PERAN KONDISI LINGKUNGAN RUMAH, STATUS GIZI, STATUS BESI, RESPONS IMUN DAN POLIMORFISME *NRAMP1* TERHADAP PROBABILITAS KEJADIAN SAKIT PADA ANAK KONTAK TUBERKULOSIS

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TUBERCULOSIS; MYCOBACTERIUM

RINGKASAN

Indonesia adalah salah satu negara dengan kasus tuberkulosis (TB) terbanyak di dunia (WHO, 2007). TB umumnya bermanifes pada usia produktif antara 15-65 tahun. Walaupun demikian tidak dapat dipungkiri bahwa anak-anak juga terinfeksi oleh kuman *Mycobacterium tuberculosis,* terutama dari orang tua yang terkena TB aktif. Di negara endemis, TB pada anak berkisar antara 12-20% dari total kasus TB seluruhnya (Donald, 2004). Berbeda dengan kasus pada dewasa yang mana kebanyakan dalam bentuk TB paru (pulmonal), maka pada anak bentuk TB bervariasi dari pulmonal maupun ekstra-pulmonal, misalnya TB meningitis, maupun TB tulang. Anak dari orang tua dengan TB aktif (anak kontak) merupakan suatu sumber dari penularan TB dimasa mendatang dengan adanya infeksi laten *M.tuberculosis* (Khan, 1995).

Lingkungan dimana anak kontak tinggal seperti lingkungan fisik rumah, kelembaban, dan ventilasi yang buruk dapat meningkatkan risiko untuk terkena sakit TB. Kepadatan dan tingginya jumlah penghuni rumah mempermudah terjadinya penularan pada anak. Hal ini ditunjang oleh data epidemiologis yang menunjukkan bahwa densitas kejadian sakit tertinggi pada umumnya berada pada area miskin yang padat dan kumuh.

Besi dibutuhkan oleh *M.tuberculosis* untuk dapat tumbuh, sehingga keseimbangan kadar besi dalam tubuh sangat penting dalam proses terjadinya infeksi. Asupan makanan yang mengandung besi cukup dapat mempengaruhi daya tahan tubuh terhadap TB, sementara infeksi parasit seperti cacingan dapat membuat tubuh menjadi kekurangan besi dan anemis. Protein *natural resistance associated macrophages protein* (NRAMP) adalah protein yang mengatur transport besi masuk dan keluar dalam fagosom yang mempunyai peranan yang besar dalam kerentanan tubuh terhadap infeksi TB. Oleh sebab itu status besi pada anak-anak sangat penting dalam kaitannya dengan infeksi TB. Variasi pada gen yang menyandi protein *NRAMP1* dilaporkan dapat berdampak pada kerentanan terhadap infeksi TB. Beberapa polimorfisme pada gen *NRAMP1* telah dilaporkan di berbagai etnis di dunia dengan hasil yang berbeda-beda.

Adanya infeksi *M.tuberculosis* pada makrofag akan memberikan signal kepada sel T untuk memproduksi IFN-γ yang kemudian dapat mengeliminasi *M.tuberculosis.* Pada kondisi makrofag gagal, maka upaya tubuh selanjutnya adalah membentuk granuloma untuk melokalisir kuman. Kapasitas sel T yang tertekan dengan produksi IFN-γ yang rendah membuat *M.tuberkulosis* dapat berkembang biak dengan baik, dan tahap ini disebut sakit TB. Kegagalan makrofag ini ditandai dengan rendahnya sitokin pro-inflamasi IFN-γ dan adanya kerusakan jaringan yang ditandai dengan tingginya sitokin

anti-inflamasi IL-10. Suatu studi longitudinal melaporkan bahwa rasio IFN-γ dan IL-10 dapat merupakan prediktor untuk terjadinya sakit TB (Hussain, 2007).

Infeksi laten dapat terjadi bertahun-tahun selama fungsi makrofag adekuat melawan upaya *M.tuberculosis* untuk berkembang biak. Tes tuberkulin digunakan untuk membuktikan adanya infeksi laten. Tes *Interferron Gamma Release Assay* (IGRA) menggunakan prinsip kerja sel T secara *ex vivo*. IFN-γ yang diproduksi oleh sel mononuclear darah tepi setelah dikultur dengan fragmen *M.tuberculosis* spesifik ESAT-6 dan CFP10 dapat diukur. Diharapkan tes IGRA ini dapat lebih spesifik mendeteksi adanya infeksi laten. Tes ELISPOT juga menunjukkan adanya aktivitas sel T dalam memproduksi IFN-γ yang digambarkan dengan adanya spot yang mewakili sel T yang aktif. Adanya *M.tuberculosis* dalam sel dapat dilakukan oleh pemeriksaan 16S rRNA pada sel mononuclear darah tepi. Penelitian disertasi ini bersubjek anak-anak yang tinggal serumah di sekitar penderita TB dewasa (kasus indeks) dengan sputum basil tahan asam yang positif selama minimal 8 minggu berturut-turut. Dari 56 kasus indeks penderita TB dewasa, didapatkan 73 anak kontak yang berasal dari sosial ekonomi rendah. Pada anak kontak dilakukan pemeriksaan klinis, pemeriksaan radiologis dan tes tuberkulin/IGRA/ELISPOT. Anak kontak tersebut dikelompokkan dalam kelompok 'sakit' bila terdapat gejala klinis, dan kelompok 'tidak sakit' bila tidak terdapat gejala klinis. Jumlah angka kesakitan TB pada anak kontak yang didapat pada penelitian ini sebesar 42,4% dengan jumlah yang terinfeksi sebesar 57,5%. Selain itu, anak kontak juga digolongkan dalam kategori TB yang dibuat dengan menilik hasil tes tuberkulin/IGRA/ELISPOT, yaitu kelompok TB kelas 1 (hanya kontak saja), kelas 2 (kontak dan bila tes tuberkulin/IGRA/ELISPOT positif), atau kelas 3 (kontak, bila tes tuberkulin/IGRA/ELISPOT positif serta dicurigai melalui bukti pendukung bahwa terdapat kerusakan jaringan). Berdasarkan gabungan tes tuberkulin maupun IGRA/ELISPOT angka infeksi ditemukan pada anak kontak tersebut adalah 43,8% sampai 53,4%. Pemeriksaan 16S rRNA kuman TB dilakukan secara random pada 43 pada sampel mononukelar darah tepi. Seluruh sampel yang diperiksa menunjukkan hasil positif. Dengan demikian, terjadi kolonisasi kuman TB pada makrofag dengan maupun tanpa reaksi tubuh inang. Anak terinfeksi dapat jatuh sakit atau bila bertahan pada kondisi terinfeksi maka akan menjadi infeksi laten.

Pada penelitian ini, kondisi lingkungan rumah yang berperan dalam terjadinya reaksi terhadap infeksi dan atau sakit adalah kelembaban dan jumlah penghuni, sedangkan kedekatan ruang tidur dan densitas kuman kasus indeks tidak terkonfirmasi peranannya. Kedekatan genetik derajat pertama dengan orang tua sebagai kasus indeks meningkatkan reaksi positif terhadap infeksi 3-4 kali lipat pada anak kontak, dibandingkan bila kedekatan genetik dengan kasus indeks derajat dua atau lebih.
Faktor ini juga didapati berbeda bermakna diantara kelas TB, baik menurut tes tuberkulin maupun IGRA/ELISPOT.

Status gizi pada umumnya kurang, walaupun demikian status besi pada umumnya normal. Anemia hanya ditemukan pada 2 dari 73 anak kontak (2.7%). Dari 50 sub sampel yang diperiksa faecesnya, tidak ada satupun anak kontak yang cacingan. Polimorfisme *NRAMP1*, D543N dan 3 UTR pada kasus maupun kontrolnya ditemukan dalam keseimbangan Hardy-Weinberg, tetapi tidak terkonfirmasi berperan dalam kejadian sakit.

Respons imun anak kontak secara desktriptif berbeda dengan orang dewasa dimana kadar IFN-γ plasma cukup tinggi, namun tidak terkonfirmasi berperan terhadap kejadian sakit. Meskipun tidak menampakkan perbedaan bermakna, namum tampak

kecenderungan bahwa IFN-γ tertinggi pada anak kelas 3, sedangkan IL-10 tampak tidak terlalu mengalami perubahan, cenderung statis pada berbagai kelas. Sementara itu rasio IFN-γ dan IL-10 menunjukkan perbedaan bermakna di antara kelas TB.

Untuk memprediksi apakah anak kontak dapat terkena TB, suatu model prediksi sakit dibuat dengan regresi logistik ganda. Berat badan menurut umur, jumlah penghuni serumah dan hasil tes tuberkulin dalam model prediksi sakit dengan regresi logistik ganda, menghasilkan prediksi 75,3%, dengan koefisien determinasi (pseudo R) sebesar 46,7%.

Penelitian ini memberikan fakta untuk pertamakali bahwa semua anak kontak telah terinfeksi. Bila infeksi tidak berlanjut menjadi sakit, kemungkinan besar kuman *M.tuberculosis* tetap bertahan dalam makrofag (Lillebaek dkk, 2002). Hal ini menjelaskan mengapa TB paru dewasa pada umumnya merupakan kasus sekunder, baik reinfeksi maupun reaktivasi. Penelitian ini menggeser penggunaan tes tuberkulin, IGRA maupun ELISPOT sebagai penanda infeksi. Bila kasus indeks diketahui, maka baik tes tuberkulin, IGRA maupun ELISPOT lebih tepat diinterpertasi sebagai penanda reaksi tubuh terhadap infeksi yang telah terjadi atau lebih jauh sebagai prediktor perkembangan penyakit. Reaktivasi *M.tuberculosis* dapat menyebabkan kasus baru TB paru dewasa. Dengan demikian, penanganan TB anak adalah suatu hal yang strategis untuk menurunkan insidens TB paru dimasa yang akan datang.

SUMMARY

Indonesia is one of the countries with the largest tuberculosis (TB) cases in the world, affecting people in the productive age between 15 to 65 years old. In endemic countries, TB in children account for 12-20 % from all TB cases (Donald, 2004). TB pulmonal is the most prevalent form of TB in adult, while in children the form of TB varies from pulmonal TB to extra pulmonal TB, ranging from meningitis TB to TB in bones. Children living with active TB parents, termed as contact children, are infected by *Mycobacterium tuberculosis,* the causative pathogen of TB disease. These children form as reservoir of *M.tuberculosis* and may form as source of infection for TB later in the future when they become adult (Khan, 1995). The environment where these contact children live, such as the housing, humidity and bad ventiliation can increase the risk factor for TB disease. Moreover, the density of population in the house and the number of household members further increase the infection rate from active TB cases in adult, termed as index case, to children. According to epidemiology data the high prevalence of TB occured in the area with low social economy condition.

Iron is an obligatory for *M.tuberculosis* to grow suggesting that iron homeostasis in the cell play a significant role in developing of TB infection. Sufficient iron content food influenced the immunity, while parasitic infection in children such as helminth infection may reduce the iron storage and children become anemic. Co-infection with helminthes in childhood may negatively influence outcome of BCG vaccination. Natural resistance associated macrophage protein as iron transporter through phagosom membrane might paly a role in susceptibility to TB. Therefore, the iron status in children has an important value. Variation in *NRAMP* gene are reported to play a role in susceptibility to TB and studies on polymorhpisms in *NRAMP* gene across different ethnicities are well examined. Much of the host infection mechanisms and TB pathogenesis remain unclear. Host defense mechanism in TB is dependent on cellular

mediated immunity, involving macrophages, T cells and type-1 cyotkines. From the first exposure of *M.tuberculosis,* a seris of host response is triggered. After proccessing of mycobacterial protens intro smalles fragments, macrophage activate T cell to produce proinflammatory cytokines with IFN-γ as a key cytokine. When macrophage failed, granuloma is formed as a host defense mechanism to contain the mycobacterium by walling off from further immune response. The capacvity of T cell in active TB is depressed and the production of IFN-γ in decreased, making the *M.tuberculosis* grow well. In the other side of the coin, antiinflammatory cyotkine IL-10 is increased. A longitudinal study reported tthat ratio of IFN-γ over IL-10 might serve as a good predictor for active TB (Hussain, 2007). Identifying children with latent infection of TB, known as LTBI faces many challenges. LTBI can last for years as long as macrophages function well to contain *M.tuberculosis* to grow. Tuberculin skin test (TST) or Mantoux test is until recently used to detect LTBI. New test based on IFN production of T cell *ex vivo* such as *Interferron Gamma Release Assay* (IGRA) is in the market. The IFN-γ is produced after stimulation of a highly specific MTB antigen for latent TB e.g. ESAT-6 and CFP10. Another assay to detect IFN-γ response is by counting the IFN-γ producing T cells that has been developed in the enzymed like immunoassasy ELISPOT. With new sophisticated method of 16S rRNA examination, *M.tuberculosis* containing cells in peripheral blood mononuclear cells can be detected. In this dissertation, children have been living with active TB adult consecultively for at least 8 weeks. Of 56 index cases of active TB adult who live in low socio economc condition, 73 contact TB children could be recruited. These children were examined for radiology and TST/IGRA/ELISPOT. These children were grouped as *sick* when they showed clinical signs (42.4%) indicating they have TB disease, or otherwise grouped as *not sick* (57.5%) After adjusting with TST/IGRA/ELISPOT, contact children were grouped as TB class 1 (TB contact, but all tests were negative), class 2 (TB contact with one of tests was postive) and class 3 (TB contact, with one of test was positive and clinical/radiological signs suggesting lesion in lung or lymphnodes. Morbidity rate according to this class is 43,8% to 53,4%. Surprisingly, all 16S rRNA *M.tuberculosis* examination in a subset randomized of 43 samples showed positivity for the mycobacterium, suggesting that all contact TB children are infected with *M.tuberculosis.* Environmental condition of the houses played a role in this study, such as humidity and the number of the members in house. The distance of bedroom and the density of *M.tuberculosis* in the sputum were, however, not confirmed with higher risk for developing of TB disease. Nutritional status was generally poor, however, the iron status was within normal range. Anemia in these children were only found in 2 of 73 (2.7%). Of 50 sub sample no helminth infection were found. *NRAMP* polymorhpisms in D543N and 3 UTR showed no significant different between these children with TB and without TB disesase. Proinflammatory cytokine IFN-γ production in plasma as a read out in immune response in *M.tuberculosis* was higher in TB class 3, however, this was not significantly different. In the other end, antiinflammatory cytokine IL-10 were tended to be stable. The ratio of IFN-γ over IL-10 were significantly different as indicated in study conducted previously. To predict whether contact TB children can develop TB later in the life time, a model had been developed using multiple regression analyses. The predictive value of TB disease using parameters such as weight to age, the number of members in house and positive TST test, resulted in predictive value of 75,3 % with determinat coefficient (pseudo R) 46.7%. This disertation have showed for the first time that TB infection is happened on all children living with an active TB case. *M.tuberculosis* infection are contained in

macrophage efficiently until it evades the host defense mechanisms to grow later in the life time. This give a fact that TB in adult is a secondary infection, as a result of reinfection or a reactivation. Result in this reseacrh shift the TST//IGRA/ELISPOT that have been used as LTBI marker. When there is a TB contact, the TST/IGRA/ELISPOT test can be determined as a host response in infected children or more to predict development of disease. Morever, later reactivation of TB infection in childhood may increase further the new TB cases. TB assesment in children is essential to prevent new TB cases later in adult.

ABSTRACT

Role of home environmental condition, nutritional status, iron status, *NRAMP1* polymorphisms and immune response in probability to disease on children living with active tuberculosis patients

Pudji Lestari

Indonesia is one of the countries with the largest tuberculosis (TB) cases in the world. In endemic area, TB in children account for 12-20% from all TB cases. Children living with active TB adult cases are at higher risk for infection and disease development. Identifying children with latent infection of TB may be of potential use. Environmental factors play significant roles and host responses to mycobacterium are essential.

This research aimed to explore environmental factors, including humidity and number of people living in the same house, and host factors, including nutrition status iron status, and genetic background of variation in *NRAMP* gene which play role in disease development.

In a cross-sectional study design, children living with active TB adult for at least 8 weeks were recruited. Clinical and radiological examinations were conducted. Tuberculin skin test (TST), *Interferon Gamma Release Assay* (IGRA), and ELISPOT were examined. Mycobacterial 16S rRNA in cell was studied.

Of 56 index cases with active TB adult who lived in a low socio economic condition, 73 contact TB children were recruited. These children were examined for chest radiology and TST/IGRA/ELISPOT. These children were classified as *sick* when they showed clinical signs (42.4%), indicating they had TB disease, or otherwise classified as *not sick* (57.6%). After adjusting with TST/IGRA/ELISPOT, morbidity rate is 43.8% to 53.4%. Surprisingly, all mycobacterial 16S rRNA examination in a subset randomized of 43 samples showed positive results, suggesting that all contact TB children were infected with *M.tuberculosis.* Environmental conditions of the house played roles in disease development i.e humidity and the number of people living in the same house. Shared bedroom and the density of *M.tuberculosis* in the sputum were, however, not confirmed with higher risk for developing of TB disease. Nutritional status was generally poor, however, the iron status was within normal range. Anemia in these children was only found in 2 of 73 (2.7%). Of 50 sub sample examined, no helminthes infection was found. *NRAMP1* polymorphisms in D543N and 3 UTR showed no significant different between *sick* and *not sick* groups. Result on Interferon gamma concentration in serum showed the highest concentration on *sick* group, while Interleukin-10 remain stable between groups. To predict whether contact TB children can develop TB later in the life time, a model had been developed using multiple regression analyses. The predictive value of TB disease using parameters such as weight to age, the number of member in house and positive TST test, resulted in a predictive value of 75,3% with determinant coefficient (pseudo R) 46,7%.

This study has showed for the first time that in contact with open case for 8 weeks, 100% children are infected (using 16S rRNA examination). Nutrition status, number of people living in the same house and positive tuberculin test are predictor for disease development. TB assessment in children is essential to prevent new TB cases later in adult.

Key words: children tuberculosis, humidity, 16S rRNA *Mycobacterium spp*.