

Polymorphism of Natural-Resistance-Associated Macrophage Protein 1 (NRAMP1) D543N Gene and Expression of NRAMP1 on Lung Tuberculosis Patients and Nurses in Surabaya

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19

POLYMORPHISM OF NATURAL-RESISTANCE-ASSOCIATED MACROPHAGE PROTEIN 1 (NRAMP1) D543N GENE AND EXPRESSION OF NRAMP1 ON LUNG TUBERCULOSIS PATIENTS AND NURSES IN SURABAYA

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ABSTRACT

Tuberculosis caused by *Mycobacterium tuberculosis* which can cause death, where the situation is more compounded when the cause is caused by genetic factors. The purpose of this study will evaluate the role of gene polymorphisms D543N natural-resistance-associated macrophage protein 1 (NRAMP1) and the expression of NRAMP1 in nurse and patient with pulmonary tuberculosis. NRAMP1 gene polymorphism D543N occur because there are nucleotide changes at codon 543 in exon 15, which displacement causes a change to aspartic acid asparagin. NRAMP1 gene is expressed not only in macrophages also on the blood cells known as peripheral blood mononuclear cells (PBMC). NRAMP1 gene encoding a protein, transport protein NRAMP1 is a bivalent metal ion that serves as a channel for divalent ions including Fe^{++} ions. Fe^{++} ions can inhibit the growth of *M. tuberculosis* and will eventually kill *M. tuberculosis* (Blackwell, 2001). If because there is a mutation in the NRAMP1 gene produces proteins that are not functional or NRAMP1 mutations cause NRAMP1 protein expression decreased, so that the growth inhibition of *M. tuberculosis* will be lost or reduced, causing the germs will breed freely in pulmonary alveolar macrophages. Results variant genotype A/A polymorphism D543N occurred only in patients with pulmonary tuberculosis. Average yield of protein expression of NRAMP1 in nurses is higher than pulmonary tuberculosis patients. In conclusion, the polymorphism D543N A allele tended to cause an average of NRAMP1 expression decreased, so that the variant genotype A/A gene NRAMP1 D543N polymorphism is gene causes susceptibility to infection of *M. tuberculosis*.

Keywords: NRAMP1 gene polymorphisms D543N, expression NRAMP1, pulmonary tuberculosis

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INTRODUCTION

Tuberculosis (TB) is an infectious disease that is still difficult to control. In the world, the incidence of TB in 1990 is estimated to occur around 1.7 billion or about one third of the world's population infected with *Mycobacterium tuberculosis*. Incidence of TB occurred eight million cases and mortality occurred three to four million cases per year, therefore the WHO declared TB as one of the most important infectious disease to be considered and addressed throughout the world (WHO, 2002). In Indonesia, the incidence of TB in 1999 is estimated to WHO, every 100,000 population there are 130 new patients with lung tuberculosis AFB (acid resistant bacteria) is positive (Indonesian Department of Health, P2TB Guidelines, 2002). The global TB situation in the year 2005, there were approximately 9 million new TB cases and the estimated 1.6 million

people (27/100.000) died from TB. Indonesia, including 22 countries with the number of TB cases (WHO, 2007). Pulmonary TB disease transmission through droplets that enter through respiratory air into the lung alveoli, then the inflammatory response arising from the accumulation of macrophages and neutrophils, followed by migration to regional lymph nodes form the primary complex. Basil in the lung tissue or lymph glands ingested by macrophages and multiply within macrophages. Primary lesion healing occurs as the result of exudates inflammation and destruction. If bacteria can survive to reach the lymph and blood flow to other organs.

This phenomenon occurred in the field, there is a group of people easily catch and on the other hand there are groups who are exposed to droplets from pulmonary tuberculosis patients but not pulmonary TB disease. In

Room Inpatient pulmonary tuberculosis patients in Dr. Soetomo, nurses who treat patients with pulmonary TB for years, showed no clinical symptoms of pulmonary TB. Results of various studies show that the number of people infected with TB only about 10% who are sick, partly because they are known to host due to infection immunocompromized Human Immunodeficiency Virus (HIV) or Diabetes Mellitus. The remaining alleged to be caused by environmental or genetic factors (Kim, 2003; Zhang, 2005). *M. tuberculosis* is a proliferative tuberculosis bacteria in the cells mainly in the macrophages. Based on these facts the authors suspect there is a strong genetic linkage to the immunity in the macrophages with the incidence of susceptibility (or immunity) against pulmonary TB.

NRAMP1 gene is expressed not only in macrophages also on blood cells, known as peripheral blood mononuclear cells (PBMC). These genes encode a protein that serves as a channel for divalent ions including Fe ions⁺⁺. Fe⁺⁺ ions can inhibit the growth of *M. tuberculosis* and eventually kill *M. tuberculosis* (Blackwell, 2001). If due to a mutation in the NRAMP1 gene, it will produce no functional NRAMP1 protein or mutation causes decreased protein expression, hence the growth inhibition of *M. tuberculosis* will be lost or reduced, so the germs will breed freely.

Of the various genes NRAMP1 polymorphisms, namely: (GT) n; 136del9; 274C/T, 469 +14 G/C (INT4); 577-18G/A, 823C/T, A318V; 1465-85G/A, D543N; 1729 +55 del4 (3'UTR); (CAAA) n/(CA) n (Camstock, 1978), only a few are associated with susceptibility (or immunity) against *M. tuberculosis* is polymorphic 5 '(GT) n, 274C/T, INT4, D543N, and 3'UTR (Bellamy, 1998; Dalgado, 2002). Of the five polymorphisms were also found there was lack of conformity between the findings of the investigators, ie there are expressed D543N polymorphism raises immunity (Dalgado, 2002; Kim, 2003) which states there is also cause susceptibility (Bellamy, 1998; Gao, 2000; Liu, 2004, Zhang, 2005), so that should be investigated the role of genes NRAMP1 D543N polymorphism and expression of NRAMP1 in nurse and pulmonary tuberculosis patients in Surabaya.

4 MATERIALS AND METHODS

This is an observational cross sectional analytic study which takes places in Dr. Soetomo General Hospital, Tembok Dukuh Hospital, and Institute of Tropical Disease Airlangga University Surabaya. Samples of this study are 69 men with pulmonary tuberculosis patients aged between 18-55 years, smear positive, positive sputum culture of *M. tuberculosis*, photos Thorax

positive pulmonary tuberculosis, HIV negative, Hb, GDA, BUN, creatinine within normal limits; and 43 nurses who care for pulmonary tuberculosis patients aged ≥ 2 years with between 18-55 years, smear negative, culture negative sputum *M. tuberculosis*, photos Thorax normal, HIV negative, Hb, GDA, BUN, creatinine within normal limits.

Determination of Genotypic Variants of Genes NRAMP1 D543N

Isolation of PBMC from EDTA blood

Two ml EDTA blood centrifuged 3000 rpm for 30 minutes, then pipetted with buffycoat (white layer between the deposition of blood and serum) or PBMC as many as 200 in Eppendorf tubes, then stored frozen-20°C until use.

Genotyping

29
Isolation of DNA with a "High Pure PCR Template Preparation Kit" Roche as stated in the brochure guidelines in the kit. Interpretation of isolated DNA by electrophoresis using agarose LE (Roche), 1% (0.2 g LE 1 Ethidium bromide. D543N agarose 0.5 X TBE plus 20ml mixed with 5 genotype variant DNA amplification using the "Fast Start PCR Master" Roche as guidelines stated in the brochure in the kit uses a pair of primers D543N: 5'-GCA TCT CCC CAA TTC ATG GT-3' and 5'-AAC TGT CCC ACT CTA TCC TG-3'. PCR performed with the following conditions: stage denaturated in temperature of 95°C conducted for 5 minutes, then run as many as 35 PCR cycles with each cycle stages as follows: denaturation for 30 seconds at a temperature of 95°C, annealing for 30 seconds at a temperature of 60°C, and its extension for three minutes at a temperature of 72°C. Final Extension conducted over seven minutes at a temperature of 72°C. After the cycle is complete, the temperature cooled to 40°C. The results of DNA PCR is amplicon gene NRAMP1 D543N genotype and can be stored at 4°C or -20°C until used for RFLP examination.

27 PCR-RFLP

Analysis of restriction fragment length polymorphism (RFLP) using endonuclease restriction enzyme *AvaII* to get different variations of the individual pieces of the D543N variant genotype. RFLP results will be obtained variant G/G, G/A, and A/A which is performed as follows, 1 μ l PCR enzyme *AvaII* in tube 10xSuRE/Cut plus 2 μ l Buffer A, then added water and the last 9 μ l added 8 μ l template DNA. Then the tubes were incubated in 37°C incubator overnight. The next day the reaction carried out with incubated in a waterbath

inactivation for 5 minutes at a temperature of 70°C. Results piece of DNA was detected by electrophoresis using 2% agarose LE (0.4 g LE agarose plus 20 ml TBE 0,5X which is mixed with 5 µl ethidium bromide 10 mg/ml). At the time of application, 20 µl pieces of DNA (result from RFLP) was mixed with 2µl loading buffer followed by the marker (molecular weight marker VIII, Roche), and electrophoresis equipment run on 100 volts for 30 minutes. The result is read by UV light using a Polaroid and made documentation.

Determination of protein expression of NRAMP1

Making Slide PBMC from EDTA Blood

Blood is inserted into a tube containing EDTA and then centrifuged 3000 rpm for 30 minutes. Dipipet buffycoats (PBMC): a white layer between blood cells and plasma, and then abolished in the surface of the glass object. Once dry container immersed in buffer containing 10% formalin (PZ + Formalin solution of 3.8% with a ratio of 9:1). Once dry stored inside the box to continue preparations Immunocytochemistry examination.

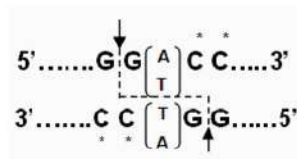
Immunocytochemistry Test

Inspection Immunocytochemistry indirect method using "Ultra Vision Detection System Anti-Polyvalent, HRP/DAB as follows, which already contains a slide smear PBMC were washed with PBS 3x @ 10 minutes, then carried permeabilization with Igepal CA-630 solution 0.5% in PBS for 10 minutes. To reduce the effects of background staining, slides dropped 0.3% H2O2 for 10 minutes. The slides were washed with PBS 2x @ 10 minutes. Goat polyclonal NRAMP1 antibody (E-20) dropped (containing 200 tg of IgG in 1 ml PBS) with a 1:50 dilution. The slides were incubated for 2 hours at room temperature. Slides were washed in PBS 3 x @ 10 minutes. Second antibody (Biotinylated Goat Anti-Polyvalent) dropped and the slides were incubated for 30 minutes at room temperature. Slides were washed in PBS 3 x @ 10 minutes. Peroxidase Streptavidin slides and the slide dropped incubated for 30 minutes at room temperature. The slides are washed with PBS 3 x @ 10 minutes. Slide a working solution dripped DAB chromogen (DAB Plus Chromogen + DAB Plus Substrate with a ratio 1:50) were incubated for 15 minutes at room temperature. The slides are washed with deionized water for 1 minute. Slide shed CounterStain (Hematoxyline Mayer), and the slides were incubated 10 minutes at room temperature. The slides are washed with deionized water for 10 minutes. The slides are dried in the air and mounted (entelan + cover glass). Read the slides under a microscope with a

magnification of 40x as many as 20 field of view using USB PC Camera 301P.

RESULTS

PCR-RFLP from the results of PCR amplifikasi D543N nurses and pulmonary tuberculosis patients. Allele G and allele A contained in the sequence of bases in 1703, where allele G/GAC (Asp/aspartic acid) and allele A/AAC (Asn/Asparagine) contained in Codon 543 in exon 15. RFLP technique was carried out using restriction enzymes *AvaII*, which will cut at the site-specific cuts in a series of bases of DNA molecules of the composition as follows:



Before cut :

126bp
TTGAAGAG|GACCAGAAAGGG
AACTTCTCCTG|GTCTTTCCC

After cut with *AvaII* enzyme :

TTGAAGAG and ACCAGAAAGGG
AACTTCTCCTG GTCTTTCCC

At the cutting with the enzymes *AvaII*, the cutting is called "sticky end, because there are sections of DNA molecules that have a single stranded form (not in pairs), the GAC and CTG. RFLP genotyping results showed variations of pieces as follows: allele G (Asp) showed three bands with the size of DNA nucleotide bases: 126 bp, 79 bp and 39 bp and allele A (Asn) showed two DNA bands with the size of nucleotide bases: 201 bp, and 39 bp. D543N genotype RFLP genotyping results of gene NRAMP1 on: individual G/G homozygotes called the cuts the ribbon will be visible DNA at 126 bp and 79 bp, the individual G/A will be called heterozygotes visible place ribbon cuts DNA at 201 bp, 126 bp and 79 bp, individual A/A homozygotes called the piece will look at the 201 bp DNA band. RFLP genotyping results were visualized on 2% LE agarose containing 10mg/ml ethidium bromide by electrophoresis. RFLP genotyping results were visualized on 2% LE agarose containing 10mg/ml ethidium bromide by electrophoresis.

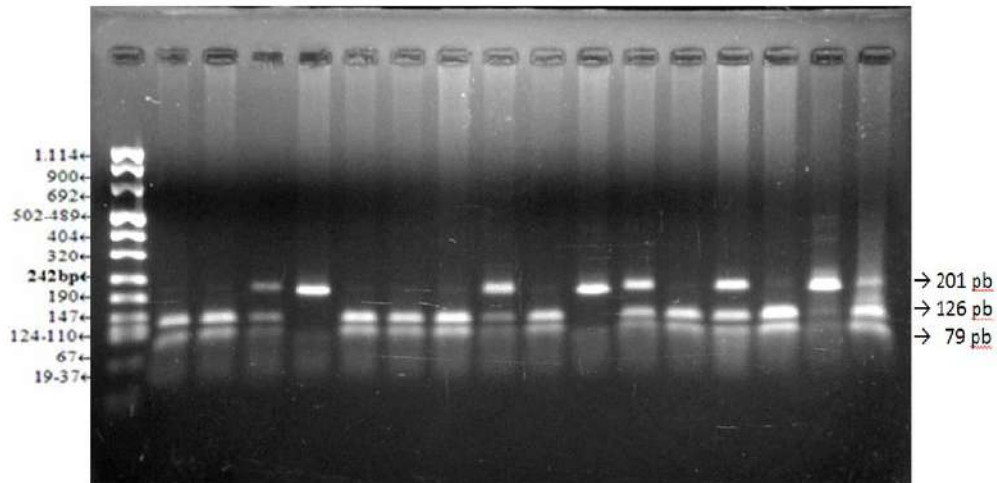


Figure 1. D543N genotype RFLP genotyping results NRAMP1 gene in pulmonary tuberculosis patients. Line 1: Marker; Line 2, 3, 6, 7, 8, 10, 13, 15: variant G/G which showed two bands: 126 bp, 79 bp. Lane 4, 9, 12, 14, 16, 17: variant G/A, which showed three bands: 201 bp, 126 bp, 79 bp. Line 5, 11: variant A/A which shows a band: 201 bp.

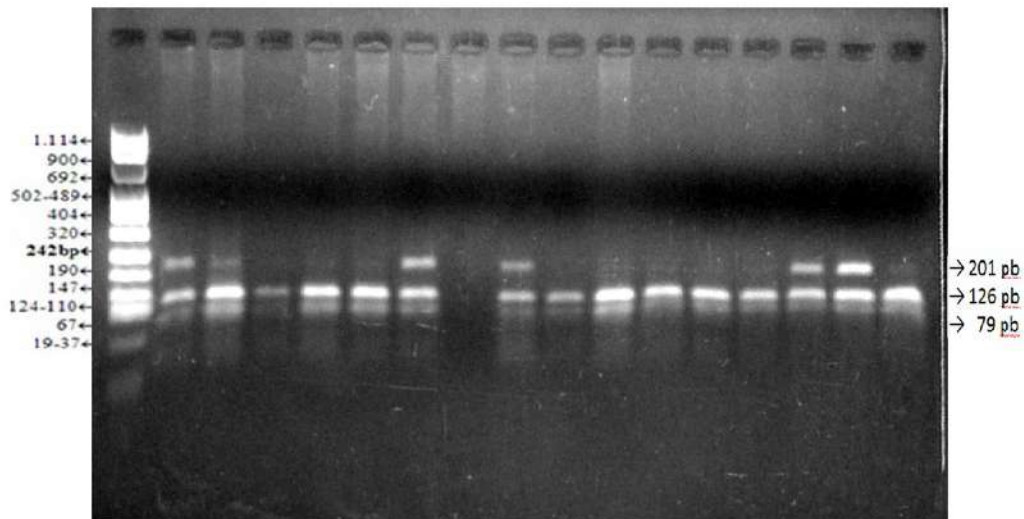


Figure 2. RFLP genotyping results of gene NRAMP1 D543N genotype on nurses. Line 1: Marker; Line 2, 3, 7, 9, 15, 16: variant G/G which showed two bands: 126 bp, 79 bp. Line 4, 5, 6, 10, 11, 12, 13, 14, 17: variant G/A, which showed three bands: 201 bp, 126 bp, 79 bp. Line 8: failed.

Result Analysis of Differences in The Frequency Distribution of Variant Genotypes Of G/G, G/A, A/A Gene NRAMP1 D543N Polymorphism Between Groups Of Nurses and Patients With Pulmonary TB

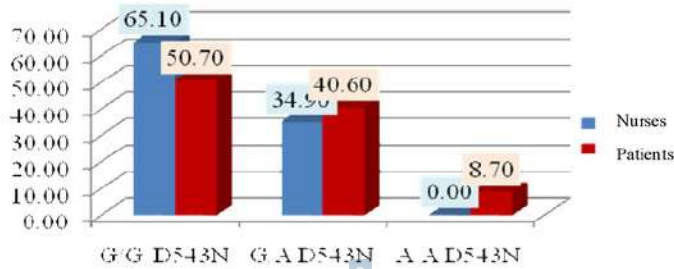


Figure 3. Frequency distribution diagram of genotype variant G/G, G/A, A/A polymorphism D543N between groups of nurses and pulmonary tuberculosis patients.

Here can be seen that, the picture description variant genotype G/G greater for nurses 65.1% (28/43) compared to pulmonary tuberculosis patients 50.7% (35/69), and the variant genotype G/A is greater in patients pulmonary TB 40.6% (28/69) compared to

nurses 34.9% (15/43), and the description descriptions of variant genotype A/A is greater in patients with pulmonary tuberculosis 8.7% (6 /69) than nurses 0% (0/43).

Table 1. Analysis of Fisher's Exact Test to analyze differences in the frequency distribution of variant genotypes of G/G, G/A, A/A polymorphism D543N between groups of nurses and pulmonary tuberculosis patients

D543N polymorphic variant genotype	TB Patients (n=69)	Nurse (n=43)	Fisher's Exact Test	P value
G/G	35 (50.7%)	28 (65.1%)	9.438	0.098
G/A	28 (40.6%)	15 (34.9%)		
A/A	6 (8.7%)	0 (0%)		

The result of Fisher's Exact Test test to analyze differences D543N polymorphism frequency distribution among the group of nurses and patients with pulmonary tuberculosis. Distribution of variant genotypes of G/G, G/A, and A/A on nurses and pulmonary tuberculosis patients with acquired Fisher's Exact Test p-value = 0.98 ($p > 0.05$), then there is no significant difference.

Immunocytochemistry Test

Immunocytochemistry test conducted on 43 samples of PBMC preparations nurses and 69 samples of PBMC preparations in pulmonary tuberculosis patients to detect NRAMP1 protein expression in monocyte cytoplasm. Immunocytochemistry test using a commercial kit that tests sensitivity and specificity, so that the user only according to instructions given by the factory. NRAMP1 antibody was purchased from Santa Cruz Biotechnology, Inc. Europe is produced through a process of purification of polyclonal goat antibody

binding to the peptide mapping of the human NRAMP1 derived from chromosome mapping of human 2q35. Immunokimia test is not recommended by the manufacturer to conduct a chessboard titration, because the concentration of NRAMP1 optimal antibody dilutions already obtained against several kinds of tests, such as Western Blotting suggested in dilution 1/200 (1/100-1/1000), immunocytochemistry suggested in dilution 1/50 (1/50-1/500).

In this study used the dilution 1/50. Kit UltraVision Detection System Anti-polyvalent, HRP/DAB (Ready to Use) to detect antigen NRAMP1. In this study prior to the enzymatic reaction, slides containing PBMC were soaked with cold buffer permeability of Igepal CA-630 solution 0.5% to enlarge the monocyte cell membrane pores that can be passed by NRAMP1 antibody that resulted in antigen-antibody binding in the cytoplasm NRAMP1 monocytes, PBMC then moistened with 0.3% H2O2 solution over 10 minutes to avoid going nonspecific background staining effects of endogenous

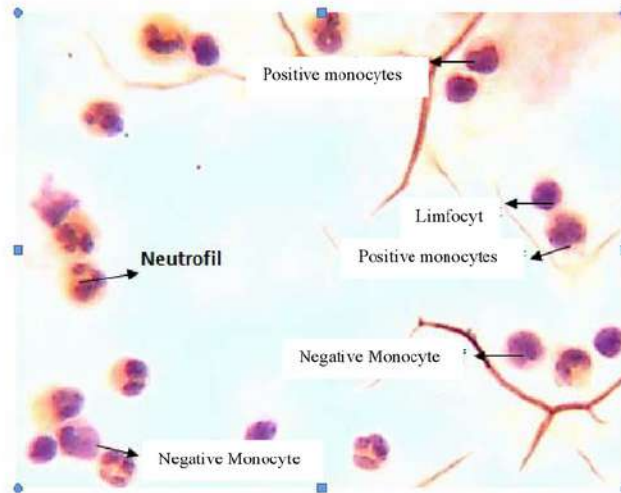
peroxidase activity, it is intended to avoid painting a false positive result or a false negative.

Interpretation of staining results showed the number of monocytes immunocytochemistry will be positive in the 20 field of view with a magnification of 40x using USB

PC Camera 301P, then the percentage of positive monocytes of total monocytes (monocytes positive + negative monocytes). Obtained values in percent of positive monocytes.

Immunocytochemistry Test Results in PBMC Nurses Group (A) and Pulmonary Tuberculosis Patients (B)

(A)



(B)

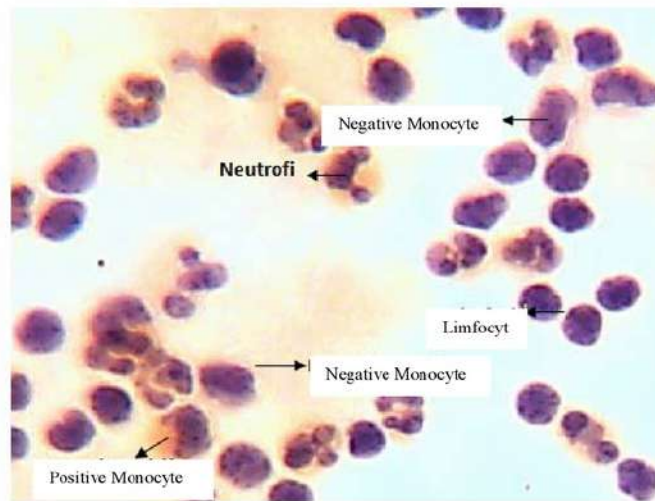


Figure 4. Preview results in PBMC nurses Immunocytochemistry (A) and pulmonary tuberculosis patients (B) with the DAB method Indirect Enzyme Immuno Assay. Positive monocytes in cytoplasm seen with protein NRAMP1 brownish yellow color, whereas the negative monocyte cytoplasm is colorless.

Results of Independent T-Test to Analyze Differences in Protein Expression Of NRAMP1 among A Group Of Nurses and Patients with Pulmonary Tuberculosis

Figure 5. Description of average NRAMP1 protein expression between groups of nurses and pulmonary tuberculosis patients.



Table 2. Independent T-test to analyze differences in protein expression of NRAMP1 among a group of nurses and patients with pulmonary TB

Expression of NRAMP1 Protein	Nurses (n=43)	TB Patients (n=69)	F count	P value
NRAMP1	69.51%	59.15%	14.668	0.002

Based on the average picture of NRAMP1 protein expression between nurses and patients with pulmonary tuberculosis. On average nurses 69.51%, and in pulmonary tuberculosis patients an average of 59.15%. Results The average difference analysis NRAMP1

protein expression between groups of nurses and pulmonary tuberculosis patients got value of 14.668 and probability of F test 0.002 ($p < 0.05$), so that there are significant differences.

Results One Way ANOVA Analyzing the Difference Between NRAMP1 Protein Expression with the Variant Genotype G/G, G/A, A/A Gene NRAMP1 D543N Polymorphism in the Nurse Group (A) and Pulmonary Tuberculosis Patients (B)

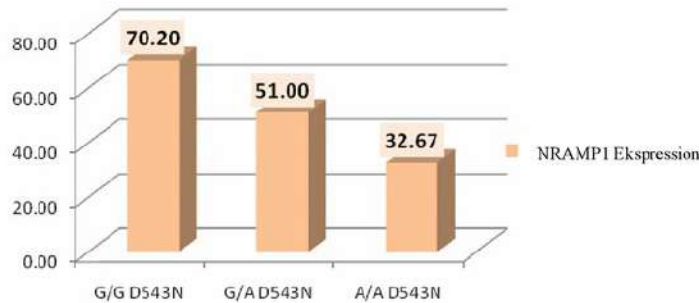


Figure 6. Description between NRAMP1 protein expression with the variant genotype G/G, G/A, A/A gene NRAMP1 D543N polymorphism on the nurse group (A) and pulmonary tuberculosis group (B).

Table 3. Oneway ANOVA to analyze differences in protein expression with the variant NRAMP1 genotype G/G, G/A, A/A gene NRAMP1 D543N polymorphism in nurse and patient with pulmonary tuberculosis

Expression of NRAMP Protein	Variant G/G D543N	Variant G/A D543N	Variant A/A D543N	F count	P value
NRAMP1 Nurses	73.75%	61.60%	0%	11.341	0.002
NRAMP1 TB Patients	70.20%	51.00%	32.67%	16.532	0.000

Based on the average picture NRAMP1 protein expression in the Figure 6 (A) variant genotype G/G (73.75%), variant genotype G/A (61.60%), and the variant genotype A/A (0%) of the polymorphism D543N NRAMP1 gene in the group of nurses. In (B) variant genotype G/G is 70.20%, variant genotype G/A is 51.00%, and the variant genotype A/A is 32.67% of the genes NRAMP1 D543N polymorphism in the group of patients with pulmonary tuberculosis.

Results of analysis of protein expression differences with the variant NRAMP1 genotype G/G, G/A, A/A gene NRAMP1 D543N polymorphism in the group of nurses there are significant differences with the calculated F value of 11.341 and $p = 0.002$ ($p < 0.05$) and in group pulmonary tuberculosis patients got value, calculated F value of 16.532 and $p = 0.000$ ($p < 0.05$), so that there are significant differences.

DISCUSSION

From the results of the determination of gene NRAMP1 D543N polymorphism by PCR-RFLP, found the frequency distribution of variant genotypes of G/G polymorphism D543N, higher in healthy individuals in China (Zhang) 81.3% (74/91), China (Liu) 96 % (231/240), Korea (Ryu) 85.4% (164/192), and in Indonesia, 65.1% (28/43). Distribution frequency of variant genotype G/A polymorphism D543N, higher in patients with pulmonary tuberculosis in China (Zhang) 29.1% (37/127), China (Liu) 9% (11/120), Korea (Ryu) 22, 4% (43/192) and Indonesia 40.6% (28/69). Distribution frequency of variant genotype A/A polymorphism D543N, higher in patients with pulmonary tuberculosis in China (Zhang) 0.7% (1/127), Korea (Ryu) 1.6% (3/192), Cambodia 3.4 % (12/358), and Indonesia 8.7% (6/69).

In Cambodia variant genotype G/G polymorphism D543N higher in pulmonary tuberculosis patients 64.2% (228/358), nor with the variant genotype G/A in Cambodian higher in healthy individuals 46.2% (49/106). Variant genotype frequency differences A/A polymorphism D543N in the four countries (China

(Zhang) 0.7% (1/127), Cambodia 3.4% (12/358), Korea (Ryu) 1.6% (3/192), and Indonesia 8.7% (6/69) for more results occurred in patients pulmonary TB and rarely occurs in healthy individuals, except in Korea (Ryu 2000) was one case among 192 cases (0.5 %). This can be inferred that the variant genotype A/A polymorphism D543N can be parameters of *M. tuberculosis* susceptibility to infection.

Results D543N polymorphism distribution between China, Cambodia, Korea, and Indonesia there are differences. Is this difference due to the criteria of healthy individuals are used as samples different?, In which healthy individuals in China comes from individuals who donate blood at the Hua Shan Hospital, with the individual criteria are not infected or infected but not sick. Healthy individuals used in Cambodia is in addition to pulmonary tuberculosis patients with PPD (+) > 10mm who came to the same hospital sampling pulmonary tuberculosis patients. Healthy individuals used in Korea the same as in China, namely individuals who come to donate blood to the same hospital with a pulmonary TB patient samples, whereas the healthy individuals used in this study was the nurse who has cared for patients with pulmonary TB ≥ 2 years with test results PPD (+) > 10mm, are individuals who have been infected and have immunity against *M. tuberculosis* infection.

The Result of the Determination of Protein Expression of NRAMP1 in the Group of Nurses and Patients with Pulmonary Tuberculosis

The results of this study found that the NRAMP1 protein expression imonositokimia test between nurses and patients with pulmonary tuberculosis showed significant difference with $p = 0.002$ ($p < 0.05$), which is in line with the conceptual framework, that the NRAMP1 protein expression increased in macrophages of TB patients a nurse rather than macrophages lung. According to the research Ciro Estrada Chávez in Mexico in 2001 with a Westem Blot test TB granuloma tissue of cows and peripheral blood cells (PBC) cows. The results of this study indicate that cows appear to NRAMP1 protein expression in cows that experienced

abundant granulomas than NRAMP1 protein expression in caseous necrosis encountered cows NRAMP1 protein expression only slightly and almost none.

The results of this Estrada when compared with the results of this research from immunocytochemistry test results, obtained in which there are similarities NRAMP1 expression was higher in nurses as having granuloma and delayed type of hypersensitivity (PPD (+)> 10mm). According to Ulrichs (2004) in healthy individuals who gave a positive TST result > 10 mm indicates a process of delayed-type-hypersensitivity (DTH). DTH reaction was temporary and will disappear when the antigen is activated by monocytes lost, but when a similar reaction took place continuously in a location with mycobacterial persistence as a consequence micobacteria alive and not immediately destroyed, then the antigenic collected will form a granulomatous reaction at the site of bacterial life these.

In this study the protein expression of NRAMP1 in nurses who treat patients with pulmonary TB ≥ 2 years with the PPD (+)> 10mm average yield NRAMP1 protein expression by 69.51%, higher than average NRAMP1 protein expression in patients pulmonary tuberculosis by 59.15%, and the results are statistically significant differences with $p = 0.002$ ($p < 0.05$).

Govani on research in 1996, variants resistant/resistant variant was replaced with the replacement of susceptible rats with adenine to guanine bases in position 596 of the gene, resulting in replacement of glycine to aspartic acid amino acid position 169 in transmembrane region-fourth of the protein becomes susceptible gene (Nramp1D169). Due Nramp1D169 is unstable and rapidly broken, then the mice with this homozygous variant becomes susceptible to infection. This is similar to the result, Vidal (1995) in rats with functional variants that have resistance through recombinant homologous deletion, these mice became susceptible to infection. When examined closely the analysis of gene NRAMP1 D543N polymorphism when associated with protein NRAMP1 expression obtained in the group of nurses with the variant genotype G/G is 73.75%, variant genotype G/A is 61.60%, and the variant genotype A/A is 0% with $p = 0.002$ ($p < 0.05$).

Similarly, results in the group of pulmonary tuberculosis patients with the variant genotype G/G is 70.20%, variant genotype G/A is 51.00%, and the variant genotype A/A is 32.67% with $p = 0.000$ ($p < 0.05$, 2005). This shows that the average NRAMP1 protein expression of variant G/G, G/A, and A/A polymorphism D543N contained in the nurse and pulmonary tuberculosis patients found a significant difference. So NRAMP1 protein expression in nurse and a decrease in

pulmonary tuberculosis patients is influenced by both the variant A allele genotype G/A or A/A polymorphism of NRAMP1 D543N gene. Variant genotype A/A occurred only in patients with pulmonary tuberculosis, so it can be concluded that homozygous variant genotype is (A/A) could reduce the NRAMP1 protein expression, so that individuals who have the variant genotype A/A will be increased vulnerability to infection of *M. tuberculosis*.

CONCLUSIONS

In conclusion, the polymorphism D543N A allele tended to cause an average of NRAMP1 expression decreased, so that the variant genotype A/A gene NRAMP1 D543N polymorphism is gene causes susceptibility to infection of *M. tuberculosis*.

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REFERENCES

- Bellamy R, Ruwende C, Corrah T, McAdam KPWJ, Whittle HC, Hill AVS, 1998. Variation in the NRAMP1 gene is associated with susceptibility to tuberculosis in West Africans. *N Engl J Med* 338: 640-644
- Blackwell JM, Goswami T, Carlton AWE, Dean S, Natalie P, Jacqueline KW, Susan S, E Nancy M, Christopher SP, Hiba M, and Muntaser I, 2001. SLC11A1 (Formerly NRAMP1) and disease resistance. *Cellular Microbiology*. Vol.3 Issue 12 Page 773
- Delgado JC, Baena A, Thim S, and Goldfeld AE, 2002. Ethnic-Specific Genetic Associations with Pulmonary Tuberculosis. *The Journal of Infectious Disease* : 186:1463-8
- Ryu S, Park YK, Bai GH, Kim SJ, Park SN, Kang S, 2000. 3'UTR polymorphisms in the NRAMP1 gene are associated with susceptibility to tuberculosis in Koreans. *Int J Tuberc Lung Dis* 4: 577-580
- Liu W, Cao WC, Zhang CY, et al., 2004. VDR and NRAMP1 gene polymorphisms in susceptibility to pulmonary tuberculosis among the Chinese Han population: a case-control study. *Int J Tuberc Lung Dis*; 8:428-434

- Estrada-Chavez C, Pereira-Suarez AL, Meraz MA, Arriaga C, Garcia-Carranca A, Sanchez-Rodriguez C, Mancilla R, 2001. High-level expression of NRAMP1 in peripheral blood cells and tuberculous granulomas from *Mycobacterium bovis*-infected bovines. *Infect Immun* 69:7165–7168
- Gao PS, Fujishima S, Mao XQ, Remus N, Kanda M, Enomoto T, et al, 2000. Genetic variants of NRAMP1 and active tuberculosis in Japanese populations. International Tuberculosis Genetics Team. *Clin Genet* 58: 74–76
- Camstock GW, 1978. Tuberculosis in twins: a re-analysis of the Proffit study. *Am Rev Resp Dis*; 117:621
- Kim JH, Lee SY, Lee SH, Sin C, Shim JJ, In KH, Yoo SH, Kang KH, 2003. NRAMP1 genetic polymorphisms as a risk factor of tuberculous pleurisy. *INT J TUBERC LUNG* 7(4): 370-375
- Ulrichs ES, Kaufmann SHE, 2004. Cell-Mediated Immune response. In: *Tuberculosis*. Rom WN, Garay SM, 2nd edition. Philadelphia. Lippincott Williams & Wilkins. 251-334
- Vidal S, Tremblay M, Govoni G, et al, 1995. The *Ity/Lsh/Bcg* locus: natural resistance to infection with intracellular parasites is abrogated by disruption of the *Nramp1* gene. *J Exp Med*; 182:655–66
- WHO Report, 2002. Global tuberculosis control: surveillance, planning, financing. *WHOCDSTB* ; 295 : 21
- WHO Report, 2007. Global Tuberculosis Control.
- Zhang WH, Shao L, Weng X, Hu Z, Jin A, Chen S, Pang M, and Chen ZW, 2005. Variants of the Natural Resistance-Associated Macrophage Protein 1 Gene (NRAMP1) are associated with Severe Forms of Pulmonary Tuberculosis. *Clinical Infection Disease*;40:1232-1223.

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Walid Ben-Selma, Hedi Harizi, Mahmoud Letaief, Jalel Boukadida. "Age- and gender-specific effects on NRAMP1 gene polymorphisms and risk of the development of active tuberculosis in Tunisian populations", International Journal of Infectious Diseases, 2012

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PAGE 1

PAGE 2

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