RESEARCH NOTE

NRAMP1 POLYMORPHISM AND SUSCEPTIBILITY TO LUNG TUBERCULOSIS IN SURABAYA, INDONESIA

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Abstract. The aim of this study was to evaluate the role of three polymorphisms (D543N, TGTG deletion in 3' UTR, INT4 G/C) of *NRAMP1* to susceptibility to lung tuberculosis (TB) disease. The results showed that homozygous TGTG deletion in 3'UTR of *NRAMP1* was found more frequent in lung tuberculosis patients than in healthy nurses working in a TB ward. This 4 base deletion might cause malfunction of NRAMP1 protein so that it fails as Fe^{2+} ion transporter causing macrophage unable to eliminate *Mycobacterium tuberculosis*.

Keywords: *NRAMP1* gene polymorphism, lung tuberculosis disease, Indonesia

INTRODUCTION

Lung tuberculosis (TB) disease is an infectious disease, which is still difficult to be controlled. WHO estimates that the largest number of new TB cases in 2008 occurred in the Southeast Asia region, which accounted for 34% of incident cases globally. An estimated 1.3 million people died from TB in 2008. The highest number of deaths was in the Southeast Asia region, while the highest mortality per capita was in the Africa region (WHO, 2010).

In Indonesia TB has re-appeared as a prominent cause of death. The results of a family health survey (SKRT-Study) revealed that TB is the third cause of death after cardiovascular and respiratory tract diseases in all age and is the most leading cause of death in infectious disease (Ratgono *et al*, 2005).

Lung TB spreads through droplets that enter via air breathing to lung alveoli, which evoke an inflammation response through accumulation of macrophages and neutrophils, which then migrate to regional lymph nodes to form primary complex (Grosset *et al*, 2000). The bacilli in lung tissues or lymph nodes are engulfed by macrophages and multiply inside these cells. The healing of primary complex happens as an inflammation exudate and destruction of bacilli. If the microorganisms can survive they will reach lymph circulation and blood stream to infect other organs. In human, *NRAMP* gene is expressed in macrophages, lymphocytes and lung tissue. The gene encodes a protein that functions as a divalent ion channel (Samantha and Phillip, 2000). Fe^{++} ion can inhibit growth of *Mycobacterium tuberculosis* (Blackwell *et al*, 2001). When

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a mutation of *NRAMP1* gene yields a non functional NRAMP1 protein, there is inhibition of intracellular killing mechanism of *M. tuberculosis* in macrophages. Bellamy *et al* (1998) have reported in Gambia that polymorphisms in 5'(GT)n, INT4, D543 and 3'UTR of *NRAMP1* affect susceptibility to *M. tuberculosis*.

In the pulmonary TB ward in Soetomo Hospital, Surabaya, nurses who have close contact with TB patients for years did not show any signs of having lung TB (Nugraha and Handojo, 2005). Likewise many other studies also revealed that people who have been infected with TB, only about 10% become ill, and that the latter group belongs to immunocompromised subjects, as a result of HIV (human immune deficiency virus) infection, or suffer from diabetes mellitus, and the rest is considered less susceptible because of genetic and environment factors (Kim *et al*, 2003; Zhang *et al*, 2005).

The aim of this study was to determine whether there were any associations between the three polymorphism (D543N, TGTG deletion in 3' UTR, INT4 G/C) of *NRAMP1* and susceptibility to lung tuberculosis.

MATERIALS AND METHODS

This sample study was an observational cross-sectional study conducted at Dr Soetomo Hospital, Karang Tembok Hospital and Institute of Tropical Disease, Airlangga University, Surabaya, Indonesia. Whole EDTA-blood samples were taken from 69 lung TB patients and 43 pulmonary ward nurses who filled the criteria (age, AFB, thorax photo, HIV, Hb, RBG, BUN, creatinine). PBMC DNA was isolated using HighPure PCR Template Preparation Kit (Roche, Mannheim, Germany), and 5 l were taken for polymerse chain reaction (PCR) using *Fast Start PCR Master* kit (Roche, Mannheim, Germany). Exon 15 together with 3'UTR region was amplified using forward primer 5' -GCA TCT CCC CAA TTC ATG GT -3' and reverse primer 5' - AAC TGT CCC ACT CTA TCC TG -3'. PCR thermocycling was performed as follows: 95°C for 5 minutes; 35 cycles for 30 seconds; 60°C for 30 seconds, and 72°C for 3 minutes; a final step at 72°C for 7 minutes. The amplicon had a size of 244 bp. Restriction fragment length polymorphism (RFLP) using *Ava* II (Roche) to identify D543N mutation was done as follows. One l of *Ava II*, 2 l of buffer A, 9 l of water, 8 l of template DNA were incubated at 37°C overnight and the reaction was stopped at 70°C for 5 minutes. The results were electrophoresed in 2% LE agarose (Roche) and stained with ethidium bromide. For *Ava II* digestion of Asp allele there are 3 bands namely 126, 79 and 39 bp, and for Asn allele 2 bands of 201 and 39 bp.

FokI (Roche) was used to digest 3'UTR for RFLP analysis. One l of *FokI*, 2 l of Buffer A, 13 l of water, 4 l of template DNA were incubated at 37°C overnight and reaction stopped at 70°C for 5 minutes. The results were analyzed as described above. *FokI* cuts TGTG+ allele into 2 bands of 211and 33 bp, and TGTG del produced only the 240 bp band.

For detection of INT4 polymorphism G to C base in intron 4, PCR was done using *Fast Start PCR Master* kit (Roche) and INT4 forward primer 5' - TCT CTG GCT GAA GGC CTC TCC - 3' and reverse primer 5' - GAG GCT CAA ACT GAT AGC ACA - 3', which yields a product of 628 bp. RFLP using *ApaI* (Roche) to detect INT4 was conducted as follows. Two l of *ApaI*, 2 l buffer A, 12 l of water and 4 l of template DNA were incubated at 37°C overnight and stopped at 70°C for 5 minutes.

Table 1
Frequency of D543N, 3'UTR, and INT4 polymorphisms in lung TB patients and healthy nurses.

Polymorphism	Genotype	Lung TB patient (n = 69)	Healthy nurse (n = 43)	Chi-square	p-value
D543N	G/G	35 (51%)	28 (65%)	9.348	0.098
	G/A	28 (40%)	15 (35%)		
	A/A	6 (9%)	0 (0%)		
3'UTR	TGTG+/+	35 (51%)	28 (65%)	10.048	0.007
		14 (20%)	13 (30%)		
		29 (29%)	2 (5 %)		
INT4	G/G	67 (97%)	40 (93%)	1.033	0.370
	G/C	2 (3%)	3 (7%)		
		0 (0%)	0 (0%)		

Results were analyzed as described above. *ApaI* does not cut G allele in intron 4 and C allele is digested into 2 fragments of 455 and 169 bp.

The results of PCR-RFLP were evaluated using chi-square statictical method. A p-value < 0.01 is considered significant.

RESULTS

The frequency of the 3 polymorphisms (D543N, 3'UTR and INT4) in lung TB patients and healthy nurses group are summarized in Table 1. INT4 and D543N polymorphisms did not show any correlation with susceptibility to TB infection, but 3' UTR polymorphism did show significant correlation with susceptibility to TB infection.

DISCUSSION

Our results are in concordance with the previous study from Poland (Dubaniewics, 2005) showing that INT4 polymorphism is not associated with TB infection but is strongly correlated with autoimmunity, but both D543N and 3'UTR polymorphisms are associated with susceptibility to infection. The homozygous deletion in 3' UTR may have affected the addition of poly-A tail to the 3' end of mRNA which is necessary for mRNA stability. These data should be useful in the future for prediction of susceptibility to TB infection, so that appropriate action to prevent TB infection can be initiated.

REFERENCES

Bellamy R, Ruwende C, Corrah T, McAdam KPWJ, Whittle HC, Hill AVS. Variation in the *NRAMP1* gene is associated with susceptibility to tuberculosis in West Africans. *N Engl J Med* 1998; 338: 640-4.

Blackwell JM, Goswani T, Carlton AWE, *et al. SLC11A1* (Formerly *NRAMP1*) and disease resistance. *Cell Microbiol* 2001; 3: 773.

Dubaniewics A, Jamiesson SE, Dubaniewics-Wybieralska M, *et al.* Association between SLC11A1 (formerly NRAMP1) and the risk of sarcoidosis in Poland. *Eur J Hum Genet* 2005; 13: 629-34.

Grosset J, Truffpt-Pernot, Cambau E. Bacteriology of tuberculosis. In: Reichmann LB, Hershfield ES, eds. Tuberculosis – A comprehensive international approach. 2nd ed. New York: Marcel Dekker, 2000: 157-85.

Kim JH, Lee SY, Lee SH, *et al*. *NRAMP1* genetic polymorphisms as a risk factor of tuberculous pleurisy. *Int J Tuberc Lung* 2003; 7: 370-5.

Nugraha J, Handojo I. Possible use of ESAT-6 antigen in diagnosing latent tuberculosis. Konas Petri XI, PIT PAPDI II Cabang Surakarta, 2005.

Ratgono A, Kushandoyo S, Adhor D, *et al*. Program Penanggulangan Tuberkulosis di Jawa Timur. Simposium TB. Tropical Disease Center (TDC) Unair, 2005.

Samantha G, Philippe G. Genetic susceptibility to intracellular infections: Nramp1, macrophage function and divalent cations transport. *Microbiology* 2000; 3: 43-8.

World Health Organization (WHO). Tuberculosis. *WHO Fact Sheet* 2010; 104.

Zhang WH, Shao L, Weng X, *et al*. Variants of the natural resistance-associated macrophage protein1 gene (*NRAMP1*) are associated with severe forms of pulmonary tuberculosis. *Clin Infect Dis* 2005; 40: 1232-6.