 5 years, age above 65 years, child or adult who gets therapy aspirin long term, pregnant woman, patients with comorbid factors and immunodeficiency.[4]

LABORATORY EXAMINATION

Virology Examination

A lot of tests available to detect influenza virus. This test has different sensitivity and specificity. Including several examinations which can be seen in Table I.

Specimens can be obtained through: nasopharyngeal swab, aspirate (wash or nasal swab) swab the endotracheal lavage, bronchoalveolar lavage (BLA), combination of nasopharyngeal or oropharyngeal swab. Oropharyngeal. Tests should be done on the first week until healing (2 - 3 weeks after the onset of disease). Examination of virology should be done in patients with an indication of in-patient medium and heavy.[10,12]

Hematology Examination

DL examination (Hb, Platelets, leucocyte, count of leucocyte, a lymphocyte total). Other examination depends on identification, such as chemicals analysis of the blood. To remove the diagnosis of appeals examined IgG and IgM opposed to culture week and gall *Salmonela thyposa*.

Radiology Examination

Examination of the lateral thoracic pa and their routines; if necessary you can be intensified CT scans in accordance with the indications.

Microbiology Examination

If in thought occurring co-infection by bacteria can detect by sputurn (smear and culture), culture blood and urine based on identification.[10,12]

Table 1. Comparison of sensitivity and specificity of influenza and diagnostic test the CDC, 2009.

Influenza Diagnostic Tests	Method	AvailabILIty	Typical Processing Time[2]	Sensitivity[3] for 2009 H1N1 influenza	Distingushes 2009 H1N1 influenza from other influenza a viruses ?
Rapid influenza diagnostic test (RIDT)[4]	Antigene detection	Wide	0.5 hour	10 – 70 %	No
Direct & indirect immunofluorescence assays (DFA and IFA)[5]	Antigene detection	Wide	2 – 4 hours	47 – 93 %	No
Viral isolation in tissue cell culture	Virus isolation	Limited	2 – 10 days	-	Yes[6]
Nucleic acid amplification test (including rRt – PCR)[7]	RNA detection	Limited[8]	48 – 96 hours (6 – 8 hours to perform test)	66 – 100%	Yes

Table 2. The organisation antiviral drug use, organisation, 2009

Population	Pandemic (H1N1) influenza virus 2009	Multiple co-circulating influenza A sub-types or viruses with different antiviral susceptibILIties	Sporadic zoonotic influenza A viruses including H5N1
Mild to moderate uncomplicated clinical presentation			
At-risk[a] population	Oseltamivir or zanamivir (04)	Zanamivir or oseltamivir plus M2 inhibitor[b] (10)	Oseltamivir or zanamivir
Otherwise healthy[c]	Need not treat (03)	Need not treat (09)	Oseltamivir
a. Infants and children aged less than 5, the elderly (>65years), nursing home residents, pregnant women, patients with chronic co-morbid conditions such as cardiovascular, respiratory or liver disease, diabetes, and those with immunosuppression related to malignancy, HIV infection or other disease.			
b. Amantadine should not be used in pregnant women (recommendation 12)			
c. All those not covered by the at-risk definition above.			
Severe or progressive clinical presentation[d]			
At-risk[a] population	Oseltamivir (01) (zanamivir should be used where virus is known to be resistant to oseltamivir, or if oseltamivir unavailable) (02)	Oseltamivir plus M2 inhibitor[b], or zanamivir (05,06,07)	Oseltamivir plus M2 inhibitor
Otherwise healthy			
d. See section 2 Case Description. Would include all patients requiring hospitalization			

MANAGEMENT

Management new patients with influenza a H1N1 2009 was based on some new consideration of the degree of the sick the risk of complications and the results of laboratorium with the criteria consideration adjusted therapy the patient whether policlinic, in-patient, the icu. Almost of influenza cases caused by a new influenza virus H1N1. There is no complications, short duration and the provision of anti virus tend to be needed for the majority of patients.[13]

Management in general

1. Mild case : supportive therapy such as providing an antipyretic rehidration
2. Sufficient case : symptomatic therapy, rehydration, antiviral, antibiotic, if secondary infection was proven, fluids and nutrition therapy
3. Severe case (care ICU) : correction hypoxia with oxygene provision and ventilator assemblies for management strategy of ARDS, monitor hemodynamics for septic shock management, antiviral and antibiotic, parenteral and enteral nutrition

Table 3. Recommendations antiviral a dose of influenza a new H1N.

Oseltamivir	
Oseltamivir is indicated for treatment of patients one year of age and older. For adolescents (13 to 17 years of age) and adults the recommended oral dose is 75 mg oseltamivir twice daily for 5 days. For infants older than 1 year of age and for children 2 to 12 years of age recommended doses are as follows :	
15 kg or less	30 mg orally twice a day for 5 days
15 – 23 kg	45 mg orally twice a day for 5 days
24 – 40 kg	60 mg orally twice a day for 5 days
40 kg	75 mg orally twice a day for 5 days
Zanamivir	
Zanamivir is indicated for treatment of influenza in adults and children (>5 years). The recommended dose for treatment of adults and children from the age of 5 years is two inhalations (2 5mg) twice daily for 5 days	

Table 4. Recommendation for giving antibiotics to influenza patients

For influenza not complicated by pneumonia a. Previously well adults with acute bronchitis complicating influenza, in the absence of pneumonia, do not routinely require antibiotics. b. Antibiotics should be considered in those previously well adults who develop worsening symptoms (recrudescent fever or increasing dyspnoen). c. Patients at high risk of complications or secondary infection should be considered for antibiotics in the presence of lower respiratory features. d. Most patients can be adequately treated with oral antibiotics. e. The preffered choice includes co-amoxiclav or a tetracycline f. A macrolide such as clarithromycin (or erythromycin) or a fluoroquinolone active against *Streptococcus pneumoniae* and *Streptococcus aureus* is an alternative choice in certain circumstances.
Non-severe influenza-related pneumonia a. Most patients can be adequately treated with oral antibiotics b. Oral therapy with co-amoxiclav or a tetracycline is preffered c. When oral therapy is contraindicated, recommended parenteral choices include iv co-amoxiclav, or a second- or third-geneeration cephalosporin (cefuroxime or cefotaxime). A macrolide (erythromycin or clarithromycin) or a fluoroquinolone active against *S. pneumonia* and *S. aureus* is an alternative regimen where required, e.g. for those intolerant of penicillins. Currently, levofloxacin and moxifloxacin are the only recommended fluoroquinolones licensed in the UK.
For severe influenza-related pneumonia a. Patients with severe pneumonia should be treated immediately after diagnosis with parenteral antibiotics. b. An iv combination od a broad-spectrum β-lactamase stable antibiotics such as co-amoxiclav or a second-geneeration (e.g. cefuroxime) or third-geneeration (e.g. cefotaxime) cephalosporin together with a macrolide (e.g. clarithromycin or erythromycin) is preffered. c. An alternative regimen includes a fluoroquinolone with enhanced activity against pneumococci together with a broad-spectrum β-lactamase stable antibiotic or a macrolide. Currently, levofloxacin is the only fluoroqionolone with an iv formulation licensed in the UK.

The management of patients are not separated from the medikamentosa therapy provision:

Antivirus

Influenza virus A H1N1 sensitive to neuraminidase inhibitor (Nals) namely oseltamivir and zanamivir, but resistant to amantadine or rimantadin. The antiviral could reduce the disease alleviate symptoms, for illness, progresivity at preventing disease and death and decrease hospitalization. Based on the research the antiviral beneficial is given the maximum 48 hours after the onset of the disease, 5 days in therapy for patient hospitalization, patients with severe infections or in ICU patients could be given more therapy.[8,13]

Recommendations given of the antiviral that is hospitalized patients with the confirmation, probable or suspect, patient has a high risk, if there are complications and patients with chronic disease.

Antibiotics

Antibiotics can be given if the result of examination positive and secondary infection symptoms is caused by bacteria. Recommendation for giving antibiotic to patients with influenza depend on British Thoracic Society (BTS) can be seen in table 2

If based on clinical and radiological, influenza - the assessment of the weight of pneumonia associated pneumonia can stamp use standard score as PSI, CRUB

Table 5. Regimen antibiotics for infection by bacteria in patients after the flu.[14]

Antibiotic regimen (doses provided are for adults)	Antibacterial activity	Ecological risk
Outpatients		
Doxycydine 200mg once and then 100mg every 25h	SP, strep, H, MC, MSSA, MRSA, atypicals	Low
Co-amoxiclav 625mg every 8h	SP, strep, MC, MSSA, GNEB	Moderate
Erythromycin 500mg every 6h or clarithromycin 500mg every 12h	SP, strep, MC, MSSA, atypicals	moderate
Levofloxacin 500mg every 24h or moxifloxacin 400mg every 24h	SP, strep, H, MC, MSSA, GNEB, atypicals	High
Non-severe inpatients oral (as for outpatients [see above)]		
Non-severe inpatients, iv (for non-severe inpatients unable to take oral therapy)		
Benzylpenicillin 1.2g every 6h	SP, strep	low
Amoxicillin 500mg to 1g every 8h	SP, strep, H	moderate
Clarithromycin 500mg every 12h	SP, strep, MC, MSSA, atypicals	moderate
Flucloxacillin 1g every 6h plus clarithromycin 500mg every 12h	SP, strep, MC, MSSA, atypicals	moderate
Co-amoxiclav 1.2g every 8h	SP, strep, H, MC, MSSA, GNEB	moderate to high
Amoxicillin 500mg to 1g every 8h plus clarithromycin 500mg every 12h	SP, strep, H, MC, MSSA, atypicals	moderate to high
Cefuroxime 750mg every 8h or cefotaxime 1g every 8h	SP, strep, H, MC, MSSA, GNEB	high
Levofloxacin 500mg every 24h	SP, strep, MC, MSSA, GNEB, atypicals	high
Severe inpatients (patients who require critical care, whether a bed is available or not; all regimens to be given iv initially)		
Co-amoxiclav 1.2g every 8h plus clarothromycin 500mg every 12h	SP, strep, H, MC, MSSA, GNEB, atypicals	moderate to high
Cefuroxime 750mg every 8h or cefotaxime 1g every 8h plus clarithromycin 500mg every 12h	SP, strep, H, MC, MSSA, GNEB, atypicals	high
Levofloxacin 500mg every 24h	SP, strep, H, MC, MSSA, GNEB, atypical	high
Benzylpenicillin 1.8 – 2.4 g every 4h plus clindamycin 600mg every 6h	For proven life-threatening Lancefield group A, C or G streptococcal infection	high
MRSA (in the UK, only for patients with proven MRSA)		
Linezolid 600mg iv / oral every 12h	SP, strep, MSSA, MRSA	low
Other agenets that may be useful in specific circumstances (e.g. if microbiological results are available)		
Trimethoprim 200mg oral every 12h	H, some MSSA/MRSA, some GNEB	low
Co-trimoxazole 960mg oral every 12h (iv; 960mg to 1.44g every 12h)	SP, strep, H, MC, MSSA, some MRSA, GNEB	low to moderate
Piperacillin/tazobactam 4.5mg iv every 8h	SP, strep, H, MC, MSSA, GNEB	moderate
Tigecycline 100mg iv once then 50mg is every 12h	SP, strep, H, MC, MSSA, MRSA, GNEB, atypicals	unclear, probably low to moderate

– 63. Regimen antibiotics for bacterial infection in patients with swine flu can be seen in Table 5

Cortikosteroid

Not give a corticosteroid routine in patients by a new, H1N1 influenza based on reports of Mexico granting a corticosteroid unprofitable.A corticosteroid use higher doses will cause serious side effects due to rising replication of the virus and improve the germ opportunistic infection.[11]

PREVENTION

1. Be careful when contact with persons infected who is someone who can transmit H1N1 virus days before the onset of diseases such as 1 to 7 days, and after the onset of disease (24 hours no fever). An act done is cover nose and mouth or use a mask, wash hand soap and water or alcohol in the normal time 15 until 20 seconds,

Table 6. Antiviral prophylaxis drugs the who recommendations

Agenet	Age Groups (yrs)					
	Duration	1 - 6	7 - 9	10 - 12	13 – 64	≥65
Oseltamivir						
	Begin as soon as exposure identified and continue for 5 -7 days after last known exposure	Weight adjusted doses : - 30 mg/day for ≤15 kg - 45 mg/day for ≤15 kg - 60 mg/day for ≤15 kg - 75 mg/day for ≤15 kg			75 mg/day	75 mg/day
Zanamivir						
	Begin as soon as exposure identified and continue for 5 -7 days after last known exposure	1 – 4 yrs : NA	5 – 6 yrs : 10 mg (2 inhalations) once daily	10 mg (2 inhalations) once daily	10 mg (2 inhalations) once daily	10 mg (2 inhalations) once daily

eat nutritious food, less migration to the epidemic and pandemics.

2. Antiviral prophylaxis can be given to someone with the acts of close contact patients confirmation, case probable, infectious, suspect during the period of high risk patients experienced complications and health officers contact with confirmation, cases of patients probable, suspect infectious table during the period. [14]

Vaccination CDC identify priority to get population main vaccination are pregnant woman, all medical society, age 25 - 64 by the risk of complications and someone who care for infants under 6 months.[13]

SUMMARY

Influenza is respiratory infections caused by influenza virus a subtype H1NI. Virus H1N1 could cause a new transmission between humans. It is because a mutation in the virus.Virus H1N1 influenza totally different with the H1N1 virus seasonal.Known for its clinical symptoms I*nfluenza Like Illness* (ILI) WHO clinically divide light being, weight. Management patients with the H1N1 influenza a new year, in some degree was based on the consideration of the complications and the risk of a laboratory. Consideration also according to this criteria of patients are policlinic, inpatient, icu. Therapy medikamentosa be granted antiviral, antibiotics and therapy symtomatic. Prevention is to avoid contact with an infected, the antiviral prophylaxis, and vaccination.

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THE EFFECT OF *GENDARUSSIN* A ISOLATES OF *JUSTICIA GENDARUSSA BURM.F.* LEAF IN REVERSE TRANSCRIPTASE INHIBITION OF HIV TYPE I IN VITRO

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ABSTRACT

Screening has been done to a few extracts from the leaves Justicia gendarussa Burm.f to see the growth rate of the virus from the blood plasma of HIV patients at Dr Soetomo Hospital. It is known that J. gendarussa leaf extract inhibits HIV type 1 reverse transcriptase. In addition, its main content is gendarussin A, besides gendarussin B, JGF1, JGF2 and JGF3, which have just identified. At the beginning, extraction and fractionation were performed with 3 models that highlight the absolute methanol, 70% methanol and 70% ethanol with the release of alkaloids. Furthermore, samples of each fraction were incubated in plasma of HIV patients with a titer of 3.6 10^6 copies for 1 h in concentrations of 1.64 ppm, 4.1 ppm, 8.2 ppm, 16.4 ppm and 41.0 ppm. After incubation, examination was performed by using Nucli sens a machine, which is a combination of PCR and Elisa, thus avoiding direct contact with the highly pathogenic virus. The result showed that the activity sequence from the most potential to the weak, among others, was 1.64 ppm > 4.1 ppm > 8.2 ppm > 16.4 ppm > 41.0 ppm, each with barriers value of 0.62 10^6, 1.4 10^6, 1.6 10^6, 2.4 10^6 and 5.2 10^6 cells/ml. In conclusion, highest anti-HIV activity comes from the concentration of gendarussin A isolate at 1.64 ppm. Furthermore, after linear regression of y = -3.063 x + 81.37 was done, the IC_{50} of 10.24 ppm was obtained.

Keywords:*, Justicia gendarussa, gendarussin A,reverse transcriptase, inhibition, anti HIV*

ABSTRAK

Penelitian telah dilakukan pada beberapa ekstrak daun Justicia gendarussa Burm.f untuk melihat angka pertumbuhan virus plasma darah pasien HIV di Rumah Sakit Dr. Soetomo. Telah diketahui bahwa ekstrak daun J. gendarussa menghambat HIV tipe 1 reverse trancipitase. Selain itu, kandungan utama dari J. gendarussa adalah gendarussin A disamping gendarussin B, JGF1, JGF2, dan JGF3 yang telah diidentifikasi. Pada awalnya, ekstraksi dan fraksinasi ditunjukkan oleh 3 model yang ditandai oleh absolute methanol, 70% methanol dan 70% ethanol dengan melepaskan alkoid. Selanjutnya, sebagian dari masing-masing sampel diinkubasi ke dalam plasma HIV pasien HIV dengan titer 3.6 10^6 sebanyak masing-masing 1h dengan konsentrasi 1.64 ppm, 4.1 ppm, 8.2 ppm, 16.4 ppm dan 41.0 ppm. Setelah inkubasi, pengujian ditunjukkan menggunakan mesin Nucli sens yang dikombinasikan dengan PCR dan Elisa untuk menghindari kontak langsung dengan virus yang memiliki resiko pathogen tinggi. Hasil menunjukkan bahwa rangkaian aktivitas tersebut dari yang paling berpotensi tinggi ke yang paling berpotensi rendah diantaranya adalah 1.64 ppm > 4.1 ppm > 8.2 ppm > 16.4 ppm > 41.0 ppm dengan masing-masing nilai penghalang 0.62 10^6, 1.4 10^6, 1.6 10^6, 2.4 10^6 and 5.2 10^6 cells/ml. Kesimpulannya. Aktivitas anti-HIV tertinggi diperoleh dari konsentrasi gendarussin A yang dipisahkan pada 1.64 ppm. Selanjutnya, setelah regresi linier y = -3.063 x + 81.37 selesai, diperoleh IC_{50} of 10.24 ppm.

Kata kunci*: Justicia gendarussa, gendarussin A, reverse transcriptase, inhibisi, Anti-HIV*

INTRODUCTION

The transmission of HIV-AIDS in Indonesia continues to widen, particularly in the group of young and productive individuals. Data from the Ministry of Health showed that up to June 2008 there were approximately 6782 individuals aged 20-29 years who suffered from AIDS. The number of people with AIDS in this age group is the highest compared to other groups, the second highest were those with age 30-39 years, comprising only 3539 people. The young generation is as if being chased by this deadly disease, and, unfortunately, the spread of this disease is like an iceberg phenomenon. The problem that comes to the surface is actually just a piece of the reality in the field. Integrated Biological and Behavior Survey (Survey Terpadu Biologi dan Perilaku, STBP) regarding HIV prevalence in Indonesia in 2007 showed that about 43-56% percent of drugs (narcotics, psychotropic substances and additives) users or injection drug users in four cities, Medan, Jakarta, Bandung and Surabaya has been infected with HIV.[1]

HIV-AIDS becomes the fifth leading cause of death in population aged 25-44 years in the United States. At global level, 25 million people have died in vain since the epidemics of this infectious disease and 40.3 million people worldwide are currently living with HIV-AIDS. HIV causes AIDS, the virus attacks immune system and stay in the body that can spur the onset of infection and cancer. Generally, bacteria, yeast, and viruses seem not to become a serious illness when the immune system in healthy condition. This may be different and will be fatal in people who suffer from AIDS. HIV is found in saliva, tears, nervous tissue and spinal fluid, blood, semen (seminal fluid in ejaculate), vaginal fluid and breast milk. But only through blood, semen, vaginal secretions and breast milk generally these infections can be transmitted.[2]

AIDS begins with HIV infection. HIV-infected person may be no symptoms for 10 years or more, but remain infected and can transmit infection to others. Meanwhile, if the infection is not detected or without treatment, immune system gradually weakens, and AIDS develops. Generally, symptoms may present as flu with fever, rash, sure throat, sweats, chills, swollen lymph nodes, weakness and weight loss. HIV infection is associated with decreased CD4 cells, a type of immune cells called "T cell" or "helper cell". Indications of a viral infection is when the number of "CD4 cells" below 350 cells/ml, and, specifically in HIV infection, if CD4 cell count is below 50 cells/ml. Furthermore, for monitoring HIV patients the number of CD4 cells, known as HIV-RNA, is used.[3]

Traditional medicine contributes much to the discovery of new compounds that has anti-HIV activity. There are the plants that have proteins that can inhibit HIV reverse transcription in vitro.[4] Some isolated single chain ribosom inactivating protein (SCRIP) showed the power of the antiviral action of DNA and RNA viruses. For example, MAP30 and TAP 29 are SCRIP protein isolated from *Momordica charantia* seeds and tubers of *Trichosanthes kirilowii*. Both materials can inhibit the replication of HIV-1 infected cells and also the activity of inhibitors of HIV-1 virus associated with reverse transcriptase.[5]Water extracts and 80% ethanol extracts of the plants *Andrographs paniculata, Justicia gendarussa, Vitex trifolia* and *Tinospora crispa* have the activity of inhibitors of HIV-1 reverse transcriptase.[6] Natural materials, particularly the class of polyphenol, have anti-HIV activity, whose action can work, for example, by inhibiting HIV cycle: (1). Virus adsorption, (2) Virus-cell fusion (3) Reverse transcription, (4) Integration, (5) Proteolytic cleavage, (6) Glycosylation and (7) Assembly/release.[7]

Justicia gendarussa is a plant often used by Indonesian people as medicine, either as a drug for internal and external use. As a male contraceptive, research has been carried out on its biological and pharmacological activity, even reaching preclinical and clinical trials phase I and II.[8,9,10] From phytochemical studies, the major component of *gendarussin* A has been identified, in addition to minor components of other flavonoids. There is also alkaloid content Bhagya. Furthermore, the anti-HIV screening showed that 70% EtOH extracts *Justicia gendarussa* in 100 ppm can reduce HIV growth inhibition (viral load) to 1.47 10^5 copies/ml after incubation of 60 minutes. It is even more potential if *gendarussin* A isolates are used.[10]Due to the high potential of *gendarussin* A isolates and the needs of reconfirmation with different concentrations, it was necessary to carry out reisolation, which is quite difficult, even it has already standardized method. This effort is aimed to gain the stabil gendarusi A level in extracts. Due to humanity consideration, this research is very important, since until now there is no potential anti-HIV drugs originating from Indonesia that have been subjected to clinical trials. Therefore, this study examined the ability of the isolates of *gendarussin* A, a polyphenol compound from *Justicia gendarussa* leaves, as the activity inhibitor of HIV-1 reverse transcriptase.

MATERIAL AND METHODS

Justicia gendarussa leaf powder were obtained from Pacet Mojokerto, Methanol pa, Methanol pro HPLC, *gendarussin* A isolate, JGF1 isolate, JGF2 isolate, JGF2 isolate, and Silica Gel F_{254}.

Some equipments such as Percolator, HPLC Shimadzu LC10AD, coloumn Waters Novapack C18, EasyQ HIV-1 v2.0 Worksheet Nuclisens Magnetic Extraction, Rotavapor BUCHI R-114, Rotavapor BUCHI R-153.

Sample Extraction and Isolation

Justicia gendarussa extract were obtained by maceration of 1.5 kg of powdered *gendarussa* leaves which was soaked with 3 L. methanol for 24 hours with stirring. Extraction was repeated 2 times with the same method and solvent volume. *Gendarussin* A isolate was obtained by several times preparative HPLC running with a 200 µl per injection.

Furthermore, the collections of isolates were subjected to lyophilization with freeze dryer until constant weight was obtained.

Identification of isolate preparation

Amorphous-shaped *gendarussin* A isolate was identified and compared with standard *gendarussin* A isolates through HPLC analysis, ^{1}H-^{13}C NMR, chemical reactions and physical characterization that could be done. Inhibitions to HIV-1 replication were tested in several concentrations from 5, 10, 20, 40, 80, 100 ppm (each in triplicate).

Determination of IC_{50} of *gendarussin* A isolate

Obtained *gendarussin* A isolates was diluted in methanol with various concentrations, which could be done by making more than six points around 10 ppm, and analyzed by probit which would reveal the regression equation, so that the chart pattern would also be obtained between the concentration and the viral load (HIV), and from the extrapolation we would have the IC_{50}.

HIV Monitoring Procedure

EasyQ HIV-1 v2.0 Worksheet Nuclisens Magnetic Extraction was used and it has some steps such as lysis, binding,washing and dilution. In Lysis step**,** lysis buffer tube was centrifugated for 10 seconds at 1500 g, and then 0.1 ml or 0.5 ml or 1.0 ml sample were added into it. The mixed solution was vortexed and was incubated for 10 minutes at room temperature. Then premix solution was prepared by entering 550 CAL diluent into grain-shaped CAL and 550 ul salica solution was added and was vortexed maximum 20 minutes, premix solution must be added to the sample. In binding step, Premix solution was vortexed and 10 µl premix solution was added. The solution then was vortexed and was incubated for 10 minutes at room temperature

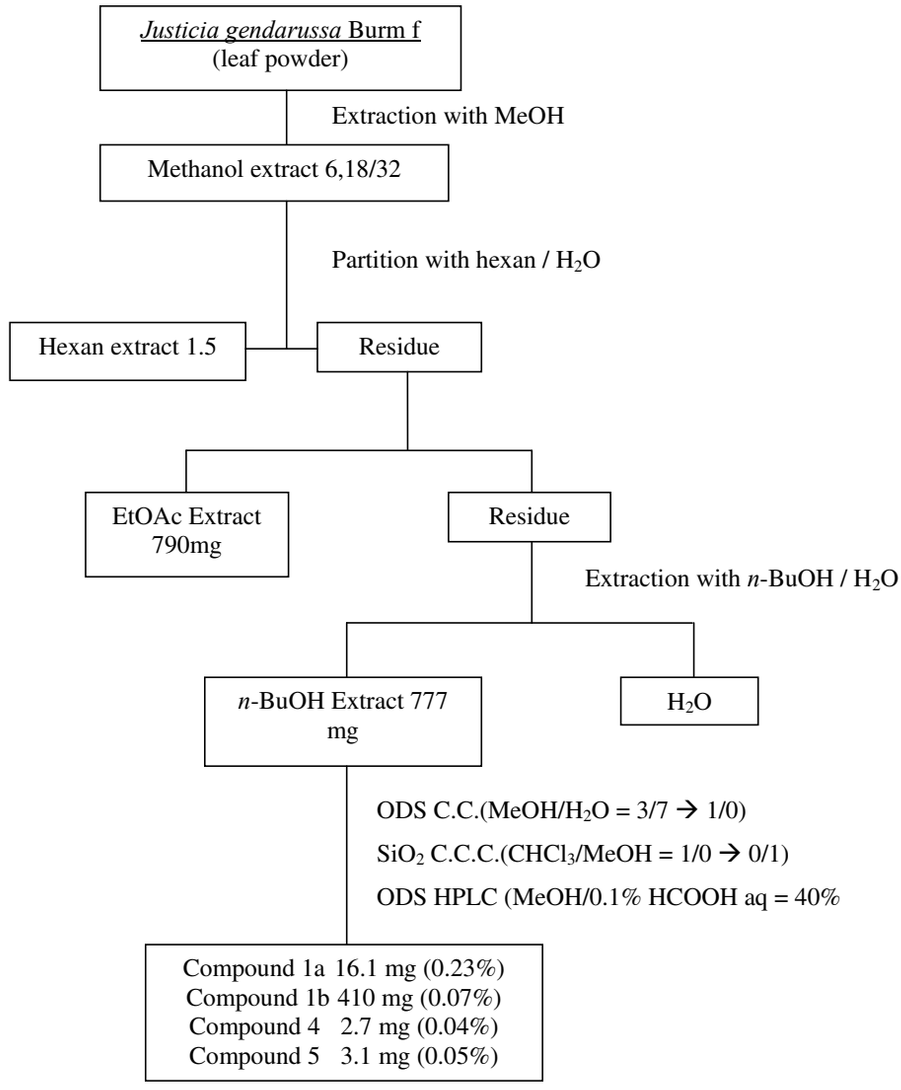

Figure 1. Scheme of flavonoids isolation

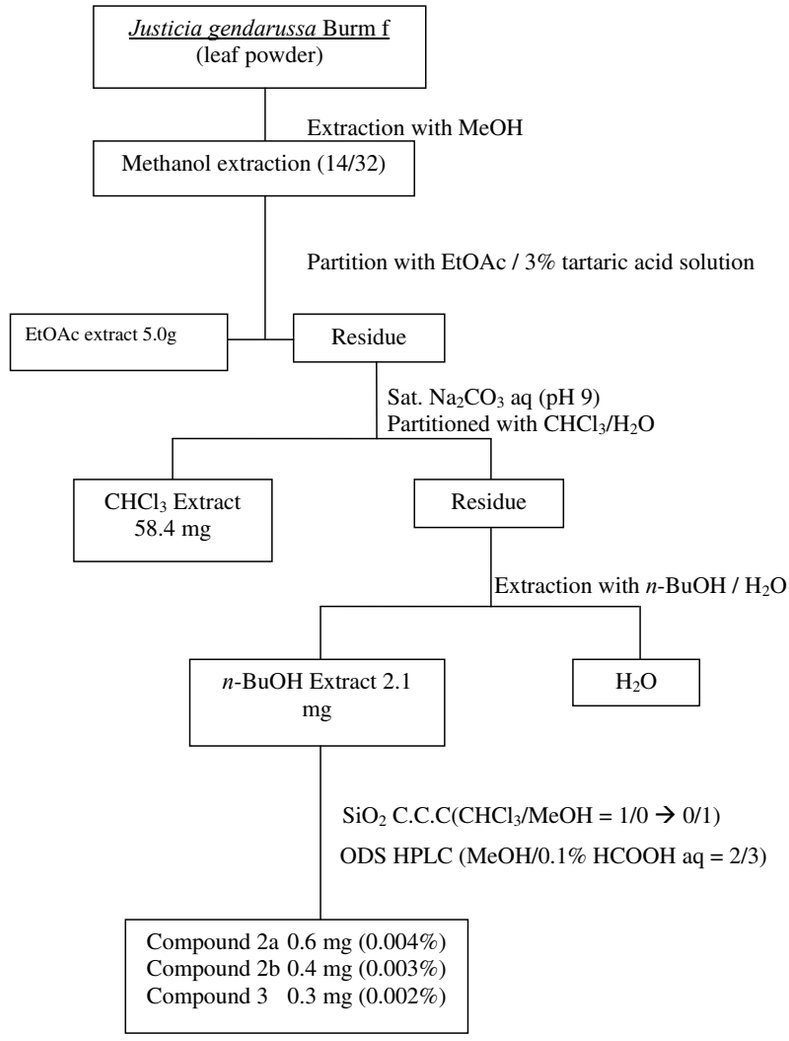

Figure 2. Alkaloid isolation scheme

without homogenization (without mixing). In washing step, the lysis buffer tube was vortexed for 2 minutes at 1500g and then was discarded the supernatant.400 µl wash buffer 1 (transparent) was added, homogenized and transfered this solution into 1.5 ml tubes and was washed for 30 seconds on the menu STEP 1 on the NucliSens mini MAG (magnetic On). After that the supernatant was discarded. 400 µl wash buffer 1 (transparent) (magnet off) was added step 4 and 5 was repeated. 500 µl wash Buffer 2 (red●) (Magnet off) was added and was repeated step 4 and 5. After that step 7 was repeated.500 µl wash Buffer 3 (yellow●) (Magnet off) was added and was washed for 15 seconds on the menu STEP 1 at nuclisens min MAG (magnet on). All supernatant/liquid (magnet on) was discarded. In dilution step, 25 µl elution buffer (yellow●) was added and was incubated for 5 min, 60°C in thermoshaker (speed 1400 rpm). After that tube was placed in magnetic rack with open tube, and 15 µl extracted samples was moved to 8 tube strips in the amplification area or store at a certain temperature.

RESULTS AND DISCUSSION

In some extraction isolation models, the type of solvent, methanol and ethanol, and alkaloid-free or not extract were influenced the quantity of fractions which were obtained. The largest amount is in the polar fraction from the methanol and ethanol in various concentrations. Whereas, in non-polar solvents, like *n*-hexane and chloroform, it was found in small amounts. It is known that the main content in the polar fraction is *gendarussin* A, which is a type of flavonoid glycosides.[11] The process of isolation in butanol fraction revealed isolates JGF1 (2 mg), JGF2 (4.2 g) and JGF3 (1.3 mg), while from chloroform fraction we obtained JGA1 alkaloids (0.96 mg). From the physicochemical analysis, it was found that JGF1, JGF2 and JGF3 are derivatives of apigenin as that in *gendarussin* A and has the same molecular weight of 534.14. They are different only in ribose, xylose and arabinose sugar structures. Then, in JGA1 alkaloid is also observed. It is the derivative of benzyl

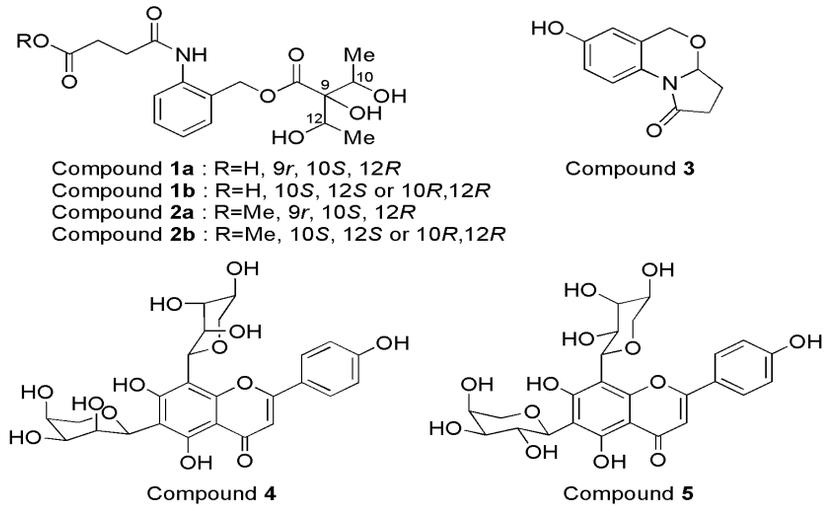

Figure 3. Alkaloid and flavonoid compounds in *Justicia gendarussa* leaves

amino alcohol bound to carboxylic acid on both sides of the core. Of the various isolates, such as *gendarussin* A, JGF1, JGF2 and JGF3 with various concentrations, anti-HIV test was done using Nucli Sens Machine (Table 1). From the results of incubation of isolates tested against human plasma HIV for 60 minutes in vitro, it was shown that the *gendarussin* A isolate provided the strongest activity (3.6 10^6) at 793 ppm compared to other isolates. In various concentrations, it was observable that anti-HIV activity is determined by the viral load. Determination of anti-HIV activity can be seen with inverse proportion between viral load and CD4 count. This indicated that the effect is positive when viral load is lower than the negative control (patients' titer) or CD4 count increases compared to a negative control. If *gendarussin* A isolate provides good effect compared to other isolates, it means that those isolates contain apegenin glycoside compounds with xylose and arabinose sugar. In tested sample 70% ethanol fraction contained 1.4% *gendarussin* A, as determined by HPLC method. In previous clinical trials, it was found that bioavailability test in plasma or blood serum detected *gendarussin* A metabolite, which also appeared in ejaculate and urine. [12] Thus, this in vitro test can then be used as a model of direct interaction with the virus and it has been known previously that the virus growth inhibition is due to the inhibition of transcriptase enzyme function of HIV type 1, whose function is to replicate itself. The certainty of viral death can be tested through the identification of proteins, since the virus is highly pathogenic.

In the HIV viral load measurement results, 200µl sample after 60 minutes incubation is showing the result sample Gendarussin A with concentration 793ppm, JGF_1, JGF_2, JGF_3 show the viral load value 3.1×10^6; 6.4×10^6; 3.1×10^6 and 8.1×10^6 subsequently. This effects of flavanoid compounds in HIV sample could be seen in the Figure 4.

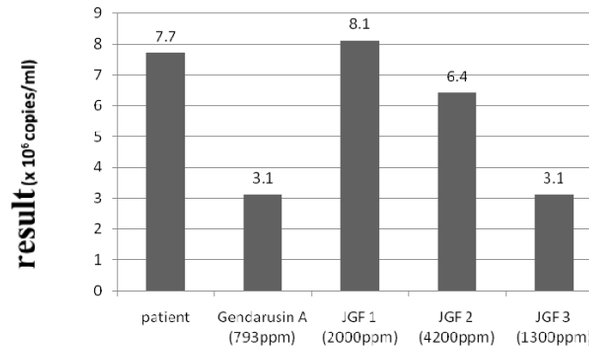

Figure 4. Diagram of the effects of flavonoid compounds in the plasma of HIV in vitro

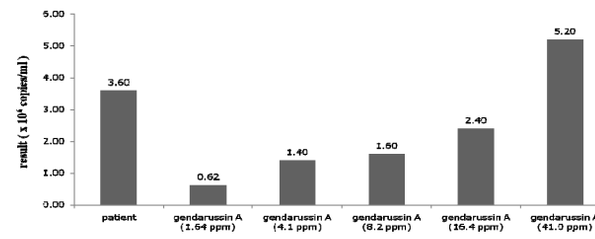

Figure 5. The Effect of Gendarussin A on Human Plasma in Patients with HIV-1

The concentration of Gendarussin A showed the tendency to be increased as high as the viral load result. The data show in Figure 5.

The isolates of *gendarussin* A, JGF1, JGF2 and JGF 3 of the leaf *J. gendarussa* at concentrations 793, 2000, 4200, and 1300 ppm produces viral load 3.1×10^6 ; 8.1×10^6 ; 6.4×10^6 and 3.1×10^6 copies/ml. The Inhibition Concentration 50% (IC_{50}) of *gendarussin* A is 235.3 ppm.

In conclusion, this fact is referred to the protency and strength of *gendarussin* A and other active compound of Jucticia gendarussa leaf in inhibition of HIV replication.

CONCLUSSION

In conclusion, this fact is referred to the protency and strength of gendarussin A and other active compound of Jucticia gendarussa leaf in inhibition of HIV replication.

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