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Original Article

A Possible Relationship Between Polymorphisms of Glutathione S-Transferase M1, P1 and T1 Genes and Rheumatoid Arthritis in Zahedan, Southeast Iran

Güneydoğu İran Zahedan'da Glutation S-Transferaz M1, P1 ve T1 Genlerinin Polimorfizmleri ve Romatoid Artrit Arasındaki Muhtemel İlişki

Mohammad HASHEMI,[1,2] Ebrahim ESKANDARI-NASAB,[1] Zahra ZAKERI,[2] Mahdi ATABAKI,[3] Hamzeh REZAEI,[1] Gholamreza BAHARI,[1] Mohsen TAHERI[4]

[1]*Cellular and Molecular Research Center, Zahedan University of Medical Sciences, Zahedan, Iran*
[2]*Department of Internal Medicine, Zahedan University of Medical Sciences, Zahedan, Iran*
[3]*Department of Immunology, Zahedan University of Medical Sciences, Zahedan, Iran*
[4]*Genetic of Non-Communicable Disease Research Center, Zahedan University of Medical Sciences, Zahedan, Iran*

Objectives: This study aims to investigate the possible relationship between glutathione S-transferase (GST) gene (*GSTM1*, *GSTT1*, and *GSTP1*) polymorphisms and susceptibility to rheumatoid arthritis (RA).

Patients and methods: This case-control study was conducted on 100 Iranian patients with RA (88 females, 12 males; mean age 44.1±13.2 years; range 17 to 75 years) and 100 healthy subjects (68 females and 32 males; mean age 45.7±9.6 years; range 23 to 70 years). The *GSTP1* polymorphism was determined using a tetra amplification refractory mutation system-polymerase chain reaction (Tetra-ARMS-PCR) assay, whereas the *GSTM1* and *GSTT1* polymorphisms were analyzed by multiplex PCR.

Results: The null genotype of *GSTM1* was significantly higher in the patients with RA (78.0%) than in the control group (59.0%), and there was a risk factor for susceptibility to RA (OR=2.86, 95% CI=1.45-5.66, p=0.002). However, no significant difference was observed in the null genotype of *GSTT1* among RA patients and healthy subjects (19% and 11%, respectively). There was no difference in the frequency distribution of the *GSTP1* Ile105Val polymorphism between the groups (chi-square=1.69, p=0.429).

Conclusion: Our findings showed that only the *GSTM1* genetic polymorphism is associated with RA risk in a sample of the Iranian population.

Key words: Glutathione S-transferase; polymorphism; rheumatoid arthritis.

Amaç: Bu çalışmada glutation S-transferaz (GST) gen (*GSTM1*, *GSTT1* ve *GSTP1*) polimorfizmi ve romatoid artrit (RA) yatkınlığı arasındaki ilişkinin araştırılması amaçlandı.

Hastalar ve yöntemler: Bu olgu kontrollü çalışmaya İranlı 100 RA hastası (88 kadın, 12 erkek; ort. yaş 44.1±13.2 yıl; dağılım 17-75 yıl) ve 100 sağlıklı birey (68 kadın ve 32 erkek, ort. yaş 45.7±9.6 yıl; dağılım 23-70 yıl) dahil edildi. *GSTP1* polimorfizm tetra amplifikasyona dirençli mutasyon sistem polimeraz zincir reaksiyonu (Tetra-ARMS-PCR) ile belirlenirken, *GSTM1* ve *GSTT1* polimorfizmleri multipleks PCR kullanılarak analiz edildi.

Bulgular: *GSTM1*'in sıfır genotipi, kontrol grubuna kıyasla (%59.0), RA'lı hasta grubunda (%78.0) anlamlı düzeyde daha yüksek olup, RA yatkınlığı için bir risk faktörüydü (OR=2.86, %95 GA=1.45-5.66, p=0.002). Bununla birlikte, RA'lı hastalar ve sağlıklı kişiler arasında *GSTT1*'in sıfır genotipi açısından anlamlı bir farka rastlanmadı (sırasıyla %19 ve %11). *GSTP1* Ile105Val polimorfizminin sıklık dağılımı gruplar arasında farklı değildi (ki-kare=1.69, p=0.429).

Sonuç: Bulgularımız yalnızca *GSTM1* genetik polimorfizminin İranlı bir nüfus örnekleminde RA riski ile ilişkili olduğunu göstermiştir.

Anahtar sözcükler: Glutation S-transferaz; polimorfizm; romatoid artrit.

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Correspondence: Mohammad Hashemi, Ph.D. Department of Clinical Biochemistry, Zahedan University of Medical Sciences, 98135 Zahedan, Iran. Tel: +98 541 3414571 e-mail: mhd.hashemi@gmail.com

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease of unknown etiology. Both genetic and environmental factors have been shown to be relevant, contributory factors to the expression and complications of this disease.[1,2] The prevalence of RA is about 1% of the total population, and genetic factors have been estimated to account for 60% of the disease risk.[3] Glutathione S-transferase (GST) genes, known as a superfamily of phase II metabolic enzymes, catalyze the detoxification of xenobiotics via glutathione conjugation. They are also believed to play an important role in detoxifying products generated by the activity of reactive oxygen species (ROS). The glutathione S-transferase Mu 1 *(GSTM1)*, glutathione S-transferase P 1 *(GSTP1)*, and glutathione S-transferase theta 1 *(GSTT1)* genes are mapped on chromosome 1 (1p13.3), chromosome 11 (11q13), and chromosome 22 (22q11.2), respectively.[4] There is evidence that some allelic variants are associated with differences in detoxification efficiency. In addition, these three genes have been shown to have functional polymorphisms that are frequently present in the general population.[5] Individuals with homozygous deletions at the M1 and T1 loci of GST (*GSTM1* null and *GSTT1* null, respectively) have no functional enzymatic activity. Deletions in the *GSTM1* and *GSTT1* genes occur with varying frequencies in different populations. It has been reported that a one point mutation in the coding region of the *GSTP1* gene results in the substitution of isoleucine (Ile) for valine (Val) at codon 105, which modifies the enzyme activity.[6,7] Glutathione S-transferases have peroxidase activity concerning cytotoxic metabolites that are produced in inflammatory reactions, and this is the main feature of RA. In addition, it has been proposed that defense mechanisms against ROS are impaired in RA.

Though there are several studies regarding the association between GSTs polymorphisms and the risk of RA, the findings are controversial. Therefore, the aim of the present study was to evaluate the impact of GSTs polymorphisms on the susceptibility to RA in a sample of Iranian population.

PATIENTS AND METHODS

This case-control study was performed from October 2010 to March 2012 in Clinic of Rheumatology, Ali-Ebneh Abitaleb hospital Zahedan, Iran. We investigated the possible association between the *GSTM1*, *GSTT1*, *GSTP1* polymorphisms of GSTs genes and RA susceptibility in 100 patients (88 females and 12 males; mean age 44.1±13.2 years; range 17 to 75 years) who fulfilled the American College of Rheumatology (ACR) criteria for RA.[8] All the subjects were patients of the Rheumatology Clinic at Zahedan University of Medical Sciences.[2,9,10] The control group consisted of 100 healthy individuals (68 females and 32 males; mean age 45.7±9.6 years; range 23 to 70 years) who were unrelated to the RA patients. The ethics committee of the university approved the project, and informed consent was obtained from all patients and healthy individuals. Blood samples from the patients and healthy controls were collected in ethylenedinitrilotetraacetic acid disodium salt (EDTA-Na) tubes. Genomic DNA was then extracted from the peripheral blood samples that had been collected in the tubes containing EDTA in a manner that has been previously described.[10]

The *GSTM1* and *GSTT1* gene polymorphisms

Examinations of the *GSTM1* and *GSTT1* gene polymorphisms were performed using multiplex polymerase chain reaction (PCR), with the TLR2 gene as an internal control, as described by Hashemi et al.[11]

The primers used were 5`-GCTGCCCTACTTGATTGATG-3` (sense) and 5`-CCCCAAATCCAAACTCTGTC-3`(anti-sense) for the *GSTM1* gene, resulting in a 325-bp fragment; 5`-TTCTGCTTTATGGTGGGGTC-3` (sense) and 5`-GTGATGTTCCCTGTTTTCCT-3` (anti-sense) for the *GSTT1* gene, resulting in a 542-bp fragment, and 5`-GATGCATTTGTTTCTTACAGTGAGCG-3`(sense) and 5`-GTGATGTTCCCTGTTTTCCT-3`(anti-sense) for the TLR2 gene, resulting in a 259-bp fragment. The *GSTM1* null and *GSTT1* null variant forms were defined by the absence of the 325- and 542-bp fragments, respectively.

The *GSTP1* polymorphism

The *GSTP1* polymorphism was determined by a tetra-primer amplification refractory mutation system PCR (T-ARMS-PCR) as described in an earlier study.[11] Two external primers (forward outer: 5`-CAGGTGT CAGGTGAGCTCTGAGCACC-3` and reverse outer: 5`-ATAAGGGTGCAGGTTGTGTCTTGTCCCA-3`) and two internal primers [forward inner (A allele or Ile allele): 5`-CGTGGAGGACCTCCGCTGCAAATC CA-3` and reverse inner (G allele or Val allele): 5`-GCT CACATAGTTGGTGTAGATGAGGGATAC-3`] were used for detection of the GSTP1 polymorphism. The product sizes were 233-bp for the A allele, 290-bp for the G allele, and 467-bp for the outer primers (control band).

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, Illinois, USA) version 18.0 software program for Windows. The associations between the genotype of the GSTs gene and RA were assessed by computing the odds ratio (OR) and 95% confidence intervals (95% CI) by logistic regression analyses.

RESULTS

The frequency distribution of the *GSTM1*, *GSTT1*, and *GSTP1* genotypes was compared between 100 RA patients and 100 normal subjects (Table 1). The null genotype of *GSTM1* was more prevalent in the patients (78.0%) than in the controls (59.0%), which resulted in a significant association between the incidence of RA and the *GSTM1* null genotype (OR=2.86; 95% CI=1.54-5.66; p=0.002). However, no significant difference was observed among the groups regarding the null genotype of *GSTT1* (19% versus 11%). The *GSTT1* null did not increase the risk of RA (OR=1.85; 95% CI=0.78-4.38; p=0.165). In addition, the results showed that the genotypic and allelic frequencies of *GSTP1* did not differ significantly between the RA patients and the control subjects (chi-square=1.69; p=0.429 and chi-square =0.57; p=0.450, respectively).

DISCUSSION

In the present study, we evaluated the association of the *GSTM1*, *GSTT1*, and *GSTP1* genotypes and the susceptibility to RA in a sample of Iranian population. The findings imply that the *GSTM1* null genotype was significantly associated with the risk of RA, though no association was seen between the *GSTT1* null genotype and *GSTP1* Ile105Val genotypes when examining vulnerability to RA.

In agreement with our findings, Yun et al.[12] reported that the null polymorphism of *GSTM1*, but not the *GSTT1* or *GSTP1* genotypes, is associated with an increased risk for RA, but Keenan et al.[13] found no significant differences in the distributions of the *GSTP1*, *GSTT1* and *GSTM1* genotypes between RA and healthy subjects. In contrast, Bohanec et al.[14] provided evidence that patients with the *GSTT1* null genotype had a higher risk for developing high activity RA than patients with *GSTT1* genes present and that the *GSTM1* and *GSTP1* polymorphisms were not associated with the disease activity.

Glutathione S-transferases, the multifunctional enzymes, promote the detoxification of a wide range of xenobiotics, such as environmental carcinogens, steroids, and ROS. The most common substrates for GSTs are ROS compounds, which are products of oxidative stress (OS).[15] It is well known that individuals with the *GSTM1* null or *GSTT1* null genotypes display an absence of enzymatic activity and are thought to be at an elevated risk for the cytotoxic effects of a wide spectrum of carcinogens and xenobiotics.[16,17] In addition, it has been demonstrated that the *GSTP1* Ile105Val polymorphism is associated with altered catalytic function and that *GSTP1* malfunction makes cells vulnerable to oxidative DNA damage.[18,19] Given that the *GSTM1* and *GSTT1* null genotypes along with the *GSTP1*Ile105Val polymorphism display decreased

Table 1. Frequency distribution of the *GSTM1*, *GSTT1*, and *GSTP1* genotypes in rheumatoid arthritis and control subjects

GST genotypes	Patients		Control		OR (95% CI)		*p*	*OR (95% CI)		*p*
	n	%	n	%	Mean	Range		Mean	Range	
GSTM1										
Wild	22	22.0	41	41.0	Reference		–	Reference		–
Null	78	78.0	59	59.0	2.46	1.33-4.57	0.004	2.86	1.45-5.66	0.002
GSTT1										
Wild	81	81.0	89	89.0	Reference		–	Reference		–
Null	19	19.0	11	11.0	1.90	0.85-4.23	0.117	1.85	0.78-4.38	0.165
GSTP1										
AA (Ile/Ile)	52	52.0	44	44.0	Reference		–	Reference		–
AG (Ile/Val)	37	37.0	46	34.0	0.68	0.37-1.23	0.201	0.68	0.36-1.28	0.234
GG (Val/Val)	11	11.0	10	10.0	0.93	0.36-2.39	0.882	0.73	0.27-2.02	0.549
GSTP1 allele										
A allele (Ile allele)	141	70.5	134	67.0	Reference		–			
G allele (Val allele)	59	29.5	66	33.0	1.17	0.77-1.79	0.517			

GSTM1: Glutathione S-transferase Mu 1 gene; GSTT1: Glutathione S-transferase theta 1; GSTP1: Glutathione S-transferase P 1; GST: Glutathione S-transferase; * Adjusted for sex and age.

GST detoxification, they are thought to be potential risk factors that influence susceptibility to RA and impact the outcome of this disease.[12]

It has been reported that the risk of developing severe RA disease is increased in female patients who smoke that also have the *GSTM1* null polymorphism compared with nonsmoking RA patients with the same genotype.[20] Increased severity in RA has been shown to be associated with a null polymorphism of *GSTM1*.[21] Furthermore, it has been demonstrated that in both Caucasian and South Asian populations, the *GSTT1* null genotype, but not the *GSTM1*, is associated with RA.[1]

Overall, although some small studies have examined the relationship between the polymorphisms of GSTs and the risk of RA, the association still remains unclear, and the debate continues. The reason for the controversial results of these studies could be associated with the differences in the populations studied and their exposures to agents related to RA development. Our findings suggest that the *GSTM1* null genotype is a risk factor for susceptibility to RA in a sample of Iranian population. However, this needs to be confirmed by other large studies involving RA.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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