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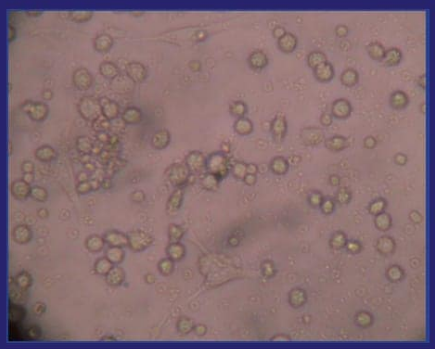

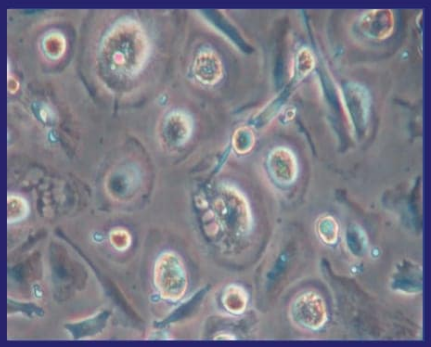

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Research Report

PRODUCTION AND CHARACTERIZATION OF IMMUNOGLOBULINE YOLK AS ANTI ANTIGEN MEMBRANE *TOXOPLASMA GONDII*

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ABSTRACT

Toxoplasma gondii is an obligate parasite intracellular which can infection human and other mammalian. Immunoglobulin Y technology offers several advantages better than antibody production in mammals. This research is aimed to get immunoglobulin Y from egg yolk, and to find the characterization of immunoglobuline Y according to molecular weight by SDS PAGE and targeted protein with antibodies using Western Blot. This research divided from many step: culture tachyzoites of T. gondii fromintraperitoneal fluid, preparation of membrane antigen tachyzoite of T. gondii, then immunization laying hens with membrane antigen, extraction and purification immunoglobuline Y from egg yolk and then protein analyzed by SDS PAGE and Western Blot. The result of this research showed that immunoglobulin Y from egg yolk can produced antibody against protein membrane of T. gondiiandprofile protein immunoglobuline Y according SDS PAGE has molecular weight 179,8 kDa. Immunoglobuline Y was analyze by Western Blot can recognize antigen epitope of T.gondii on molecular weight 35,7kDa and 78,8 kDa.

Keywords: *Toxoplasma gondii, anti membrane T.gondii, immunoglobulin Y anti membrane*

ABSTRAK

Toxoplasma gondii merupakan parasit obligate intraselluler yang dapat menginfeksi manusia dan mamalia lain. Pemanfaatan immunoglobulin Y memberikan beberapa keuntungan dari pada antibodi yang diproduksi mamalia. Tujuan dari penelitian ini adalah mendapatkan immunoglobulin Y dari kuning telur dan menemukan karakterisasi immunoglobulin Y berdasarkan berat molekul dengan SDS PAGE dan penargetan protein dengan antibody menggunakan WESTERBLOT. Penelitian ini dibagi menjadi beberapa tahapan yaitu kultur takizoit Toxoplasma gondii dari cairan intraperitoneal, preparasi antigen membran Toxoplasma gondii, imunisasi ayam dengan protein membrane Toxoplasma gondii, ekstraksi dan pemurnian kuning telur untuk mendapatkan Ig Y dan karakterisasi Ig Y dengan SDS Page serta penargetan protein westrn blott. Hasil SDS PAGE Ig Y ditemukan pita protein 178 kDa- 7038kDa, kemudian analisa penargetan protein dengan westrn blottdapat mengenali antigen epitop Toxoplasma gondii pada berat molekul 35,7kDa dan 78,8kDa.

Kata kunci: *Toxoplasma gondii, membran anti T. gondii, immunoglobulin Y anti membran*

INTRODUCTION

Toxoplasma gondii is an obligate intracellular parasite that can infect humans. Definitive host of this parasite is the cat, while the intermediary hosts include mammals, birds and reptiles nation even fish. In the life cycle of this parasite can infect a host by 3 ways: through ingestion of tissue cysts by bradizoit, by ingestion of oocysts and congenital infection with tachizoit.[1] Cats infected with *Toxoplasma gondii* in all excretions will spend millions of oocysts. When the oocyst is ingested by an intermediate host such as humans, cows, goats on the various tissues will be established intermediate host groups tropozoit actively dividing to form the rest of the stadium in the form of cysts

(bradizoit) on the network. At the intermediate host is not formed sexual stage but only just asexual stage. When cats eat mice containing cysts are formed in the sexual stage in the cat's intestine.[2]

Humans infected with the *T. gondii* occurs not only on those who keep cats or dogs but can also occur in other people who like to eat the food of undercooked meat containing tissue cysts, drinking fresh milk undercooked, water contaminated with raw vegetables and raw contaminated by disease-causing agents toxoplasmosis.[2] Incidence of toxoplasmosis has not significant changed in recent years, caution and attention to these diseases has increased dramatically. About 30-50% of the world population is estimated have been infected by *Toxoplasma gondii*. According to Chandra, Gandhahusada research that conducted in 1995 showed that prevalence toxoplasmosis in humans ranges between 2–63%, 35–70% on cat, 75% in dogs, 11–61% in goats, 11–36% in pigs, and less than 10% on the cow.[3] Research results from Fitria, showed that 46,66% pork intersection in RPH Surabaya positive toxoplasmosis.[4]

The negative impact on the human is very detrimental to the failure of pregnancy and abortion. In human and animal therapy for this disease is very expensive, the impact of livestock on the economic loss due to a decline in production. The administration of drugs such as pyrimethamine and a sulfonamide can kill tachizoit of stadium *T. gondii*, but these treatments are not effective on stage bradizoite. In addition, these drugs are toxic, so is not recommended for use in the long term. Prevention by vaccination not fully provided protection. Using antibodies for controlling is start assess, one of them is making antibodies from the egg yolk. Antigens which used as the immune system host stimulation can come from different parts of the body *T. gondii*. One of them that can be used as an antigen is membrane of stadium tachizoit *T. gondii*. Specific antigen in tachizoit surface are P30, SAG-1, P22 (SAG-2), P35, while the same protein membrane between tachizoit and bradizoite are P23 and P43 (SAG-3).[5] This membrane has immunogenic protein. Some protein major of tachizoit such as P22, P23, P30 and P45 have molecular weight around 20–43 kDa. Membrane protein is able to provoke both cellular immune response (lymphocyte cell, NK cell) and humoral (immunoglobulin), so that many use as diagnostic kit and vaccine.[6] In chronic toxoplasmosis cases, one of the immune system that had the play role is immunoglobulin G (IgG). Some efforts had done to multiply both polyclonal and monoclonal antibody using animal trial. Serum from this animal should be taken to get its antibody and this animal should be sacrificed. It becomes consideration both animal welfare aspect and economics.

The research of IgY usage as passive immunization had been done by Chalghoumi *et al*., on Wilkie, by using *Salmonella enteridis* that was changed into antigen and given to layer hens.[7] The advantage of IgY is not activated the complement, IgY did not bind A and G protein, IgY did not bind the mammals antibody, such as rheumatoid factor which is similar with auto-antibody that react with Fc receptor in IgG and HAMA (Human Anti-Murine Antibodies), IgY did not bind Fc receptor in the surface. The character of IgY which is similar with IgG in mammals, both from its structure and its function.

MATERIAL & METHOD

This research included in laboratory explorative research. Research design was used descriptive analysis. The animals that were used for collecting IgY were layer hens strain with 20 weeks of age, 5 hens were adapted during 2 weeks. *Toxoplasma gondii* passage and cultivation in *Mus musculus* strain Balb/C with 3 months of age. *Toxoplasma gondii* strain RH was used in this research, from Department of Parasitology, Medical Faculty, Universitas Gajah Mada.

Tachizoit *T. gondii* cultivating and harvesting were done by infecting mice with *T. gondii* RH strain isolation by 10^3 for doses in 50 mice Balb/C through intraperitoneally. Protein membrane tachizoit of *T. gondii* was isolated by sonication and centrifugation. Protein concentration was interpreted using spectrophotometry with 595 nm.[6]

In Vitro cultivating and harvesting of tachizoit *T. gondii* were done by using infected mice with isolate *T. gondii* strain RH through 1 x 10^3 on 50 Balb/C mice by intraperitoneal. Isolation of protein membrane tachizoit *T. gondii* used sonication and centrifugation.[6] Protein concentration reader used spectrophotometry with 595 nm. Protein was aliquot and saved on -20° C until it was used.[6] Protein membrane tachizoit of *T. gondii* was analyzed using SDS PAGE.

The chicken that immunization using antigen from protein membrane tachizoit of *T. gondii* by 50 µg that was diluted on PBS and emulted using *Freund's Complete Adjuvant* through 1:1 of ratio until homogenous. Emulsion injected through intra-muscular (on femur). Immunization was repeated twice with 14-days interval. On Repetition immunization, 50 µg of antigen was emulted using *Freund's Complete Adjuvant* then 14 days after second repetition.

Isolation of anti-toxoplasma antibody on yolk used combination of chloroform and ammonium sulfate precipitate method was the chosen method that produce antibody with high purity level.[8] Purification was done by using precipitation of ammonium sulfate 40% and using ratio between IgY supernatant: ammonium sulfate 40% was 1:1. Solution was precipitated one night on 4° C and was centrifuged with 10,000 rpm for an hour.[9] Precipitate was taken for re-suspension using PBS then it was purified and analyzed. Immunoglobulin Y precipitation was covered with specific plastic for sonication, then added 0.5M PBS and stirred using *magnetic stirrer* for 24 hours in 4° C. Characterization was done by reacted antibody from purification with antigen membrane using ELISA method and *Western Bloth*. IgY antibody titter measurement using ELISA.

Immunoglibulin Y protein analyzed using SDS PAGE. Antigenic membrane protein of *T. gondii* was identified using *Western Bloth* method. IgY antibody titter measurement using ELISA.

RESULT AND DISCUSSION

The result of chicken immunization was read using Optical Density level on indirect ELISA. IgY measurement was done after the third immunization booster. ELISA was done on two samples, blood serum and immunized yolk by membrane antigen. Sample consist of yolk that taken before immunization and on the 7th, 14th and 28th day after immunization. The result of ELISA on yolk between before and after immunization of *T. gondii* membrane antigen shows that OD level increased and it significant difference between before and after immunization ($p < 0.005$). The 7th and 14th day after immunization, there were no differences. Result of ELISA OD level can be seen on Table 1.

Immunoglobulin Y was gotten from egg yolk that was extracted with chloroform and precipitated with ammonium sulfate 40% then the protein was analyzed using SDS PAGE.

Marker used for SDS PAGE of immunoglobulin Y could detect protein with molecular weight between 10 kDa-260kDa. Protein molecular weight assessment had done using regression analysis between Rf and BM log. On this research, protein band on marker had line equation $y= -2.501x^5 + 2.920x^4 – 2.202x^3 + 3.467x^2 – 3.253x + 2.597$. On the 2nd column, immunoglobulin without dilution, showed 6 protein bands with molecular weight 179.8kDa, 130.4kDa, 70.6kDa, 59.1kDa, 38.6kDa and 25 kDa. On the 3rd column, immunoglobulin Y with dilution ration 1:5 showed 4 protein bands, 179.8 kDa, 67.4 kDa, 61.6 kDa and 38.6 kDa. On the 4th column, immunoglobulin Y with dilution ration 1:10 showed 4 protein bands, 179.8 kDa, 67.4 kDa, 61.6 kDa and 38.6 kDa. Column 5th, immunoglobulin Y with dilution ration 1:20, showed protein bands with molecular weight were 179.8 kDa dan 67.8 kDa. Concentration of immunoglobulin Y protein from preparation was 0.16 µg/µl.

The result of *Western Blott*, using antigen that were *T. gondii*, was reacted with polyclonal antibody from egg yolk which were Ig Y. Then, it compared with antibody from rabbit that was already immunized using *Toxoplasma gondii* proteins, IgG. Used marker on SDS PAGE could detect protein with 20 kDa–120 kDa of molecular weight. Molecular weight assessment of immunoglobulin Y had been done using regression analysis between Rf and BM log. On this research, protein band on marker had equation as $y = -3.103x^4 + 5.506x^3 – 2.515x^2 – 0.936x + 2.246$. The 1st column was antigen of *T. gondii* membrane which was reacted with rabbit Ig G. This column showed protein band with molecular weight 35.7 kDa. The 2nd and 3rd column were antigen of *T. gondii* membrane that was reacted with chicken IgY and showed protein band with molecular weight 35.7 kDa and 78.8 kDa.

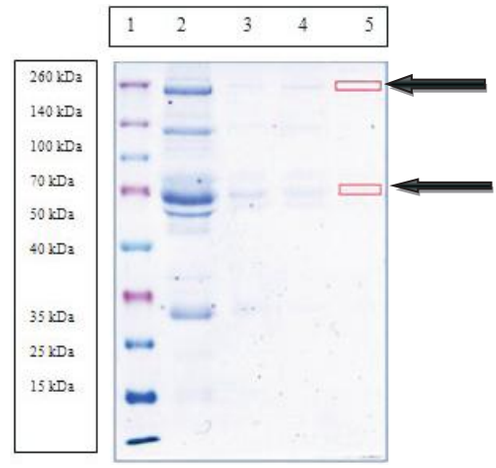

Figure 1. The result of Immunoglobulin Y protein preparation using SDS PAGE

Note: 1st marker
2nd immunoglobulin without dilution
3rd immunoglobulin with dilution ratio 1:5
4th immunoglobulin with dilution ratio 1:10
5th immunoglobulin with dilution ratio 1:20
On the 5th column, there is protein with 67.4kDa and 179.8kDa of weight

Preparation of membrane antigen protein of *Toxoplasma gondii* on tachizoit stadium was continued with characterization using (SDS PAGE) method. This method was the common used of electrophoresis method. Electrophoresis method was used for protein characterization based on molecular weight. The result of SDS PAGE analysis of *T. gondii* membrane protein showed 35.4 kDa, 59.8 kDa; 66.8 kDa; 81.9kDa; 86.8kDa which were more than 118 kDa (Figure 2). Based on Chalghoumi, *et al.,* (2009), 10-100 kDa protein for vaccine were needed

Table 1. Averages of IgY OD level on yolk that was immunized with *T. gondii* membrane antigen

Yolk	OD Level Average	OD Level Deviation
Before Immunization	0.9856[a]	0.5424
7th day after the 3rd immunization	1.5332[b]	0.2201
14th day after the 3rd immunization	1.8873[b]	0.3788
28th day after 3rd immunization	1.8303[b]	0.3640

Different superscript on the same column show significant difference ($p < 0.005$)

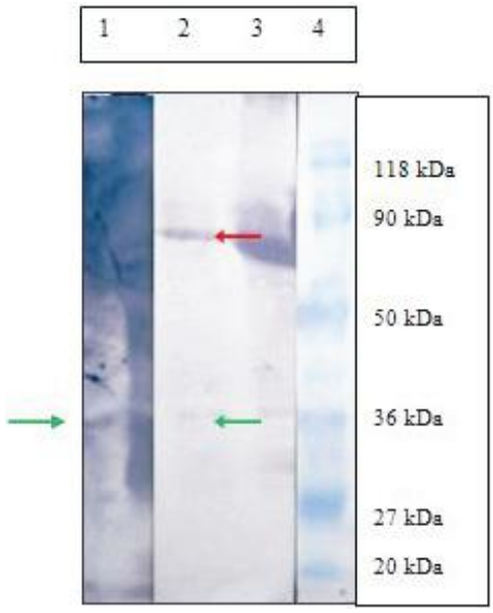

Figure 2. The result of *T. gondii* membrane protein characterization using *Western Blott*. 1st column was *T. gondii* antigen which was reacted with rabbit igG antibody, 2nd and 3rd column were *T. gondii* antigen which were reacted with chicken igY antibody and the 4th column was marker. The red sign was protein with 78.8 kDa of molecular weight, green sign was protein with 35.7 kDa of molecular weight.

to provoke immune response. It fulfill requirement for immunization so hopefully could provoke immune response.[7] From this result was in accordance with the Suwanti's research[6] that major protein on membrane had molecular weight between 60 kDa–200 kDa. 66 kDa–70 kDa of protein also found in membrane and roptry *T. gondii* was proven by Bonhomme et al. (1990) which was citated by Suwanti.[6] It was proven by protein characterization result of P22 recombinant from tachizoit membrane that showed protein band between 35 kDa–40 kDa.[5] Molecular weight of protein band above 118 kDa could not be determined using regression equation between log of molecular weight and Rf from marker, since those protein out of regession line.

Antibody was produced from induced egg yolk with immunization from membrane *T. gondii* protein. On this research, immunization was done via intra-muscular for three times using mix of *Complete Freund Adjuvant* at the 1st immunization and *Incomplete Freud Adjuvant* at the 2nd and 3rd immunization. The aim of CFA and IFA given was for induced the bigger immune response. It caused on CFA consist of protein from death micobactery or component from cell wall of bacteria which had ability to induce both cellular immune response and humoral response against injected protein antigen. Therefore, CFA addition hopefully can form antibody against *T. gondii* antigen membrane.

This research used indirect ELISA model for detected Ig Y, thereby anti-IgY conjugate was needed. The form of IgY similar with IgG was monomer, so the system of IgY also had high affinity against antigen. Fab (antibody fragment) on IgY could recognize antigen epitope more than on IgG. Structure of heavy and light chain of IgG and IgY was relatively similar which was two heavy chains on IgY had molecular weight 67–70 kDa on each chain and two light chain with molecular weight 25 kDa on each. The differences of IgG and IgY only on CH4 chain on Fc.[7,10] OD level on ELISA result both on yolk and on serum which was immunized with antigen of *T. gondii* membrane showed that there was significant different between before immunization and after the 3rd immunization. It showed that antigen of *T. gondii* membrane was immunogenic. Based on Abbas,[10] forming of immunoglobulin antibody would increase on the 2nd antigen exposure with the same antigen type.[11] B cell would produce immunoglobulin after 5 days after antigen exposure and immunoglobulin level would be kept on 23 days.[7] If booster was done every 14 days, the increasing of antibody would occur after the 3rd immunization. On the screening of both yolk and serum on the 7th day and 14th day after the 3rd immunization did not show significantly difference. It indicated that between 7th day and 14th day after the 3rd immunization immunoglobulin Y production was relatively constant on the chicken body. On the 28th day after immunization did not show significantly difference. Antibody testing on chicken serum was done until the 14th day after the 3rd immunization. On this research, protein band with molecular weight 25 kDa and 38.6 kDa might be fragment from Fab IgY on light chain, while protein with molecular weight 59.1 kDa, 61.6 kDa, 67.4 kDa and 70.6 kDa were fragment from Fab IgY on heavy chain. This was in accordance with Michael et al.,[8] who said that two heavy chains on IgY had molecular weight 67–70 kDa on each and two light chain with molecular weight 25 kDa on each.[8] Protein band which had molecular weight 130.4 kDa, 179.8 kDa was probably the fragment from complex bond of Fc receptor Igy (FcR-IgY) and the whole molecule from IgY. This was in accordance with He and Bjorkman, which was said that FcR-IgY complex and whole IgY had molecular weight between 150 kDa–180 kDa.[10] The result of Western Blot using antigen from *T. gondii* membrane which was reacted with polyclonal IgY antibody showed protein band reaction with molecular weight 35.7 kDa and 78.8 kDa (Figure 2). On 35.7 kDa of Western Blot showed that IgY antibody could recognize antigen apitop, that was shown by band reaction between protein from antigen which was 35.7 kDa (SAG 1) *T. gondii* with 38 kDa of IgY molecule (light chain Fab IgY). P30 *T. gondii* (SAG1) was the major protein on RH strain and had molecular weight around 30–38 kDa. Surface antigen (SAG) was protein which took role on attachments. SAG was protein on the tachizoit surface that consist of glicosilphosphatidilinolsitol (GPI) and helpfully gave signal on attachment process between SAG and ligan on the cell surface that would be infected.[12] Western Blott result which had reacted with primary antibody of rabbit (IgG) also showed protein band with molecular weight 82 kDa. It proved that the recognizing epitope of *T. gondii* antigen against Fab IgG of

rabbit was occure. Fab IgG of mammalian on heavy chain had molecular weight 67-70 kDa and light chain 25 kDa.[8] On the result of Western Blott using rabbit IgG antibody showed 35.7 kDa of molecular weight. It meant that Fab IgG could recognize protein of tachizoit membrane and proved there was similarity of Fab structure from IgG and IgY. Fragment from molecular antibody was antigen binding fragment and Fc was crystalizable fragment (constant) as biology effector. On aves, IgY Fc receptor was known as FcRY. In fact, aves FcRY had similarity with FcRn IgG on mammals, whereas FcRn also act as MHC1 which could bind with antigen peptide for T cell. The similarity of FcRY and FcRn was could bind with immunoglobulin molecule on pH ≤ 6 and did not bind on pH ≥ 7. FcRY which bind with the whole IgY molecule had dimer structure with N terminal chain and cyctin receptor for binding peptide from antigen. FcRY bonded on IgY CH4 chain. The differences between FcRY and FcRn were on recognizing ligand receptor of CH3-CH4 IgY and CH2-CH3 IgG whereas IgY ligand had double ability than IgG ligand. Antigen epitope could be recognized by more IgY molecule than mammalian immunoglobulin.[10]

CONCLUSIONS

To sum up briefly, the result from profil analysys of membrane protein of tachizoit *T. gondii* was protein with molecular weight 35.4 kDa, 59.8 kDa, 66 kDa, 81 kDa and 86 kDa. Immunoglobulin Y from egg yolk could produce antibody anti protein of *T. gondii* membrane. Based on Western Blot result, could be concluded that protection mechanism of immunoglobulin Y was on Fab which could recognize epitop of *T. gondii* antigen with molecular weight 35.7 kDa and 78.8kDa

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Case Report

KERION TYPE OF TINEA CAPITIS TREATED WITH DOUBLE PULSE DOSE TERBINAFINE

Tinea Capitis Treated with Double Pulse Dose Terbinafine

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ABSTRACT

Background: *Tinea capitis is a common dermatophyte infection affecting hair and skin which always requires systemic treatment to get a clinical and mycologic cure, preventing relapse, and infection spread. Griseofulvin has been the antifungal therapy of choice for tinea capitis, but it often requires higher doses and a longer duration than recommended. Thus, effective alternative antifungal with good oral tolerability and shorter course of treatment are therefore required. The objective of this report is to evaluate the effectiveness of double pulse dose terbinafine for tinea capitis alternative therapy.* **Method:** *A case of kerion type of tinea capitis in a two-year-old girl was reported. Diagnosis was established based on clinical manifestations of alopecia, presented as erythematous macule with pustules, hemorrhagic crusts, and scales on the scalp, accompanied with occipital lymphadenopathy. Fungal culture showed growth of Microsporum canis (M. canis) colonies. Patient was treated with doubled pulse dose terbinafine 125 mg/day and 2% ketoconazole shampoo for two months.* **Result:** *Clinical improvements were found on 35th day of follow up, while mycologic cure was achieved on 60th day of follow up. Tolerability was excellent and no side effects observed.* **Conclusion:** *Double pulse dose terbinafine is effective for kerion type of tinea capitis*

Key words: *double pulse dose, kerion, M. canis, terbinafine, tinea capitis*

ABSTRAK

Latar Belakang: *Tinea kapitis merupakan infeksi jamur pada folikel rambut dan kulit yang membutuhkan terapi sistemik untuk mencapai kesembuhan klinis dan mikologis, mencegah kekambuhan, dan penyebaran infeksi. Griseofulvin merupakan terapi pilihan untuk tinea kapitis. Namun, griseofulvin seringkali membutuhkan dosis lebih tinggi dan durasi pengobatan lebih lama dari yang direkomendasikan. Oleh karena itu, terapi oral antijamur alternatif yang efektif dengan toleransi baik dan jangka pengobatan lebih pendek sangat diperlukan. Laporan kasus ini bertujuan untuk mengevaluasi efektivitas terbinafin sebagai terapi alternatif untuk tinea kapitis.* **Metode:** *Dilaporkan satu kasus tinea kapitis tipe kerion pada anak perempuan berusia dua tahun. Diagnosis ditegakkan berdasarkan gambaran klinis alopesia dengan permukaan kulit kepala berambut berupa makula eritema dengan pustula, krusta sanguinolenta, dan skuama, disertai limfadenopati oksipital. Kultur jamur menunjukkan pertumbuhan koloni Microsporum canis (M. canis). Pasien mendapat terapi dengan terbinafin dosis denyut ganda 125 mg/hari dan sampo ketokonazol 2 % selama dua bulan.* **Hasil:** *Perbaikan klinis tampak pada hari ke-35, sedangkan kesembuhan mikologis didapatkan pada pengamatan hari ke-60. Terbinafin dapat ditoleransi dengan baik tanpa ada efek samping yang terjadi.* **Kesimpulan:** *Terbinafin dosis denyut ganda efektif untuk tinea kapitis tipe kerion.*

Kata kunci: *dosis denyut ganda, kerion, M. canis, terbinafin, tinea kapitis*

INTRODUCTION

Tinea capitis is a common dermatophyte infection affecting hair and skin which frequently caused by *Trichophyton* and *Microsporum* species.[1,2,3] Human, animal, and fomite (i.e. object or article of clothing or dish that may be contaminated with infectious organism and serve in their transmission) contact spread are potential sources of infection.[4,5] Clinical appearance of tinea capitis may varied, including inflammatory and noninflammatory type.[6,7,8] Kerion is one of tinea capitis type[2,5] which represents its inflammatory form.[2,3]

Griseofulvin has been the gold standard for tinea capitis since the late 1950s.[2] Griseofulvin recommended duration for tinea capitis is 6-12 weeks[2,5] or until the patient tests negative for fungi.[2] The increased failure rate necessitating higher doses and longer treatment course required that will increases the risk of nonadherence[3] has lead to consideration of newer antifungal agents.[7]

Terbinafine is an allylamine antifungal agent [9,10,11] which has been approved by Food and Drugs Association (FDA) as tinea capitis alternative therapy in children aged two years [12] or older.[8,12,13] Side effects of terbinafine are uncommon[2,13] and include gastrointestinal symptoms, rashes, and headache.[2]

Terbinafine daily dose is 62.5 mg/day for children weighing less than 20 kg, 125 mg for weighing 20–40 kg, and 250 mg for those weighing more than 40 kg.[9,10,14] The pulse therapy consisted of one week treatment duration followed by three week period without treatment.[11] Double dose administration in this case is twice standard dose that is given to children based on the body weight. This report will describe a case of kerion type of tinea capitis in a two-year-old girl, weighs 16 kg, treated with doubled pulse dose terbinafine 125 mg/day for two months.

CASE

A two-year-old girl was taken by her parents to Dermato-Mycology Division, Department of Dermatology and Venereology, Dr. Hasan Sadikin General Hospital, Bandung, Indonesia with the chief complaint of alopecia on occipital area, presented as erythematous macule with pustules and pruritus. History of outside activities and frequent contact with soil were denied, but history of contact with cat which appeared to have skin problem was admitted. Patient took bath twice a day using water from the well, liquid soap, and shampoo. Patient also used personal towel, comb, and rarely used hat. Previous similar history was denied.

On physical examination, there was one cm in diameter of occipital lymphadenopathy, rubbery, and nontender on palpation. On the right occipital scalp area, there was an erythematous macule, 6x7cm, irregular-shape, clear border alopecia, with pustules, hemorrhagic crust, and scales on the skin surface.

Dermatological State

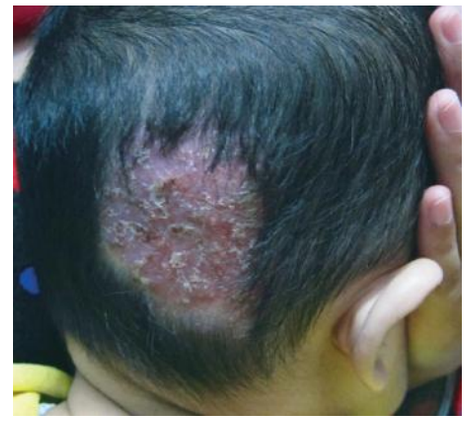

Figure 1. Lesion on occipital area of the scalp. Note the 6x7 cm solitary area with irregular-shape, clear border alopecia, with erythematous macule, pustules, hemorrhagic crust, and scales on the skin surface.

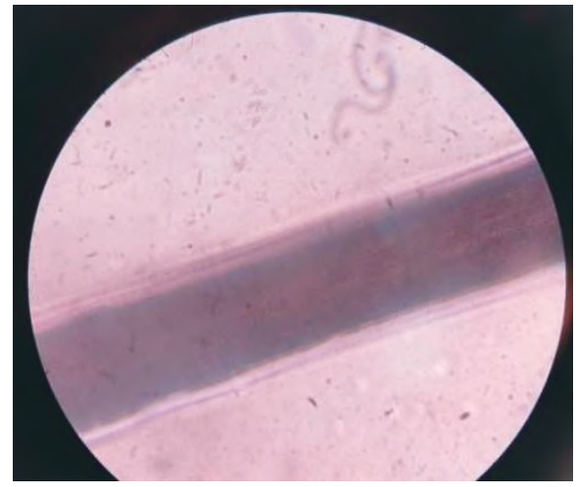

Figure 2. Direct microscopic examination of hair and skin scraping from scalp lesion using KOH 20 % + blue-black Parker® ink solution revealed no hyphae nor spores on identification. were identified.

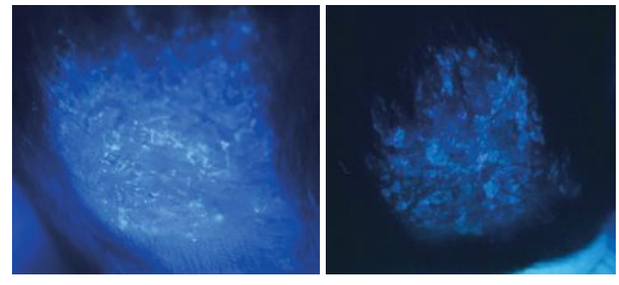

Figure 3. Wood's lamp examination showed no fluorescency

Direct microscopic examination of hair and skin scraping from scalp lesion using potassium hydroxide (KOH) 20 % added with blue-black Parker® ink solution revealed no hyphae nor spores. Direct microscopic examination from pustule on the scalp lesion using Gram

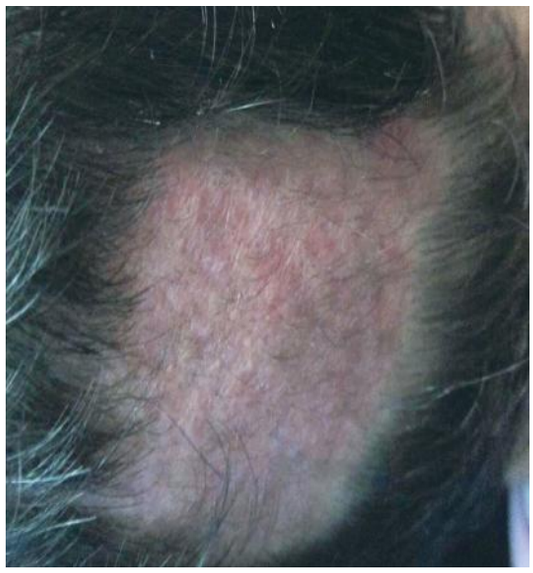

Figure 4. Lesion on 35th day: Note the improvement with decreased erythema and normal hair growth in almost all area of the scalp skin surface. Patient felt no more itchy at these moment.

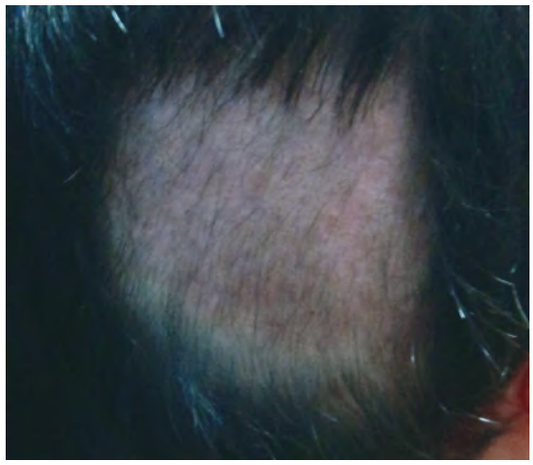

Figure 5. Lesion on 60th day of follow up

staining demonstrated epithelial cells, polymorphonuclear (PMN) cells, and Gram positive cocci. Wood's lamp examination showed no fluorescence. Fungal culture revealed *Microsporum canis (M. canis)* growth.

In this case, patient was treated with terbinafine 125 mg/day for one week-followed by three drug-free-weeks, topical ketoconazole 2% shampoo applied on scalp 3x/ week, cetirizine 1x1/2 tea spoon/day, and amoxycillin clavulanic acid 3 x ½ teaspoon. Clinical improvements were seen as erythema on scalp and itching were decreasing after one month of therapy. Treatment was well tolerated with patient has no experienced any side effect along the therapy regimen. Normal hair growth over the alopecia area has begun and the culture gave negative result on day-60.

DISCUSSION

Tinea capitis commonly affects children [9,15] aged less than 12 years old with a peak incidence at 3–7 years old.[16] In this report, the patient is a two-year-old girl. On the basis of host preference and natural habitat, the fungal causes may be anthropophilic, zoophilic, or geophilic.[2] The source for most tinea capitis infections in children and infants are human and animal.[2,5] The patient in this case had a contact history with cat and no similar complaint in her family. Thus, it can be speculated that this infection spread is *zoophilic*.

Kerion is an inflammatory type of tinea capitis [2,3] with painful mass [6] that can be accompanied by malaise,[3] fever,[2,3] and occipital lymphadenopathy.[2] Kerion lesion may take form as nodules[2] with induration,[3] pustular mass,[2,3,8] and vesicles[3] Infected scalp may be inflammed with pustule eruptions,[2] but secondary infection may also exist.[12]

Kerion diagnosis in children is often delayed, especially when pustular symptoms are misdiagnosed as bacterial infection. On physical examination, bacterial folliculitis may mimic kerion with tender lesion of erythematous plaques associated with pus. However, carbuncle rarely causes alopecia,[17] because the infection does not reach the hair bulb.[18] Delay in diagnosis and/or improper treatment may lead to complication and infection to other individuals.[5]

Patient's history taking and physical examination supported the diagnosis of kerion. The patient in this case had clear border alopecia, irregular shape, presented as erythematous macule, and itch, accompanied with pustules, hemorrhagic crust, and scales. Wood's lamp and microscopic examinations demonstrated negative results. Somehow, fungal identification through culture is necessary to establish the diagnosis and etiology of tinea capitis.[2,3,5]

Wood's lamp examination may help diagnosing tinea capitis, but it has poor sensitivity.[3] Microscopic examination of inflammatory type tinea capitis may also give negative result.[9,16] Thus, fungal culture for identification of tinea capitis etiology should be done.[3,19] If the clinical index of suspicion is high, therapy should be initiated after the culture specimen is obtained because it take time for confirming the culture results to establish the diagnosis.[8] The result of patient's fungal culture showed *M. canis* growth. Based on the result, we can conclude the diagnosis and etiology of this patient is kerion type of tinea capitis which is caused by *M. canis*.

Tinea capitis requires systemic treatment because topical antifungal could not penetrate to the deepest part of the hair follicle [2,20] nor eradicate the infection. Furthermore, the use of topical antifungal treatment alone may contribute to develop a carriers and cause transmission, since symptoms and clinical signs are minimal, but mycologic cure has not been achieved.[20] Adjunctive topical therapies have been shown to decrease the viable spores responsible for the

disease contagiousness, reinfection, and may shorten the duration of therapy courses. Ketoconazole shampoo should be applied three times weekly until the patient is clinically and mycologically cured. [21]

Some considerations in systemic therapy administration for tinea capitis are high efficacy level of treatment, low relapse rate, time and cost effectiveness, as well as safety. Griseofulvin is considered to be the treatment of choice for tinea capitis[10] for its effectiveness and safety to dermatophyte infection. Somehow its main disadvantage is the long duration of treatment required which may lead to reduced compliance.[2] Griseofulvin therapy duration which is recommended for tinea capitis is 6-12 weeks or until clinical and mycologic cure are achieved.[2] Also, griseofulvin requires continuous administration because it has low affinity for keratin.[22]

Terbinafine offer a shorter therapy duration and a less variable absorption compared to griseofulvin. Terbinafine absorption is not altered when taken with food, so the administration is easier compared to other systemic antifungals, such as griseofulvin and itrakonazole, which should be given with food.[15] Terbinafine is also very lipophilic and keratinophilic, so it can be distributed to adipose, epidermis, dermis,[2] hair,[2,10] nail,[2] and it can persist until one month after the treatment was stopped.[23]

Terbinafine also has less side effect compared to griseofulvin[5] and safe, including in pregnancy. Moreover, terbinafine rarely interacts with other medication.[24] Since its availability in 1991, terbinafine has been approved for the management of tinea capitis in many countries including Australia, New Zealand, China, Japan, Holland, and India.[15]

Terbinafine has fungicidal effect to dermatophytes since it inhibits squalene epoxidase[2,24] that leads to a decrease in ergosterol which is an essential component of fungal cell membranes.[15,24] Panagiotidou et al.[14] studied the efficacy and tolerability of terbinafine use for eight weeks in children with tinea capitis caused by *M. canis*. In that study, highest mycologic cure rate (97,1 %) was gained in dosage use of 7-12,5 mg/kg/day, followed by cure in 91,3% patients with dosage between 6-7 mg/kg/day, and 2,7 % in patients with dosage 3,3-6 mg/kg/day. Koumantaki et al.[25] experimented on terbinafine dosage for tinea capitis and stated that oral terbinafine should be given at a daily dose according to body weight: 125 mg for patients weight 10-25 kg and 250 mg/day for patients weight > 25 kg. Terbinafine can be administered in pulsed and continued dose. An advantage of terbinafine pulse therapy for tinea capitis over the continuous regimen is that it allows the physician to individualize the treatment schedules so that just sufficient therapy is administered to gain a cure. The decision to give a second or third pulse of terbinafine was based on the clinical appearance of the lesion prior to the time-point at which the next pulse was due.[11]

Patient in this case was treated with terbinafine 125 mg/day for one week and followed by 3 drug-free-weeks, ketoconazole 2% shampoo applied 3x/week, cetirizine 1x1/2 tea spoon/day to decrease itching, and amoxycillin clavulanic acid 3 x ½ teaspoon for the secondary bacterial infection. Clinical improvement were seen as erythema and itching were decreasing after one month of therapy. More over, normal hair growth over the alopecia area has begun and the culture result came out negative on follow up day-60, so therapy can be discontinued.

Tinea capitis is not life-threatening, but kerion type of tinea capitis may cause scarring and permanent alopecia.[2] Treatment of the animal source of infection is the effort should be made in tinea capitis case.[3] In conclusion, we feel that as regard griseofulvin to remain the antifungal drug of choice in tinea capitis, terbinafine may constitute an alternative drug which is well tolerated and safe.

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Literature Review

CLINICAL MANIFESTATION APPROACH OF DENGUE VIRAL INFECTION

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ABSTRACT

Currently by an estimated 50-100 million dengue fever cases per year in worldwide, 500.000 were in the form of a disease is heavy Dengue Hemorraghic Fever (DHF) and Dengue Shock Syndrome (DSS). Survey serology in Indonesia show that DEN-1 and DEN-2 are the dominant serotipe virus until the end of the 1980s but the recent shift has occurred epizoötic where viruses DEN-3 dominant. (Dos Santos, 2004; Malavige, 2004; Stephenson, 2005). Dengue virus infection induces transient immune aberrant activation of CD4/CD8 ratio inversion and cytokine overproduction, and infection of endothelial cells and hepatocytes causes apoptosis and dysfunction of these cells. The aberrant immune responses not only impaire the immune response to clear the virus, but also result in overproduction of cytokines that affect monocytes, endothelial cells, and hepatocytes. Dengue-virus-induced vasculopathy and coagulopathy must be involved in the pathogenesis of hemorrhage, and the unbalace between coagulation and fibrinolysis activation, and prolonged duration of shock increase the likelihood of severe hemorrhage in DHF/DSS. Capillary leakage is triggered by the dengue virus itself or by antibodies to its antigen. To date, there are no effective strategies to prevent the progression of DHF/DSS. The control of dengue will be possible only after an efficient vaccine has been developed.

Key words: *aberrant immune, capillary leakage, hepatocytes, fibrinolysis, Dengue Shock Syndrome*

ABSTRAK

Saat ini diperkirakan sekitar 50-100 juta kasus demam berdarah per tahun di seluruh dunia, 500.000 adalah DBD. Survei menunjukkan bahwa serologi di indonesia dan yang dominan adalah DEN-1 DEN-2 serotipe virus hingga akhir tahun 1980-an tapi akhir-akhir ini telah terjadi pergeseran epizootic di mana virus DEN-3 dominan (Dos Santos, 2004; Malavige, 2004; Stephenson, 2005). Infeksi virus dengue yang aktif dapat menginduksi kekebalan tubuh dan rasio jumlah perbandingan CD4/CD8 dan kelebihan produksi cytokine, infeksi sel-sel endotel serta menyebabkan disfungsi hepatosit dan apoptosis sel-sel ini. Penyimpangan respon imun yang terkait tidak hanya respons kekebalan tubuh untuk membersihkan virus, tetapi juga mengakibatkan kelebihan produksi sitokin yang memengaruhi monosit, sel endotel, dan hepatosit. Virus dengue menginduksi vasculopathy coagulopathy dan terlibat dalam kegiatan patogenesis dari pendarahan, dan durasi shock sehingga dapat meningkatkan kemungkinan pendarahan yang parah pada DBD. Kebocoran kapiler terjadi karena virus dengue itu sendiri atau oleh antibodi terhadap antigen DBD. Tidak ada strategi efektif untuk mencegah perkembangan DBD, pengkontrolan DBD hanya mungkin setelah vaksin yang efisien telah berhasil dikembangkan.

Kata kunci: *Penyimpangan imun, kebocoran kapiler, hepatosit, fibrinolisis, Dengue Shock Syndrome*

INTRODUCTION

Dengue fever (DF) is an acute infectious disease, caused by dengue virus that have four type of serotipe (DEN-1, DEN-2, DEN-3 dan DEN-4). With traits that is spatially biphasic, fever myalgia, headache, pain in some part of the body, rash, limphadenopati and leucopenia. In most case, DF is self limited, but there is a risk of the development of being dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). DHF is a febrile disease with traits abnormality hemostasis and increase of vascular permeability as well as the development of progressive can be DSS. DSS is a condition shock hipovolemic, are clinically associated with hemoconcentration and can cause the death if adequate handling is not given. A mechanism that involved in pathogenesis hemorrhagic fever, a viral infection particularly regarding DHF/DSS unresolved.[1]

Currently, it has been estimated 50-100 million DF cases per year in worldwide, 500,000 were in the form of a disease is heavy DHF and DSS. Survey serology in Indonesia show that DEN-1 and DEN-2 are the dominant serotipe virus until the end of the 1980s but the recent shift has occurred epizoötic where viruses DEN-3 dominant.[2,3,4]

Based on data cases report in September 1998, cases DHF in adults (≥ 18) most widely is Jakarta (13,813), overtaken West Java (10,730), East Java (8,546), Central Java (6,879) and Jogjakarta (3,257).[5]

WHO is categorized dengue as one of the main international public health problem because wider geographical distribution both virus and its vector, the increased frequency of epidemics, co-circulation of various serotypes of the virus and the emergence of DHF in new places.[6]

Currently, there is no specific therapy against DHF. Provision of adequate fluid, can decrease mortality due to DHF. Control of the main vector (*Aedes aegypti*) costly and often ineffective is the only method of prevention is still available to this day. Therefore, the basic comprehension of the molecular pathogenesis dengue infection became very important for the development of diagnosis and therapeutic as well as facilitate the protective vaccine production, rather than aggravate the disease.[4,7]

The Body Response Against Infections Hemorrhagic Fever

After infected mosquito is bite human, replication of the virus in regional lymph gland and spreading to the lymphatic system, blood and other tissues. Replication in reticuloendotelial system and bark yield viremia.[6,8]

The immune system has a strong defense against bacteria or viruses invasion. The components that contribute to viral infections are the antibodies, phagocytes, IFN, NK cells and T cells. When a virus infects a cell, viral proteins are broken down into specific peptides were then expressed with the help of MHC-I molecules on the cell surface. Then the peptide will be known by Th1 cells which in turn activate effector cells or Tc CTC can destroy virus-infected cells by direct (lethal hit). NK cells have Fc receptors (Fcγ - R) plays a role in ADCC.[9]

Dendritic cells (DCs) (<1% of total blood cells in peripheral blood) derived from bone marrow, have a central role in the development of an immune response to both natural and adaptive. At least two subsets of myeloid DCs in humans, namely DCs (MDCS) and plasmacytoid DCs (PDCs). Both have strong antigen presentation capacity and capability stimulate Ag-specific T cell responses. PDCs are CD123+, with appropriate stimuli can migrate directly from the peripheral blood and lymphoid organs to limphonoduli. PDCs have the characteristics that the potential for a strong Ag presentation, lymphoid morphology and strongly stimulate the secretion of IFN-α -mediated stimulation of CD40 as a virus. So, MDCS and especially PDCs assist in anti - viral innate immunity and the formation of Th1 adaptive immune response against viral pathogens.[10]

Interferon response is the first line to inhibit viral infection. Interferon (IFN) is a cytokine that is secreted by the immune system is the first virus to infect target cells. IFN binds to the IFN receptor on the cell surface and activates the JAK target - STAT (transcription factors) signaling pathway to inhibit translation of viral RNA and protein synthesis, thus the virus will be eliminated. However, the dengue virus has its own strategy for tackling defense mediated by IFN. Dr. Eva Harris at the University of California, Berkeley, showed that treatment with IFN-α and IFN-γ before infection can reduce the replication of dengue virus in human cells. Same treatment when administered after infection have no effect at all, it shows that the dengue virus can suppress IFN signaling. Dr. Adolfo Garcia - Sastre, Mount Sinai School of Medicine, New York showed that the NS4B protein of dengue virus can inhibit the activation of STAT1 in cell culture systems, so this explains some of the mechanisms that can be suppressed IFN signaling through STAT1 barriers. Whether the same mechanism also occurs in vivo in patients with dengue, is still unclear. Although IFN and ribavirin combination therapy can be applied to HCV, which is classified with dengue virus (family Flaviviridae), is not effective when it comes to the treatment of dengue infection.[11,12]

Dengue virus infection causes lifelong imunity protective against the homologous serotype but only partial or temporary protection against subsequent infection by the other three serotypes. Already a generally accepted concept that secondary infection or multiple major risk factors occurrence of DHF/DSS as antibody - dependent enhancement. Other factors that play an important role in the pathogenesis of DHF have been formulated include the virulence of the virus, the genetic background host, activation of T cells and autoantibodies.[4,13]

CHARACTERISTIC OF DENGUE VIRUS

Dengue virus consists of a single strand of RNA included in the family Flaviviridae. The first discovered by Albert Sabin in 1944, there are four serotypes are classified according to biological and immunological criteria. The length of the viral genome is about 11 kb. Mature virion consists of three structural (core, premembrane and envelope) and seven non - structural proteins, namely NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS (Figure 1). Envelope protein required for various major biological functions for the virus, which binds to receptors on the surface of the host cell, allowing the virus to enter target cells. Envelop proteins are also associated with erythrocyte hemagglutination, induces the formation of antibodies and protective immune response. Non - structural proteins (NS1 - NS5) expressed as a membrane -associated and secreted forms also have an impact on the pathogenesis of severe disease. Unlike other viral glycoprotein, NS1 does not form a component of the virion but is expressed on the surface of infected cells. Levels of NS1 (sNS1) secreted in plasma associated with viral titers, being higher in patients with DHF compared with DF. Furthermore, increased levels of free sNS1 within 72 hours of the start of the illness, indicating the risk of patient become DHF. NS1 that very high levels of protein detected in the acute phase blood samples from patients with secondary dengue, rather than the primary infection. This suggests that NS1 has contributed to the formation of immune complexes in the circulation are likely to play an important role in the pathogenesis of severe dengue infection.[2,3,7,14]

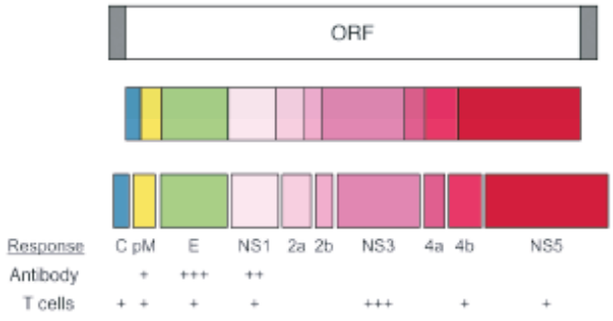

Figure 1. Dengue virus genome, a protein produced and the location of the main targets of the immune response.[7]

Latest Hypotheses About the Pathogenesis of Dengue Virus Infection

Several hypotheses on the pathogenesis of dengue virus infection have been proposed. Among these are the antibody-dependent enhancement (ADE) of infection, which has long been considered an important role. ADE hypothesis formulated to explain the finding that severe manifestations in DHF/DSS occurs when an infection to dengue virus with two different serotypes of dengue virus infection before. Antibodies are formed against a previous viral infection does not seem to be able to neutralize but rather aggravate the infection in vitro. Serum that taken from children before an infection which then progress to DHF/DSS further describe an ADE than just being DF. Epidemiological studies support an association between DHF/DSS with secondary dengue virus infections. Thus, ADE hypothesis is reinforced by the findings that: 1) Prospective cohort study of dengue epidemic, indicating that the majority of DHF/DSS occur during infection to two; 2) DHF/DSS in infants less classical than 1 year in the first dengue infection, the presence of antibodies to dengue virus factor of maternal circulation; 3) In vitro, non - neutralizing IgG antibodies can bind to dengue virus and attached to the Fc receptor and enhance dengue virus infection in monocytes or macrophages in the peripheral blood; 4) An increase in viremia was noted in vivo in animal models of rhesus macaques on secondary infection by DEN – 2; 5) In humans, high levels of viremia correlate with disease severity. It is not known how the addition of a viral infection caused by dengue enhancing antibodies can lead to DHF/DSS, is the proliferation of dengue virus infects or signal amplification through Fc receptors still require explanation. Due to the absence of animal model for DHF/DSS causal relationship between ADE with DHF/DSS is still no verification.[11,15,16,17]

Serotype crossreactive antibodies that resulting from previous infection binding with virions without neutralization and the increase of virus entry into monocytes, so that the number of virus-infected monocytes increased. As a result the number of activated T cells increased. This reflects the increased antigen presentation, increase in dengue - virus -specific T cells on subsequent infection and activation and proliferation faster than memory T cells. The T cells produce cytokines such as IFN-γ, IL - 2 and TNFα, as well as lysis of dengue virus -infected monocytes. TNFα is also produced by activated monocytes. Complement cascade is activated by a virus - antibody complexes and the release of several cytokines through C3a and C5a are also having a direct effect on vascular permeability. The synergistic effect of IFN-γ, TNFα and activation complement proteins trigger plasma leakage from endothelial cells in secondary dengue virus infections. However, some problems still can not be explained by this theory. Not all cases of DHF/DSS is secondary infection. Complement activation may be caused by severe disease and not the cause of DHF/DSS.[15,16]

Mongkolsapaya *et al* in 2003, did a study on the response of dengue virus specific T cells. In patients with DHF and DSS found some CD8+ T cells are virus - responsive most of undergoing apoptosis. This study is demonstrated the phenomenon of "original antigenic sin" that has been described a few years ago, the antibody responses to secondary viral infection is dominated by the activation of memory B cells cross reacting induced by primary infection. Activation of memory B cells will produce low affinity antibodies against the virus that causes secondary infections. So the levels of activated T cells with rapid death and domination of cellular immune responses by cells with low affinity to viral infections may inhibit viral clearance

and lead to high viral loads and increase imunopatologis (Mongkolsapaya, 2003; Stephenson, 2004). In late 2004, a study of 48 Vietnamese adults with secondary dengue virus infections showed that NS3 (epitopes targeted T cells) and T-cell responses cross-reactive responses do occur, but the magnitude was not significantly associated with clinical grading of disease.[18]

Virulence of Virus

The virulence of the virus, the ability to cause disease in the host, is an alternative hypothesis on the pathogenesis of DHF/DSS. Different manifestations of DF, DHF and DSS may be caused by dengue virus variants with different degrees of virulence. The risk for the occurrence of DHF/DSS higher in secondary infections with dengue virus serotype 2 when compared with other serotypes. Structural differences of the virus also found in isolates of DF and DHF patients. Furthermore, it has been reported that in viremia with high titers of dengue virus increases the severity of disease. Peak virus titer reached 100-1000 -fold higher in patients with DSS than in DF patients Thai children infected with dengue virus. Patients with secondary antibody response are likely to be DHF is twice than that has a primary antibody response. Evidence available to date do not all support the hypothesis of viral virulence.[15]

The analysis of individual genotypes in a single population showed little evidence that there are differences between the isolates DF patients with DHF/DSS, although exceptions have been reported. So the evidence of a link between viral genotype with clinical manifestations is still weak.[13]

Vulnerability Host

Some researchers have proposed the existence of predisposing DHF or DSS located on the human histocompatibility (HLA) haplotypes, but which locci that linked is still unclear or other genetic factors as the cause of a severe form of dengue is also unclear. Very few studies have been reported regarding the sensitivity of the host, especially regarding natural immunity. The latest scientific articles in Nature Genetics (Sakuntabhai, Nature Genetics, 2005) have reported the existence of genetic variants in the DC -SIGN promoter region is associated with severe forms of dengue infection. However, the molecular mechanism which in the genetic variants that affecting viral replication or trigger an excessive immune reaction on DHF and DSS still difficult to understand.[13,17]

Clinical and Pathological Manifestations of Dengue Virus Infection

DF symptoms include fever accompanied by headache, retroorbital pain, myalgia, arthralgia, race, mild leucopenia and thrombocytopenia. Pharyngeal hyperemia is almost in 97% of patients with DHF. Biphasic heat and the race was the most prominent characteristic of classical dengue fever. The symptoms will improve within 2–7 days. DHF is a syndrome of acute vascular permeability accompanied by abnormalities of hemostasis. Clinical features include plasma leakage, bleeding tendency and liver involvement. Capillary leak occurs rapidly in the period of a few hours, close to or at the time of the heat period ends when the classical DF symptoms subside. Pleural effusion, ascites, and hemoconcentration is indicative of a loss of intravascular volume. It can progress to shock if the patient did not receive fluid resuscitation. Manifestation hemorrhagic cranging from a positive torniquet test until spontaneous bleeding from the nose or gastrointestinal tract. Hemoconcentration and thrombocytopenia are the two main characteristic features of DHF/DSS. Liver involvement is common in dengue virus infection with a slight increase in serum transaminases. The liver is often enlarged, soft and slightly painful on palpation but usually no jaundice. Generalized lymphadenopathy obtained approximately in 50% of cases. Three organ systems (hematologic, vascular and hepatic) are involved in the pathological changes in DHF/DSS. Dengue virus infection causes dysfunction of the system either directly or indirectly, lead to the manifestation of DHF/DSS.[1,8,14]

Effects of Dengue Virus Infection on Blood Cells

Immune aberrant activation during dengue virus infection

In the analysis of blood samples collected during the outbreak of dengue virus serotype 3 from November to December 1998 in Southern Taiwan describe a disorder of immune status in patients with dengue. In the peripheral blood of uninfected persons, the number of CD4 + T cells more than CD8+. In the peripheral blood of patients with dengue, the number of CD8 + T cells more than CD4+, so the CD4/CD8 ratio decreased < 1. This phenomenon is not only found in patients with DHF/DSS alone, but also on the patient DF. Of the 21 patients with DF and 8 patients with DHF/DSS, found CD4/CD8 ratio is inverted in 10 cases. The frequency of CD4/CD8 ratio is inverted is higher in DHF/DSS (5/8) of the DF (5/21, $p < 0.05$). Kinetic Analysis imunofenotip CD4 + T cells and CD8+, indicating that the CD4/CD8 ratio reverse occurs during acute infection (6-14 days after the onset of heat). CD4/CD8 ratio is slowly returning to normal after a day to 15. It also found $CD4^{dim}$ monocytes and CD8dim, the percentage in peripheral blood mononuclear cells (PBMCs) were higher or highest on days 6–7, then down to the lower level healing moment later. Atypical lymphocytosis reached a peak on days 8-10, and then disappeared rapidly after day 12. Initial activation of mononuclear cells was confirmed by expression of the early activation marker, CD69, on day 4 after the onset of heat. CD69 stained on the surface of lymphocytes, as well as on monocytes, but more widely expressed in CD8+ T cells of the CD4+. With the description of atypical lymphocytosis and dynamic changes in CD4/CD8 ratio, an indication of the occurrence of immune activation during dengue virus infection aberan.[1,8]

Excessive Cytokine Production During Dengue Virus Infection

During acute dengue infection, mononuclear cells

activated excessively so expected that increased levels of cytokines can be found in the plasma. High levels of markers of T-cell activation such as soluble IL-2 receptor, soluble CD4, soluble CD8, IL-2 and IFN-γ, as well monokin eg TNFα, IFN-β and GM - CSF, all detected in children infected with dengue, and markers are higher in patients with DHF/DSS than in DF. High levels of cytokines in the serum inhibitors such as IL-10 or soluble receptors (soluble TNF receptor) sTNFRI and sTNFRII were also found in patients with DHF. In dengue patients in the study, cytokines such as RANTES, IL-8 and IL-6 levels are increased after infection with dengue virus. Levels of IL-6 and IL-8 in serum were higher in patients with DHF/DSS than in DF patients. IL - 6 has a dual role, as a mediator of pro-inflammatory or anti - inflammatory. In the kinetic analysis showed large variations at points different times and in different individuals, but increased serum levels of IL-6 high transient occurs either at day 7 or day 9-11 after the onset of heat. It is impressive when the host response to dengue virus infection through the formation of cytokines pro - inflammatory cytokines also formed simultaneously inhibitor against inflammation. The end result depends on the balance between the two.[1,19]

Thrombocytopenia and anti - platelet pntibodies

Thrombocytopenia is common in DF, and always found in DHF/DSS. Its pathogenesis is still poorly understood. Impressed that the dengue virus triggers bone marrow suppression that reduces platelet production and resulting in thrombocytopenia. One group of studies found that dengue virus-2 can bind to platelets in the presence of specific viral antibodies and this supports the role of immune-mediated clearance of platelets. Surprisingly, found IgM (not IgG) anti-platelet autoantibodies in patients with dengue. Titer was higher in DHF/DSS than in DF patients. The presence of these autoantibodies is not only triggered by complement lysis of platelets but also inhibit ADP-induced platelet aggregation. Cross-reactions of antibodies against dengue virus proteins, particularly NS1 and platelets, suggesting a role for the pathogenesis of anti-platelet autoantibody during dengue virus infection.[1,15]

Immune Deviation Caused Dengue Virus Infection

Patients infected with dengue usually experienced leucopenia for a few days during the acute infection, with the characteristics of a decrease in the absolute number of neutrophils and monocytes. Impaired T cell responses associated with PHA-stimulated monocytes $CD4^{dim}$ or $CD8^{dim}$ deficiency. Detection of early activation marker, CD69 on $CD8^+$ T cells, NK cells and monocytes, and lymphocytes are atypical shapes showed activation of lymphocytes by dengue virus infection. Dengue virus can infect Langerhans cells or immature dendritic cells and can replicate more efficiently in these cells than in monocytes or macrophages. Infected dendritic cells stimulate maturation cytokine production and TNFα and IFNα, but not IL-6 or IL-12. Levels of IL-12 in patients with higher DF than in DHF. Blunted blood PDC response to dengue virus infection associated with higher levels of viremia and is part of the natural immune response and changes cascade causes severe disease pathogenesis. In patients with DHF stage III and IV, not detected the presence of IL-12. Production deficiency of IL-12 can cause a shift to a Th2 response and mismatch generation of cytotoxic T cells. Dengue virus infection appears to strongly influence the immune response such as, monocytosis $CD4^{dim}$ or $CD8^{dim}$ early, inversion of CD4/CD8 ratio were temporary, atypical lymphocytosis with a large percentage and depressed T-cell proliferation. Immune deviation is not only slow viral clearance, but also triggers excessive production of cytokines and anti- platelet autoantibodies that started the sequence of the pathogenesis of dengue virus infection.[1,3,10]

Effects of Dengue Virus Infection of Endothelial Cells

Vasculopathy due to dengue virus

Overview of the most distinctive and best indicator of disease severity is plasma leakage. Plasma leakage due to increased capillary permeability in a diffuse manner and manifest as hemoconcentration, pleura effusion or ascites. It usually occurs on days 3-7, during the current easing of dengue fever. Plasma leakage occurs systemically, developing progressive, but it will get better in 1-2 days in patients receiving fluid resuscitation adequately. No tissue or organ function abnormalities occur. Although edema perivaskuler seems obvious, but no evidence of damage to vascular endothelial cells. Dengue virus can infect endothelial cells in vitro led to the release of cytokines and chemokines IL-6, IL-8 and RANTES. Dengue virus infection can cause endothelial cell apoptosis in vitro, but the effect is directly dependent on the dengue virus isolates were used. Endothelial cells were infected with dengue virus can activate complement and induce the expression of adhesion molecules such as ICAM-1. The expression of ICAM-1 along with the production of IL-8 and RANTES would invite polymorphonuclear cells, mononuclear cells, and then finally freed vasopermeability and trombomodulin, a marker of endothelial damage. Increased levels of circulating trombomodulin in the acute stage of DHF/DSS, indicating that the structural failure of endothelial cells in vivo. Apparently, direct viral cytopathic effects and immune -mediated leukocyte recruitment and anti - dengue virus antibodies, both of which cause structural damage to endothelial cells. Vascular leakage can be caused by infection with dengue virus, both directly and indirectly. Endothelial cells play an important role in maintaining hemostasis, therefore, endothelial cell damage due to dengue virus infection can affect either the balance of procoagulant or anticoagulant endothelium that increase the risk of bleeding. Recruitment of platelets by activated endothelial cells may also cause thrombocytopenia.[1,3]

Coagulopathy Due to Dengue Virus

Hemorrhagic manifestations caused by the dengue virus, which is more common is vascular - platelet abnormalities, but when severe bleeding can occur with

DIC. Hemostasis is maintained by a balance between coagulation and fibrinolysis. Coagulation system can be activated by the intrinsic and extrinsic pathways to form thrombin converts fibrinogen to fibrin. Fibrinolytic system on the other hand can break down fibrin. Fibrinolytic system consists of plasminogen, a proenzyme that can be turned into an active enzyme plasmin by plasminogen activators several kinds. Plasminogen activator is a major endogenous tissue -type plasminogen activator (tPA). Plasminogen activator inhibitor (PAI-1), which is produced by platelets, and endothelial liver, on the other hand is a major inhibitor of tPA. In general, secondary coagulation activation trigger fibrinolysis activation that inhibits the release of large amounts of PAI-1 rapidly.

During acute dengue virus infection, coagulation parameters such as platelet count, activated partial thromboplastin time (APTT), as well as fibrinolytic parameters tPA and PAI-1 changes. APTT elongated, while tPA increased. Both coagulation and fibrinolysis are both activated and this activation is more severe in patients with DHF/DSS than in DF. After healing increased levels of PAI-1 and the platelet count in line with decreased levels of tPA and APTT returned to normal. The ratio of tPA/PAI-1 was higher in patients with DHF/DSS than in DF patients. Elongation of APTT and increased the ratio of tPA/PAI-1 in the acute phase of dengue virus infection associated with disease severity and can be used as an early indicator of DHF/DSS. APTT and prothrombin time is an indicator of intrinsic and extrinsic pathways of coagulation. Only APTT, not prothrombin time that extending to dengue virus infection, suggesting that abnormalities in the intrinsic pathway. This can be caused by a decrease in the synthesis of specific factors or the increased use of specific factors. Disorder of liver function is responsible for the reduction in the synthesis of specific factors in the intrinsic pathway. Increased use of these factors as indicated by high levels of tPA is also associated with prolongation of the APTT but less meaningful. Thus, both a decrease in the synthesis or increased consumption of coagulation factors, both involved in the lengthening of APTT.

Hiperfibrinolisis in the acute phase of DHF/DSS, due to the increased production of tPA. Correlation and linear regression analysis showed a significant association between IL-6 serum with tPA in DHF, but not in the dengue fever. Dengue virus infection stimulates endothelial tPA and to produce IL-6. Synthesis of tPA could be blocked by anti-IL-6 antibody, suggesting that tPA production by endothelial cells is dependent IL-6. Furthermore, antibodies against dengue virus E protein, can bind to human plasminogen. It can inhibit the action of plasmin or plasminogen activation strengthens. So, both coagulation and fibrinolysis are experienced hyperactivation in the acute phase of dengue virus infection. Imbalance between coagulation and fibrinolysis may cause bleeding in DHF/DSS.

Bleeding that occurs in DHF/DSS is not directly due to the prolongation of the prothrombin time and partial thromboplastin time, nor is it just because of thrombocytopenia. The most powerful risk factor causes bleeding is the presence of prolonged shock. It shows that patient with a long shock, there has been a leakage of plasma and bleeding.[20]

Effects of Dengue Virus Infection in Liver Cells

Dengue virus is hepatotrophic. Dengue virus antigen was detected in the liver cells and virus particles found in liver biopsy specimens from patients with DHF. Dengue virus can infect the liver and cause hepatitis. Increased levels of serum transaminases in patients with dengue and degree of elevated levels of *aspartate aminotransferase* (AST) associated with bleeding events. In hepatitis due to dengue virus, the AST level higher than *alanin aminotransferase* (ALT) with a ratio of about 1-1.5, while hepatitis due to other viruses, ALT is higher than AST. By using hepatoma cell lines, dengue virus can lead to apoptosis and chemokine RANTES production through oxidative stress and activation of NF-κB. Furthermore, RANTES is typically caused by the dengue virus but not by enteroviruses in the liver cells. Patients with dengue virus infection had serum levels of RANTES were higher compared with other viral infections. RANTES is a chemokine that can lead to the recruitment of lymphocytes and NK cells to sites of inflammation. Liver is damage caused by dengue virus was due to a direct effect of viral replication or indirect effects of inflammation mediated by RANTES, still require further investigation. The balance between virus elimination and tissue damage may result in disease severity. It is known that the liver is where most of the production of coagulation factors, the decreased levels of these factors is attributed to an increase in consumption, as well as impaired synthesis. The last thing is more likely due to injury to the liver. IL-6 can lead to down regulation of the synthesis of factor XII, is the first factor that initiate clotting through the intrinsic pathway. Elongation of *activated partial thromboplastin time* (APTT) in patients with DHF are caused by a deficiency in the intrinsic pathway is likely due to impaired synthesis in the liver factor XII.[1,3,14]

SUMMARY

Dengue virus infections causes dengue fever (DF), dengue hemorrhagic fever (DHF) dan dengue shock syndrome (DSS). Current hypotheses antibody-dependent enhancement, virus virulence, and IFN-γ/TNFα –mediated immunopathogenesis are insufficient to explain clinical manifestations of DHF/DSS such as thrombocytopenia and hemoconcentration. Dengue virus infection induces transient immune aberrant activation of CD4/CD8 ratio inversion and cytokine overproduction, and infection of endothelial cells and hepatocytes causes apoptosis and dysfunction of these cells. The coagulation and fibrinolysis systems are also activated after dengue virus infection. The aberrant immune responses not only impaire the immune

response to clear the virus, but also result in overproduction of cytokines that affect monocytes, endothelial cells, and hepatocytes. Platelets are destroyed by crossreactive anti-platelets antibodies. Dengue-virus-induced vasculopathy and coagulopathy must be involved in the pathogesis of hemorrhage, and the unbalance between coagulation and fibrinolysis activation, and prolonged duration of shock increase the likelihood of severe hemorrhage in DHF/DSS. The overproduced IL-6 might play a crucial role in the enhanced production of anti-platelet or anti-endothelial cell autoantibodies, elevated levels of tPA, as well as a deficiency in coagulation. Capillary leakage is triggered by the dengue virus itself or by antibodies to its antigen. To date, there are no effective strategies to prevent the progression of DHF/DSS. The control of dengue will be possible only after an efficient vaccine has been developed.

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Case Report

A NOSOCOMIAL INFECTION MANIFESTED AS ERYSIPELAS IN PEMPHIGUS FOLIACEUS PATIENT UNDER INTRAVENOUS DEXAMETHASONE TREATMENT

A Nosocomial Infection Manifested as Erysipelas

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ABSTRACT

Introduction: *Puncture wound in diagnostic interventions permits the entry of bacteria into the skin or soft tissue, thus precipitating nosocomial infection, such as erysipelas. There are other risk factors of nosocomial infections including old age, immunosuppressive drugs, and underlying diseases. Pemphigus foliaceus (PF) is an autoimmune disease with corticosteroid treatment as the mainstay therapy, which could cause immunosuppression and predispose patients to infection. The objective of this paper was to report erysipelas as one of the manifestations of nosocomial infection in patients under immunosuppressive therapy.* ***Case:*** *A case of erysipelas acquired on the 9th day of hospitalization in a PF patient underwent intravenous dexamethasone injection, with history of puncture wounds on the previous day on the site of erysipelas was reported. The clinical findings of erysipelas were well defined, painful erythema and edema that felt firm and warm on palpation, with blisters and pustules on top. Gram staining from the pustules and blisters fluid revealed Gram (+) cocci. Patient was given 2 grams intravenous ceftriaxone for 7 days and saline wet compress. Improvement on the erysipelas was seen the day after ceftriaxone injection. The patient was discharged after 12 days of hospitalization with improvement both on the PF and the erysipelas. On the next visit 7 days later, the erysipelas lesion disappeared.* ***Conclusion:*** *Puncture wound and immunosuppresive treatment are the factors that could cause erysipelas as a nosocomial infection, and an appropriate treatment of the infection would decrease the functional disability of the patient.*

Key words: *erysipelas, nosocomial infection, immunosuppresive, pemphigus foliaceous, ceftriaxone*

ABSTRAK

Pendahuluan: *Luka suntikan pada intervensi diagnostik memungkinkan masuknya bakteri ke dalam kulit atau jaringan lunak, sehingga dapat mencetuskan infeksi nosokomial, seperti erisipelas. Faktor risiko lain untuk terjadinya infeksi nosokomial antara lain usia tua, penggunaan obat imunosupresif, dan penyakit yang mendasari. Pemfigus foliaseus (PF) adalah penyakit autoimun dengan pengobatan utama kortikosteroid, yang dapat menyebabkan imunosupresi dan membuat pasien rentan terhadap infeksi. Tujuan tulisan ini adalah untuk melaporkan erisipelas sebagai salah satu manifestasi infeksi nosokomial pada pasien yang mendapatkan terapi imunosupresi.* ***Kasus:*** *Dilaporkan satu kasus erisipelas pada pasien PF yang terjadi pada perawatan di rumah sakit hari ke-9, yang mendapatkan terapi injeksi deksametason intravena, dengan riwayat tusukan jarum sehari sebelumnya pada lokasi erisipelas. Manifestasi klinis erisipelas berupa makula eritema dan edema berbatas tegas, nyeri, teraba keras dan hangat, dengan vesikel serta pustula pada permukaan lesi. Pada pewarnaan Gram yang diambil dari isi vesikel dan pustula didapatkan bakteri kokus Gram (+). Pasien diterapi dengan injeksi seftriakson intravena selama 7 hari dan kompres terbuka dengan larutan salin. Perbaikan pada erisipelas didapatkan satu hari setelah pemberian seftriakson. Pasien dipulangkan pada hari ke 12 perawatan dengan perbaikan baik pada PF dan erisipelasnya. Pada saat kontrol 7 hari kemudian, sudah tidak terdapat lesi erisipelas.* ***Kesimpulan:*** *Luka tusukan dan terapi imunosupresi dapat menjadi faktor penyebab infeksi nosokomial berupa erisipelas. Pengobatan yang tepat pada infeksi tersebut akan mengurangi gangguan fungsional pasien.*

Kata kunci: *erisipelas, infeksi nosokomial, imunosupresi, pemfigus foliaseus, seftriakson*

INTRODUCTION

Nosocomial infections are infections acquired during hospital care, which are not present or incubating at admission, occurring more than 48 hours after admission. Important factors influencing nosocomial infection include old age, immune status, immunosuppressive drugs, underlying disease, injuries to skin, diagnostic and therapeutic interventions.[1]

Skin and soft tissue infections (SSTIs) such as impetigo and erysipelas,[2] are one of the most frequent nosocomial infections found.[1] Erysipelas is usually caused by *Staphylococcus aureus* (*S. aureus*) or beta-hemolytic *Streptococci*.[2] The etiology of SSTIs may be normal host flora transferred through a break in the barrier, such as instrumentation (eg, needles), that could permit the entry of normal skin flora and indigenous flora from the instrument of penetration.[3] Esmaili *et al*.[4] reported that *S. aureus* responsible for 93,7% of skin infections in pemphigus patients.

Pemphigus foliaceus is an autoimmune disorder, with generalized crusts and erosion as clinical findings, and immunosuppressive drugs as the mainstay of treatment.[5] Hospitalization in addition to corticosteroid therapy (with or without adjuvant immunosuppressive agents) would predispose the pemphigus patients to infection, with skin infection as one of the most frequently acquired.[4] This is a case report of nosocomial infection manifested as erysipelas in a patient under immunosuppressive therapy.

CASE

A 57 year-old man was hospitalized at Department of Dermatology and Venereology, Dr. Hasan Sadikin Hospital, Bandung, Indonesia with pemphigus foliaceous (PF). The clinical findings were generalized erythema, superficial loose blisters, erosions, and crusts, that affect 70% of body surface area. Histopathology examination showed subcorneal acantholysis, and direct immunofluorescence revealed intra epidermal IgG deposition, thus the diagnosis was established. The patient was treated with 15 mg intravenous dexamethasone injection daily, along with intravenous ranitidine injection, and antihistamine.

On the 9th day of hospitalization, the patient complained about a painful, erythematous macules and edema on the upper right arm. There was history of repeated needle puncture, due to multiple failed trials of blood aspiration the day before, just below the site of the edema. From the physical examination, besides the generalized erythema and crusts, on the right arm there were irregular, well defined, painful erythema and edema with blisters and pustules on top, measuring 10 x 15 cm, that felt firm and warm on palpation (Figure 1).

The blisters and pustules were aspirated for Gram staining, that revealed Gram (+) cocci and polymorphonuclear cells. Blood examination showed leukocytosis (28,400/mm^3).

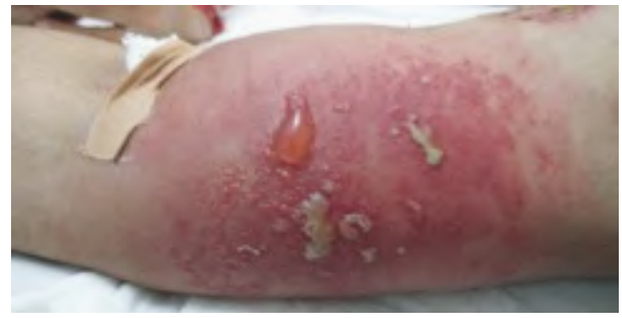

Figure 1. Erysipelas lesion

Thus the erysipelas diagnosis was added, and considered as a nosocomial infection. The patient then given 2 grams intravenous ceftriaxone injection daily for 7 days, and wet compress with saline solution was applied to the erysipelas lesion. Improvement on the erythema and edema was seen the day after.

The patient was discharged from the hospital on the 12th day, with improvement on the PF lesions, all the erosions have dried, and most of the crusts dissapeared. The erysipelas lesion was also improved, marked as decreased erythema and edema, and the pain diminished. After discharged from the hospital, the PF treatment was changed into 80 mg methylprednisolone tablet, to be tappered every 7 days. The erysipelas treatment was saline wet compress. Seven days later on the next visit, the PF lesions already became hyperpigmented macules, and the painful erysipelas lesion had already dissapeared.

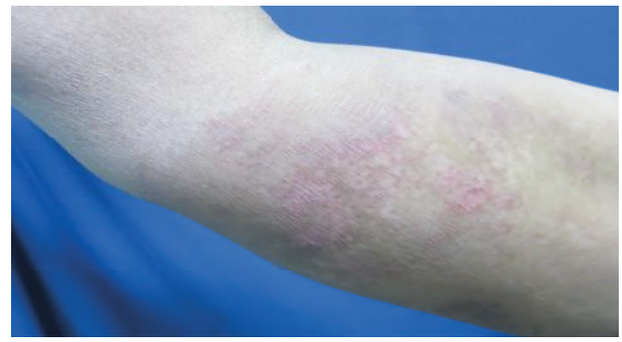

Figure 2. The erysipelas lesion on the final observation, leaving behind hypo pigmented and erythematous macules

DISCUSSION

Skin and soft tissue infection is one of the most frequent nosocomial infections found. Injuries to skin or mucous membranes due to diagnostic or therapeutic interventions could bypass natural defense mechanism of the skin. Other factors including old age that are associated with decreased resistance to infection, and immunosuppressive drugs that may lower the resistance to infection.[1]

Erysipelas is an SSTIs caused by *S. aureus* or beta-hemolytic *Streptococci*,[2] with bimodal incidence

distribution, peaking among young children and the elderly. There is also an increased risk in the immunocompromised, including patients underwent corticosteroid therapy. Erysipelas diagnosis is largely based on clinical findings, and might demonstrate leukocytosis.[6] counted 20,000/mm^3 or more.[7] The unique signs of erysipelas are well-demarcated erythema and edema, of which a diagnosis can be made confidently, and bullae or vesicles may complicate about 5% cases. The most commonly involved site was the leg, followed by the arm, and face.[8]

The clinical findings of erysipelas in this case were tender, well-defined erythema and edema that felt warm on palpation, with blisters and pustules on top. There was also leukocytosis (28,400/mm^3). In this case, erysipelas was considered as nosocomial infection, as the patient acquired it on the 9th day of hospitalization, and there were no signs of infection on admission. The predisposing factors were old age and corticosteroid consumption for PF treatment.

Conditions that lead to disruption in the skin barrier predisposed patients to erysipelas.[8] The etiology of skin and soft tissue infection may be normal host flora, with several means in penetrating the skin barrier. The most common route is through a break in the barrier, such as instrumentation (eg, needles), that could permit the entry of bacteria from the instrument of penetration.[3] In this case, multiple puncture wound from repeated trials of blood aspiration with needle the day before, on the site of erysipelas precipitated the disease.

Antibiotics are the mainstay of treatment for erysipelas and most patients experience a complete recovery after antibiotics and few experience recurrences.[6] A series of antibiotics have been suggested with highly successful clinical response, above 88%.[9] Ceftriaxone is a third generation cephalosporin, with SSTIs as one of the indications.[10] Based on the bacterial susceptibility data to antibiotics in Dr. Hasan Sadikin Hospital, ceftriaxone is still sensitive in 100% cases.[11] Pavlov and Slavova[12] reported that 3rd generation cephalosporin given in a parenteral route for 5–7 days showed good clinical resolution in severe erysipelas. Clinical improvement will be seen in 24-48 hours after treatment initiation, and several days usually needed for disease resolution.[7] In this case, the blisters are aspirated while keeping the roof intact. Intravenous ceftriaxone as the mainstay treatment. The skin lesions improved within 24 hours and complete resolution was seen on the 14th day.

Hospital-acquired infections add to functional disability and emotional stress of the patient and may, in some cases, lead to disabling conditions that reduce the quality of life. Nosocomial infections are also one of the leading causes of death. The economic costs are considerable. The increased length of stay for infected patients is the greatest contributor to cost.[1]

Prevention of nosocomial infections requires integrated, monitored programs, which includes the following key components; 1) limiting transmission of organisms between patients in direct patient care through adequate hand washing and glove use, and appropriate aseptic practice, isolation strategies, sterilization and disinfection practices, and laundry, 2) controlling environmental risks for infection, 3) protecting patients with appropriate use of prophylactic antimicrobials, nutrition, and vaccinations, 4) limiting the risk of endogenous infections by minimizing invasive procedures, and promoting optimal antimicrobial use, 5) surveillance of infections, identifying and controlling outbreaks, 6) prevention of infection in staff members, and 7) enhancing staff patient care practices, and continuing staff education.[13]

As a conclusion, puncture wound and immunosuppresive treatment could cause erysipelas as a nosocomial infection, and an appropriate treatment of the infection would decrease the functional disability of the patient.

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Case Report

NORWEGIAN SCABIES IN AIDS PATIENT: A CASE REPORT

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ABSTRACT

Scabies is a skin infection caused by Sarcoptes scabiei var. hominis. This disease may present severe clinical manifestations in immune-compromised patient, well-known as Norwegian scabies or crusted scabies.A 36-year old man with AIDS had chief complaint thick crust almost all over his body in this case. History of household member infected by scabies before was present. Clinical findings show hyperpigmented macules unsharply marginated, covered with thick scales and accompanied by papules, fissures, and erotion. T cell CD4 level was 12 cell/μL. Scraping examination showed scabies infection and so did the histopathology examination. This patient was treated by topical Permethrin 5% combined with 2-4 ointment application in between permethrin usage. Before topical scabicide was given, thick crust was previously treated by topical urea 10% and wet dressing by normal saline. On day 14 after the patient first came there was lesion improvement.

Key words: *Norwegian scabies, immunocompromised, AIDS, thick crusts*

ABSTRAK

Skabies adalah suatu penyakit infeksi kulit yang disebabkan oleh tungau Sarcoptes scabiei var. hominis. Penyakit ini bisa bermanifestasi klinis yang hebat pada pasien dengan sistem imun yang rendah dan biasa disebut "Norwegian Scabies" atau skabies berkrusta. Dilaporkan seorang laki-laki, usia 36 tahun, penderita AIDS, yang datang dengan keluhan keropeng yang tebal dan gatal pada sekujur badannya. Beberapa anggota keluarga juga menderita gatal pada malam hari, namun tidak separah pasien. Pemeriksaan klinis menunjukkan adanya bercak hiperpigmentasi yang menebal, disertai adanya erosi dan fisura pada beberapa tempat. Pemeriksaan sel Limfosit CD4 menunjukkan kadar yang rendah (12 sel/ul). Pada pemeriksaan kerokan kulit ditemukan adanya infeksi scabies dan ditunjang oleh pemeriksaan histopatologi. Pengobatan diawali dengan kompres NaCl fisiologis dan salep urea 19%, selanjutnya diberikan salep Permethrin 5% secara berkala, diselingi dengan kombinasi salep campuran asam salisilat dan sulfur (" salep 2-4 "). Setelah 14 hari diobati, lesi kulit berkurang dan menunjukkan banyak kemajuan.

Kata kunci: *Norwegian scabies, immunocompromised, AIDS, krusta tebal*

INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) is a group of clinical symptoms due to the decreasing of lymphocyte T-CD4+ cell count, caused by Human Immunodeficiency Virus (HIV) infection. This virus belongs to genus *lentivirus*, family *Retroviridae* or commonly known as the retroviral group. It destroys the lymphocyte T-CD4+ cells, causing the cell count to decrease below 200 cells/μL and the patients become prone to infection.[1] One of infection that could affect HIV/AIDS patient is scabies. Scabies is a disease caused by *Sarcoptes scabiei var. hominis* parasite infestation, family *Sarcoptidae*, class *Arachnida* on the skin.[2] This disease is one of major skin health problem in development countries associated with poverty, with estimated 300 million people worldwide are affected.[3] The prevalence of Scabies in Indonesia varies from 2–65% and it relates with geographical, seasons and communities. High prevalence are reported in certain communities (pondok pesantren,

dormitory, jails).[4] Scabies infection is very contagious and could be the source of infection to the surrounding environment through direct skin or clothing contact.[2,3] Clinical symptoms such as itchy, especially at night time accompanied by papular skin eruptions. Pathognomonic lesion in scabies infection is a burrow, which is a thin, thread-like, linear structure 1-10 mm in length. Burrow is actually a tunnel caused by the movement of the mite in the stratum corneum, with predilection at interdigital webs, periumbilical, and genital areas.[2,3]

Clinical findings of scabies infection in HIV patients is different with immunocompetent patient. Lesions manifest as thick crusts so it's called crusted scabies or commonly known as Norwegian scabies.[5] This type of infection has very enormous mite population so that it's highly contagious even through casual contact. It also affects face, scalp, nail, with minimal pruritic symptom. This uncommon and hyperkeratotic type of scabies infection tends to affect immuno-compromised person due to lack of immune system ability to maintain the mite.[6,7]

CASE REPORT

A thirty-six year old male patient admitted to the Dr. Soetomo General Hospital Surabaya with chief complaint thick crusts almost all over his body since 1 week before. Firstly it was some small papules over his thigh area, felt a bit itchy but no itchy sensation at nighttime. The papules then spread to all over his body, became thick crusts with some cracks in between and causing difficulty when moving. His wife and child ever had similar complaint 1 month before visitation, which were papules over their bodies accompanied with itchy sensation. They had been treated with permethrin cream and their lesions were getting better. Meanwhile, the patient's mother suffered from psoriasis but history of lesion on Koebner area in this patient was denied.

Before his admission, he didthe Voluntary Counseling and Testing (VCT) and HIV Rapid Test 2 months before and the result was positive, confirmed by three methods test hence he was diagnosed as AIDS. He hadcontrolled routinely to the HIV outpatient clinic and consumed antiretroviral (ARV) treatment for 1 month. At the outpatient clinic about 3 weeks before, he was diagnosed chronic dermatitis and got topical corticosteroid with emollient, and his complaint was getting better until the last complaint occurred 1 week before his admission.

Physical examination showedweak general condition was but good consciousness with normal vital sign. He had anemic conjunctiva and also slight enlargement of the liver. For the dermatological state, on auricular, axilla, colli, abdominal, inguinal, extremity (interdigitalis), and also gluteus regions there were large hyperpigmented macules, unsharply marginated and covered by thick crusts. There were also some fissures over the thick crusts, erosions, and we could see multiple papules over the eroded area over the scrotum.

The laboratory examination results were: White Blood Cells 3,600 cells/mm^3, Red Blood Cells 4.54 x 10^6 cells/mm^3, Platelet Count 128,000 cells/mm^3, Hemoglobin level 7.8 g/dl, SGOT 126 U/l, SGPT 67 U/l, BUN 7 mg/dl, creatinine serum 0.6 mg/dl, albumin level 2.1 g/dl, random blood glucose 86 mg/dl, natrium 130 mmol/l, kalium 3.8 mmol/l, chloride 100 mmol/l. The CD4 absolute count had been performed before with the result was only 12 cells/µL.Lesion scraping was done to find if there was any mite of *Sarcoptes scabiei*, there were the adult mite with some eggs.

Histopathologic examination was done to exclude psoriasis. Nevertheless, the diagnosis of scabies had been established by the scraping examination, so the therapy was soon started. First the wet dressing by normal saline solution was done to the thick crusts area in orderboth to decrease the crust andaddress the erosion, accompanied by Urea 10% cream application. The thick crusts should

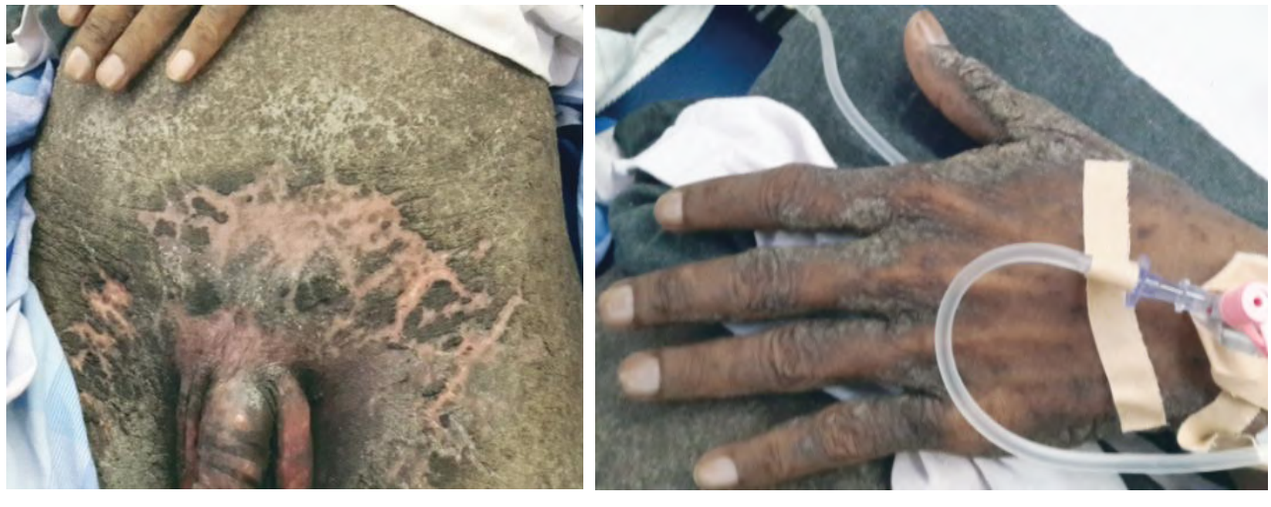

Figure 1. Thick crusted lesion over abdomino-inguinal and interdigital region.

be removed because it could inhibit topical antiscabies penetration, and the erosion should be treated soon because the applicationof topical antiscabies over the erosion could cause irritation. After 4 days of wet dressing treatment the thick crusts and the erosion were decreased, application of Permethrin 5% cream once a week at night was started, with exception wet dressing and Urea application were still done for area with thick crusts. Besides dermatology therapy, the patient also continue the antiretroviral (ARV) treatment, include terafovir, lamivudine and neviral; concomitant with supportive treatment.

Histopathology examination showed hyperkeratotic, parakeratosis, acanthosis and burrow in stratum corneum layer of epidermis. While in dermis there were capillary vessels with a little inflammatory cell, so the conclusion from the histopathology examination was scabies infection.

After hospitalized for 1 week and there were progress of his lesion, this patient was discharged from hospital. Before went home, he and his family were given education to repeat the use of Permethrin 5% cream 1 week after the first use if there was any lesion persist either crusts or papules and to visit hospital afterwards, to wash all clothes, towels, and bedding by hot water. If there were any other household members that have the same complaint, they should be treated soon.

DISCUSSION

Norwegian scabies or crusted scabies is a rare manifestation of scabies characterized by uncontrolled proliferation of mites in the skin. This disease was first described by Boeck and Danielssen among leprosy patients in Norway in 1848.[8,9] High risk groups for this infection such as they who are taking systemic glucocorticoid therapy or using potent topical glucocorticoid therapy, organ transplant recipient, having mental or physical disability, infected by HIV or human T-lymphotrophic virus-1, and also people with malignancy.[10] This severe variant

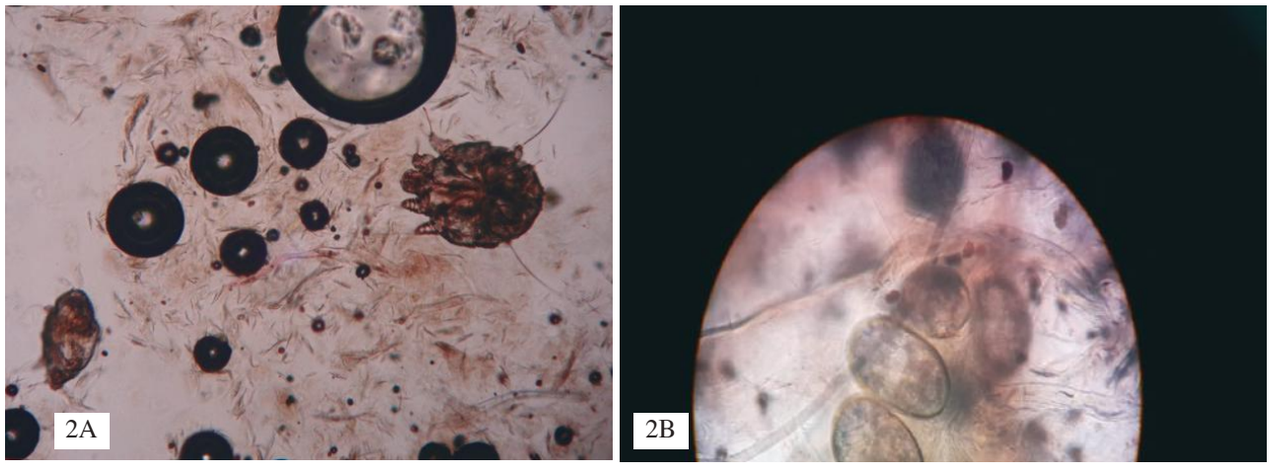

Figure 2. *Sarcoptes scabiei* mite from the lesion scraping: adult mite (2A), eggs (2B)

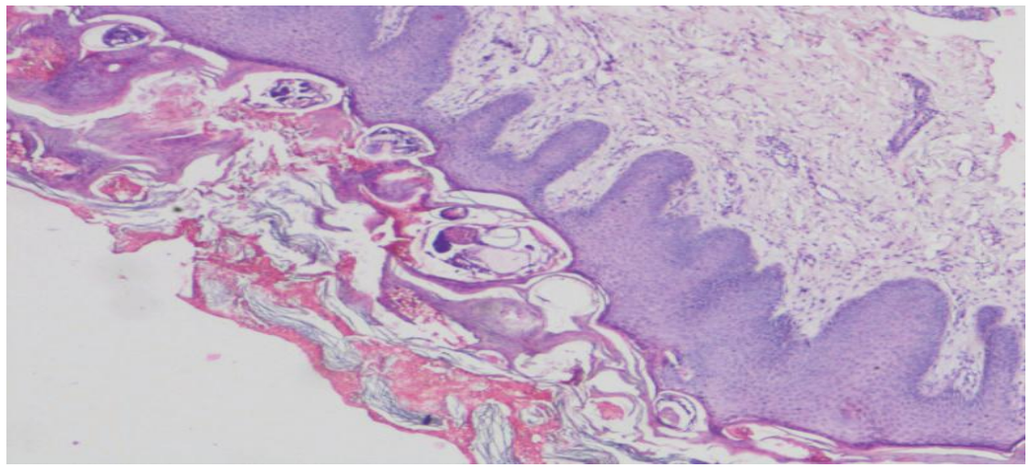

Figure 3. Histopathology slides with 40x magnification

of scabies occurs as widespread hyperkeratotic crusted lesions, hence the name "crusted scabies" is preferred as the synonym of "Norwegian scabies".[11]

The causative agent, mite *Sarcoptes scabiei var. hominis*, is an obligate parasite that lives in burrowed tunnels in the stratum corneum. In the skin, the mite survives on a diet of dissolved human tissue but does not feed on blood. It makes a sloping burrow, in the stratum corneum to the boundary of stratum granulosum every day. The mite lives in the burrow for a 30 day-period, consisting cycle as follows. The female mite lays 2 - 3 eggs daily and the eggs hatch in 10 days, then the young larva leaves the burrow to become mature adult mite in 14 – 17 days.[2,3,9] In normal patient, it is estimated that only 10% of the eggs that develop into adults with total average mite is about 11. However, the number of the mites is very enormous in crusted scabies because of uncontrolled infection.[8,9,10] Recently there is increasing occurrence of this case due to various immunosuppressive agent and increasing case of HIV patient.

Cutaneous manifestations of scabies are due to the burrowing of the female mite followed by humoral and delayed hypersensitivity of the host.[2,3] The mite antigens that trigger the immune response are probably in the mite's saliva. Combined with scratching, the immune system in the healthy host will reduce the mite load but rarely eliminates the mite. The failure of the immune system to suppress the proliferation of the mite is considered to play role in crusted scabies development, though incidence of crusted scabies in Australian aborigines with normal immunity has been reported.[13] In this case, HIV stadium IV that the patient suffered from made the level of CD4+ T cells dropped until 12 cells/μL so that the patient was susceptible to infection. While less itchy sensation occurred as the result of inadequate immune system, a huge amount of mites makes this disease very contagious.[7,8]

Definitive diagnosis of crusted scabies is equal with common scabies, which is the presence of mite, egg, eggshell or fecal material from skin lesion scraping, demonstrated by potassium hydroxide 10% solution under light microscopy examination. In this patient, we found the presence of the mite and egg so that antiscabies treatment could be initiated without waiting for the histopathology result. Later, the histopathology examination revealed that there was burrow in the stratum corneum that is surrounded by inflammation cells, showed that cellular immunity plays role in this disease's pathogenesis. Burrow was pathognomonic sign that we can find in scabies infection.[2,3]

Scabies management involves the use of scabicide agent and mite control. Antiscabies agent might be taken orally and topically as discussed above, meanwhile mite control needs education for the patient and his family. All family members that live together with the patient should be treated at the same time to prevent asymptomatic carrier's reinfestation. If possible, during the time of application of the topical scabicide, all linens, bedding, and clothing in the house that has been used should be soaked with warm/hot water before washed, and then ironed with high temperature to eradicate mite.[2,3]

This patient was treated initially with wet dressing (2-3days), using normal saline combined with 10% urea cream to remove the thick crusts. Then a 5% Permethrin cream was applied intermittently combined with ointment contains 2% salicylic acid plus 4% sulfur ("2-4 ointment) daily in between the permethrin. After 14 days application of this topical medication there is mark improvements. Theoretically oral ivermectine could be used since this drug acts on nerve synapses utilizing glutamate or γ-aminobutyric acid.[14] But this oral medication could not penetrate into the thickness of the keratinous debris and this drug is not available in Indonesia. Conventional wet dressing method using NaCl 0.9% solution was used, then followed by topical Urea 10% use for softening the crusts. After the crusts already minimal and thinned, Permethrine 5%, a topical antiscabies agent, was applied to this patient. This topical agent will be effective in such situation due to better absorption in the skin.

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