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Relationship Between Protein Tyrosine Phosphatase Non-Receptor Type 22 1858>C Gene Polymorphism with Rheumatoid Arthritis Disease Activity Score-28

Muhardani Iqbal[1], Blondina Marpaung[2], Andi Raga Ginting[2]

[1]Department of Internal Medicine, [2]Division of Rheumatology Department of Internal Medicine, Medical Faculty, Universitas Sumatera Utara, Medan, Indonesia

Corresponding Author: Muhardani Iqbal (Email: muhardani_ibal@yahoo.com)

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ABSTRACT

Background: Rheumatoid arthritis (RA) is one of the systemic inflammatory autoimmune diseases. A number of previous studies showed the potency of Protein Tyrosine Phosphatase Non-Receptor Type 22 (PTPN22) 1858c>T gene polymorphism with the incidence of rheumatoid arthritis.
Objective: To know the relationship of PTPN22 1858C>T gene polymorphism with disease activity score-28 (DAS-28) in rheumatoid arthritis patients at H. Adam Hospital Medan.
Method: 69 rheumatoid arthritis patients were conducted in this study by *consecutive sampling* who were treated at H. Adam Malik General Hospital, Medan from February-August 2022. The PTPN22 gene polymorphism was genotyped using polymerase chain reaction.
Results: Majority of the subject in the study was female, around 65 people (94.2%). The mean age of the subjects was 42.35 ± 12.28 years old. The most ethnic group was Batak with 34 people (49.3%). Mean DAS-28 score was 3.45 ± 0.79. All CC genotypes and C alleles were found in 69 (100%) subjects. Statistical analysis found no correlation between PTPN22 1858C>T gene polymorphism and DAS-28 score with p value <1.0
Conclusion: PTPN22 1858C>T gene polymorphism has no significant relationship DAS-28 in rheumatoid arthritis.

Keywords: *PTPN22 1858C>T gene polymorphism, Disease Activity Index-28, rheumatoid arthritis*

INTRODUCTION

Rheumatoid arthritis (RA) is systemic inflammatory autoimmune disease. RA patients are at risk of developing severe infection, respiratory diseases, osteoporosis, cardiovascular diseases, cancer, and death compared to general population.[1] RA affect 0.5%–1% of American adults, estimated at 1.5 million people. RA was seen more common in women and can affect to all age; though the peak incident was among 50 to 60 years. [1,2]

The most prominent feature of RA is painful symmetrical and swelling of the hands, wrists, feet, and knees (polyarthritis). Patient might as well come with complaint of monoarthritis or oligoarthritis. Some patients with RA come at later stage of disease and various manifestation in other organs (without obvious articular involvement), such as interstitial lung disease (ILD), pericarditis, pleural effusion, or bronchiectasis. [1]

RA was influenced by genetics, epigenetic and the environment. Factors that increased the risk was woman, family history with RA, factor genetic "same epitope" and exposure against tobacco smoke. Discovery of new gene polymorphisms and their relationships with vulnerability disease has added new element to clarify RA pathogenesis. One of the strongest relationships was polymorphisms in the Protein Tyrosine Phosphatase Non-Receptor

Type 22 (PTPN22) gene which was believed to be cause lower threshold for the activation of T cells and other cell immunity. Recently, studies showed that polymorphism could cause hypercitrullination because changes in PTPN22 interaction with peptidyl arginine deiminase (PAD), the enzyme responsible for citrullination, though it is still unknown whether hypercitrullination was targeted by immune response or functions that drive other processes that lead to autoimmunity. [3-5]

Previous studied have showed the potency of PTPN22 gene polymorphism with incidence of rheumatoid arthritis. West Mexico population research showed that PTPN22 1858 C>T gene polymorphism was related with rheumatoid arthritis. [6] Other cohort study in Germany also showed the relationship of PTPN22 gene polymorphisms with the incidence of rheumatoid arthritis and more was found in man. [7] However, until moment, the relationship of PTPN22 1858 C>T gene polymorphism with Disease Activity Index-28 (DAS-28) especially in Indonesian population has not yet been conducted.

MATERIALS & METHODS

A total of 69 patients who were treated at H. Adam Malik General Hospital, Medan were included in comparative analytical research using *consecutive sampling*. Patients were collected during period February until August 2022. Rheumatoid arthritis patients aged ≥ 18 years and examination of PTPN22 1858 C/T gene allele and *genotyping of* the PTPN22 1858C/T polymorphism were included in the study. Rheumatoid arthritis patients who ever get DMARD biologic therapy, had comorbid autoimmune disease (SLE, type 1 DM, autoimmune thyroid disease), chronic disease (heart failure, chronic kidney disease, and malignancy), and severe infection, were excluded from the study. All research subjects were then interviewed to obtain basic data, a physical examination was carried out, and 3 cc of blood samples were taken to examine genetic polymorphism.

This study evaluated the distribution of PTPN22 1858 C>T gene polymorphism, DAS-28 values and basic characteristic of rheumatoid arthritis patients. Demographic characteristics included sex, age, ethnicity and laboratory results, like *rheumatoid factor*, CRP, ESR, onset disease, painful joints, total swollen joint, genotype and allele the patient had. DNA extraction method followed the protocol work of purification blood on the QIAamp DNA Mini Kit (QIAGEN, 2016). *Genotyping* PTPN22 1858C/T polymorphism was performed with using tetra-primer *amplification refractory mutation system-polymerase chain reaction* (PCR) with 2 external primers (primer forward outer namely 5′-CTTCACACTCAG CTTCCCAAAGTG-3′ and outer reversal primer 5′-CAACTTTACTGATAATGTTGCTTCA ACGGA-3′) and 2 internal primers (primers forward namely 5′-CAACCACAATAAATG ATTCAGGTGTACG-3′ and reversal primer in 5′-ATCCCCCCTCCACTTCCTGGAT-3′). Size product was 213 bp for allele C and 151 bp for where the T allele size product internal control was 314 bp. PCR examination was carried out with 5x FIREPol Master Mix (Solis BioDyne, Tartu, Estonia). Condition Cyclic PCR was carried out in 5 minutes at 950 C followed by 30 cycles at 950 C, 30 seconds at 640 C, 30 seconds at 720 C, and 10 minutes at 720 C. The PCR product was then electrophoresed on 2% agarose gel and photographed. [8]

STATISTICAL ANALYSIS

Statistical analysis was performed using *Statistical Product and Service Solution* (SPSS) software. Categorical data was presented in n (percent). Numerical data was presented in mean (standard deviation) and median (min-max). Meanwhile, the Kruskal Wallis test was used to evaluate relationship between PTPN22 1858 C>T

gene polymorphism with DAS-28 in rheumatoid arthritis. The p value is said to be significant if it is less than 0.05 ($p < 0.05$).

RESULT

1.1 Characteristics of Research Subjects

The demographic characteristics of the subjects in this study were assessed based on age, gender, and ethnicity. In this study, the average age of subjects was 42.35 ± 12.28 years and was dominated by female patients, 65 people (94.2%). Based on ethnicity, the majority subjects in this study were Batak, as many as 34 people (49.3%). Complete subject characteristics were presented in table 1.1.

Table 1.1 Characteristics Demographic Research Subjects

Variable	Frequency
Age (years)	
Mean ± SD	42.35 ± 12.28
Median (minimum-maximum)	41 (18-73)
Gender	
Man	4 (5.8%)
Woman	65 (94.2%)
Ethnic group	
Batak	34 (49.3%)
Java	16 (23.2%)
Malay	10 (14.5%)
Aceh	4 (5.8%)
Minang	3 (4.3%)
Chinese	1 (1.4%)
Betawi	1 (1.4%)

1.2 Basic Characteristics of Subjects Study

Characteristics of past disease in subjects were rated based on *rheumatoid factor*, CRP, DAS-28, onset disease, painful joints, total swollen joints, genotypes and alleles. Mean *rheumatoid factor* was 10.96 ± 5.12, mean CRP was 1.02 ± 0.52 mg/dL, mean the DAS-28 score was 3.45 ± 0.79. Onset disease rate was 2.52 ± 1.945 years, mean total painful joints was 2.87 ± 2.332, mean total swollen joints was 97 ± 3.566. Whole genotype CC and allele C were found in 69 (100%) subjects.

Table 1.2 Basic Characteristics of Research Subjects

Variable	Frequency
Rheumatoid Factor	
Mean ± SD	10.96 ± 5.12
Median (minimum-maximum)	8.0 (8.0-32.0)
CRP (mg/dL)	
Mean ± SD	1.02 ± 0.52
Median (minimum-maximum)	0.70 (0.20-2.80)
DAS-28	
Mean ± SD	3.45 ± 0.79
Median (minimum-maximum)	3.50 (1.17-5.00)
Disease onset (years)	
Mean ± SD	2.52 ± 1.945
Median (minimum-maximum)	2.0 (1.0-9.0)
Painful Joints	
Mean ± SD	2.87 ± 2.332
Median (minimum-maximum)	3.0 (0-10.0)
Total of Swollen Joints	
Mean ± SD	9.97 ± 3.566
Median (minimum-maximum)	10.0 (2.0-18.0)
The Genotype the Patient Has	
CC	69 (100%)
CT	0 (0%)
TT	0 (0%)
Patient's Alleles	
C	69 (100%)
Q	0 (0%)

1.3 Relationship Between PTPN22 Gene Polymorphism with DAS-28

PTPN22 gene polymorphism was assessed by the results of genetic examination of the alleles and genotypes of each subject. Genotype and allele were presented on nominal scale, while DAS-28 value were presented on numerical scale. 100% of the study subjects only had the C allele and the CC genotype in the PTPN22 gene. Thus, in the analysis of comparative tests, there was no relationship between genotypes and alleles in the PTPN22 gene with DAS-28 value (Table 1.3).

Table 3.3 Relationship between genotypes and alleles in the PTPN22 gene with CRP and ESR

Independent variable	dependent variable	*p* value
PTPN22 gene allele	DAS-28 value	1.00 *
PTPN22 gene genotype	DAS-28 value	1.00 *

*** Kruskal Wallis test**

DISCUSSION

Based on epidemiological data, rheumatoid arthritis (RA) is found more common in women compared to man, with risk lifetime RA survival was 3.6% in women compared to with 1.7% in men. The risk of RA also increased proportionally with age, with the incident peak was among ages 65 to 80 years. [9]

The mean age of this study was 42.35 ± 12.28 (18-73) years old. Study in Jakarta found 119 patients at Cipto Mangunkusumo General Hospital (RSCM) aged 54 (21-75) years old. [10] A number of clinical score could be used for evaluate RA activity, including *Disease Activity Score* (DAS28). DAS28 integrated physical examination (total tenderness and swollen joints), reactants acute phase ((ESR) or *C-reactive protein* (CRP)), and assessment of patient 's global health alone; where the combination of these become one possible more comprehensive score showing the disease severity than variable individual.[11]

This study was dominated by female, as many as 65 people (94.2%). Previous study in 2017 with 119 patients at RSCM found that majority of patients were female, as many as 107 people (89.9%).[10] This study was also in line with descriptive study conducted in Bandung, 97 RA patients at Dr. Hassan Sadikin General Hospital. The prevalence of RA was found five times higher in women than man, which was related with the role of estrogen in activating inflammation response in RA pathogenesis. The study conducted in 2015 in 111 RA patients found that majority of patients were female, as many as 108 people, aged 55.6 years old.[13]

Based on ethnicity, majority of the subjects were Batak, as many as 34 people (49.3%), followed Java, Malay, Aceh, Minang, Chinese, and Betawi. Cross sectional study in RSCM in 119 AR patients showed that majority of the subjects were Java (28.5%), followed by Sundanese (17.6%), Batak (15.1%), Betawi (11.7%), Minang (10.9%), Chinese (5.8%), Palembang (2.5%), Bangka (1.6%), Ambonese (1.6%) and other ethnic groups (4.2%).[10]

Mean rheumatoid factor was 10.96 ± 5.12, mean CRP was 1.02 ± 0.52 mg/dL, mean DAS-28 score was 3.45 ± 0.79. Disease onset rate was 2.52 ± 1.945 years, mean total painful joints was 2.87 ± 2.332, mean total swollen joints was 9.97 ± 3.566. Cohort study on rheumatoid arthritis patients found that the median duration of RA disease was 9.93 (1-31) years, CRP 0.87 (0.02-5.61) mg/dL, mean DAS28 is 4.73 (1.21) which was proportionally associated with moderate disease activity. 2 patients (3.45%) in remission (DAS28≤2.6), 4 (6.9%) had low activity disease (2.6<DAS28≤3.2), 29 (50%) had moderate disease activity (3.2<DAS28≤5.1), and 23 (39.65%) had high activity disease (DAS28 >5). [14] The study in 2015 found that the duration of RA disease was 11 years and DAS-28(ESR) respectively significant higher from DAS28 (CRP) (4.0 vs 3.5; $p < 0.001$) and its value permanent higher after stratification based on age, gender, duration of the disease, and rheumatoid factor. [13]

Research found that RA patients were dominated by women (93%); and some patient had duration of ten years disease and the mean DAS-28 score was 4.8.[15] Another study found that the mean CRP was 6 (<4-180) mg/dL, DAS-28 was 4.8 (2.63-7.98).[16]

In this study, there was no mutation allele (C to T) or genotype (CC being CT or TT) in 100% of subjects at the PTPN22 gene position 1858. Thus, no significant relationship was found among allele nor genotype on the PTPN22 gene with CRP and ESR. This showed that there was no connection among PTPN22 gene polymorphism with CRP and ESR in the Indonesian population.

The results of the study were similar to the study conducted in Algeria where there was no relationship among PTPN22 polymorphism with incidence of RA ($p>0.05$). [17] The study was not in line with 2014 study, where frequency PTPN22 C1858T minor allele was 10.6%, 75 patients genotype CC, 18 genotypes CT and one TT genotype. [16] The PTPN22 C1858T gene was related significantly with RA incident. In Asian populations, variant polymorphic from PTPN C1858-SNP was not found in RA patients, thus no biased was observed between PTPN22 and RA incident. Studies similar in Caucasian and Asian patients showed strong relationship between 1123G>C SNP and RA. [15] PTPN22 T1858

allele was significant more often found in RA patients in Europe population compared to control (5.7% vs 3.7%, p = 0.045).[18] Different study showed that PTPN22 was related significantly with increased risk of RA in Caucasians and Africans in all genotypic models. [19]

RA pathogenesis was generally associated with infiltration of T cells, cell that was the main reason in inducing inflammation of synovial membrane. Besides that, loss tolerance against body antigens alone could be other triggers of RA. Regardless from biological factor, other predispositions factor such as genetics has been reported to play a role in vulnerability against RA. Two main gene group, including the human leukocyte antigen (HLA) locus is about 30-50% and non-HLA genes (such as the protein tyrosine gene) non- receptor phosphatase 22 [PTPN22]) is about 23%. The PTPN22 gene is located on chromosome 1p13 and encodes lymphoid protein tyrosine phosphatase (LYP), known as a negative regulator from track signal mediated through receptor T cells (TCR). [15,20] PTPN22 is member the tyrosine protein family preventing phosphatase activation spontaneous T cells with dephosphorylation and inactivation of TCR-related kinases and their substrates. PTPN22 was associated significantly with autoimmune disease in humans, including diabetes mellitus type 1, rheumatoid arthritis (RA), autoimmune thyroid disease, lupus erythematosus systemic and *inflammatory bowel disease*.[21]

CONCLUSION

This research showed characteristics of rheumatoid arthritis patients including protein tyrosine phosphatase non-receptor 22 1858C>T gene polymorphism and DAS-28 value in patients rheumatoid arthritis treated at H. Adam Malik General Hospital for the period April to August 2022. This study showed no relationship between protein tyrosine phosphatase non-receptor 22 1858C>T gene polymorphism with DAS-28 in rheumatoid arthritis.

Declaration by Authors

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