Research Article

Glutathione Peroxidase 1 Pro198Leu Polymorphism and Susceptibility to Rheumatoid Arthritis: Evidence from an Iranian Case-Control Study

Arshia Abbaszadeh [1], Mohammad Reza Asgharzadeh[1,2*], Zafar Gholinejad [2,3]

[1] *Department of Biology, Ur.C., Islamic Azad University, Urmia, Iran*
[2] *Biotechnology Research Center, Ur.C., Islamic Azad University, Urmia, Iran*
[3] *Department of Medical Laboratory Sciences, Ur.C., Islamic Azad University, Urmia, Iran*

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[*] **Corresponding Author:**

Mohammad Reza Asgharzadeh
Department of Biology, Ur.C., Islamic Azad University, Urmia, Iran.

Email:
mohammadreza.asgharzadeh@iau.ac.ir

ABSTRACT

Objectives: Oxidative stress plays a key role in the pathogenesis of rheumatoid arthritis (RA). Glutathione peroxidase (GPx) is a peroxidase enzyme that defends mammalian cells against oxidative stress. The Pro198Leu polymorphism of the GPx-1 gene, has been reported to influence its activity. This study aimed to investigate the association of this polymorphism with RA risk in an Iranian population.

Methods: In this case-control study, 45 RA patients and 45 healthy controls were enrolled. Genomic DNA was extracted from blood samples, and genotyping for the Pro198Leu polymorphism was performed using the PCR-RFLP technique. Statistical analysis was conducted using SPSS software, employing chi-square tests and ANOVA.

Results: The mean age of all participants was 55.69 years. Genotype distribution was as follows: CC (50.0%), CT (44.4%), and TT (5.6%). Chi-square analysis revealed no significant difference in genotype frequencies between patients and controls ($p = 0.779$). Furthermore, no significant association was found between genotypes and age or gender. All groups were in Hardy-Weinberg equilibrium.

Conclusion: The findings indicate that the GPx-1 Pro198Leu polymorphism is not significantly associated with the risk of developing RA in the studied population.

Keywords: Rheumatoid Arthritis, GPx-1, Pro198Leu, Single Nucleotide Polymorphism

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Introduction

Rheumatoid arthritis (RA), affecting approximately 0.5 to 1% of the world population, primarily manifested as the pain, swelling, stiffness at joints, and ultimately joint destruction and functional disability (1). The etiology of RA is considered to be multifactorial and studies have revealed that genetic predisposition and environmental factors lead to the patient susceptibility (2). Oxidative stress has been reported as an underling mechanism for both genetic and environmental factors- induced RA (3).

Oxidative stress and inflammation are tightly interconnected and play central roles in the pathogenesis of RA. In RA, activated immune cells within the synovium such as macrophages, neutrophils, and T cells, produce excessive reactive oxygen and nitrogen species (ROS/RNS), leading to oxidative stress that damages lipids, proteins, and DNA (4). These oxidative modifications not only contribute directly to joint destruction but also amplify inflammatory signaling by activating redox-sensitive pathways, including nuclear factor kappa B (NF-κB) and mitogen-activated protein kinases (MAPKs), thereby increasing the production of pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), and IL-6 (5, 6). Conversely, chronic inflammation further enhances ROS generation, creating a self-perpetuating vicious cycle. This bidirectional interaction between oxidative stress and inflammation promotes synovial hyperplasia, cartilage degradation, and bone erosion, ultimately driving the chronic and progressive nature of rheumatoid arthritis (7).

Glutathione peroxidase 1 (GPX1) is a key antioxidant enzyme that protects cells against oxidative damage by reducing hydrogen peroxide and lipid hydroperoxides using reduced glutathione (8). In the context of RA, GPX1 plays an important role in maintaining redox homeostasis within the inflamed synovium, where excessive production of ROS by activated immune cells contributes to tissue damage and amplification of inflammatory responses. Impaired GPX1 activity can exacerbate oxidative stress, leading to enhanced activation of redox-sensitive inflammatory pathways such as NF-κB and increased production of pro-inflammatory cytokines, thereby promoting synovial inflammation, cartilage degradation, and bone erosion (9, 10). In this regard, the GPX1 Pro198Leu (rs1050450) polymorphism has been associated with reduced enzymatic activity and altered antioxidant capacity (11). Carriers of the Leu allele may therefore be more susceptible to oxidative stress–induced damage and persistent inflammation, which could increase the risk of RA development or influence disease severity and progression (11). Overall, GPX1 dysfunction and the Pro198Leu polymorphism may contribute to RA pathogenesis by weakening antioxidant defenses and facilitating the vicious cycle between oxidative stress and chronic inflammation in the joint microenvironment. In the present study, we aim to investigate the association of rs1050450 polymorphism of the GPx1 gene and the RA risk in an Iranian population using a case–control study.

Materials and Methods

Study Design and Population

This study was designed as a case-control study. The study protocol was approved by the Ethics Committee of Ur. C., Islamic Azad University (ethics code IR. IAU. URMIA.REC.1404/070). The case group included 45 patients with RA who had visited a rheumatology center in West Azerbaijan, Iran. The diagnosis of the disease was confirmed by a rheumatologist and based on the ACR/EULAR 2010 classification criteria (12). The control group included 45 healthy individuals who were matched to the patient group in terms of age and gender and had no personal or family history of autoimmune, chronic inflammatory, or malignant diseases. This group had no history and current healthy problem or receive no medication for any pathological condition. Written informed consent was obtained from all participants after explaining the objectives of the study.

Rheumatoid factor (RF) and erythrocyte sedimentation rate (ESR) Measurements

Latex-agglutination test was used to measure RF in serum of the subjects. ESR was measured using an automated analyzer based on the Westergren method.

DNA extraction

From each participant, 5 milliliters of venous blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. The samples were stored at -20 ºC until DNA extraction. Genomic DNA was extracted from peripheral blood leukocytes using a DNA extraction kit, and the quality and quantity were evaluated using a Nanodrop spectrophotometer and agarose gel electrophoresis 1%.

PCR-RFLP Genotyping

The genotype of the Pro198Leu polymorphism (rs1050450) was determined using the PCR-RFLP technique. First, the target region of the GPX1 gene was amplified using specific primers: Forward primer: 5’- TCC AGA CCA TTG ACA TCG AG -3’ Reverse primer: 5’- ACT GGG ATC AAC AGGACC AG -3’. The PCR reaction was performed in a final volume of 20 µL, including 1 µL of template DNA, 1 µL of each primer, 10 µL of Master Mix containing dNTPs, PCR buffer, and Taq DNA Polymerase, and 7 µL of water. The PCR temperature conditions were as follows: initial denaturation at 94°C for 5 minutes; then 35 cycles consisting of denaturation at 95°C for 30 seconds,

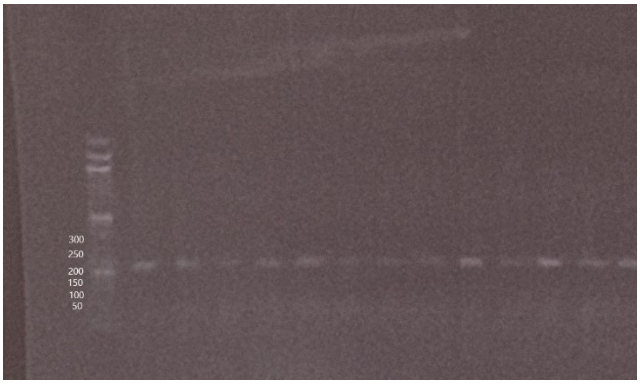

Figure 1. PCR product analysis for polymorphism (rs1050450) GPX1 on 2% agarose gel with 50 bp marker

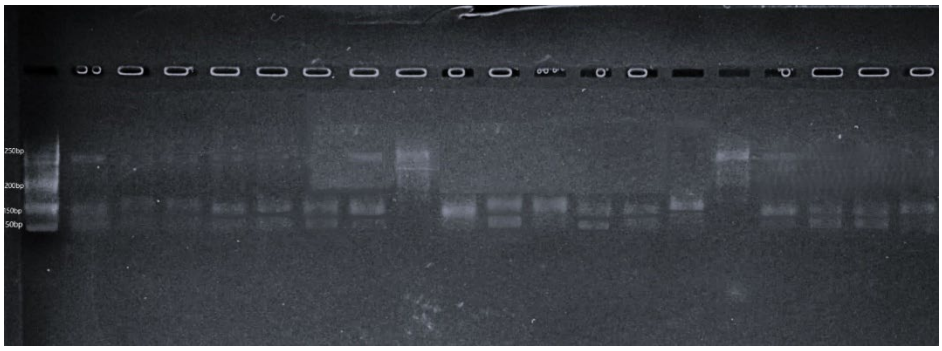

Figure 2. RFLP products for the CC, CT, and TT genotypes of the GPX1 (rs1050450) polymorphism on a 3% gel. The marker is 50 bp Sina clone: Genotype CC: Two fragments of 170 and 52 bp, Genotype TT: One uncut fragment of 222 bp, Genotype CT: Three fragments222، 170 and 52 bp.

primer annealing at 59°C for 30 seconds, and extension at 72°C for 45 seconds; and finally, final extension at 72°C for 5 minutes.

As shown in Figure 1, the PCR product was 222 base pairs (bp) in length. It was then incubated with the restriction enzyme ApaI at 37°C for 1 hour. The C allele creates a cutting site for this enzyme, while the T allele lacks this site. The enzymatic digestion results were observed on a 2.5% agarose gel electrophoresis stained with Safe Stain and are shown in Figure 2.

Statistical Analysis

The data were analyzed using SPSS version 22. The chi-square (χ^2) test was used to compare the genotype and allele frequencies between the case and control groups. Deviation from Hardy-Weinberg equilibrium (HWE) in the control group was also examined using the chi square test.

Results

Demographic characteristics

A total of 90 participants were included in this study, comprising 45 patients (37 women and 8 men) with a mean age of 54.04±13.76 years and 45 healthy individuals (22 women and 23 men) with a mean age of 57.33±13.94 years participated. The overall mean age of participants was 55.69 years (SD = 13.88), ranging from 28 to 86 years. Due to non-random sampling, no statistically significant difference was found in age and gender distribution between the two groups ($P > 0.05$). An independent samples t-test showed no statistically significant difference in mean age between the two groups ($p = 0.262$).

The allele's frequency analysis

Overall, 31 participants (34.4%) were male and 59 (65.6%) were female. The gender distribution differed significantly between groups ($\chi^2 = 11.072$, $p = 0.001$), with a higher proportion of females in the patient group. Across all participants, genotype CC was observed in 45 individuals (50.0%), genotype CT in 40 individuals (44.4%), and genotype TT in 5 individuals (5.6%). Table 1 shows the frequency of alleles between groups and Chi-square analysis revealed no significant difference in genotype distribution between patients and controls ($p = 0.779$).

Similarly, genotype distribution did not differ significantly by gender ($p = 0.464$). Hardy–Weinberg equilibrium analysis for control, patient and the total studies samples were no significant deviation from the equilibrium and the Chi-square and p values were 0.555, 0.46 ; 0.613, 0.43 and 1.01, 0.32 respectively. One-way

Table 1. Allele and genotype frequency of the Pro198Leu polymorphism in the GPX1 gene in patient and control groups

		subjects		Total
		Healthy control	Patients	
Genotype	CC	21	24	45
	CT	21	19	40
	TT	3	2	5
Total		45	45	90

Table 2. Hardy–Weinberg equilibrium analysis detail in patients' group

Patient groups	Number of Views	Percentage of Views	Number Expected	Percentage Expected
Heterozygous wild-type genotype	24	53/33	24/94	55/42
Heterozygous genotype	19	42/22	17/12	38/05
Homozygous genotype	2	4/44	2/94	6/53
C allele	67	74/44	K square value:0.541	
T allele	23	25/56	P value:0.461	

Table 3. Distribution of RF level in genotypes of the Pro198Leu polymorphism in the GPX1 gene in patient and control groups

		RF				Total
		Negative	+1	+2	+3	
Genotype	CC	16	3	0	5	24
	CT	15	0	1	3	19
	TT	1	1	0	0	2
Total		32	4	1	8	45

Table 4. Distribution of ESR level in genotypes of the Pro198Leu polymorphism in the GPX1 gene in patient and control groups

		ESR			Total
		Normal	30-40	40-60	
Genotype	CC	18	3	3	24
	CT	14	3	2	19
	TT	2	0	0	2
Total		34	6	5	45

ANOVA indicated no statistically significant difference in mean age across different genotypes ($p = 0.655$ for the total sample). The same non-significant result was found within the patient group ($p = 0.641$) and the control group ($p = 0.496$).

The Chi-Square tests indicate that there is no statistically significant association between genotype and either RF (Rheumatoid Factor) or ESR (Erythrocyte Sedimentation Rate) levels. For the relationship between genotype and RF, the Pearson Chi-Square value was 8.092 with 6 degrees of freedom, resulting in a p-value of 0.231. Similarly, for genotype and ESR, the Pearson Chi-Square value was 0.803 with 4 degrees of freedom and a p-value of 0.938. Both p-values are well above the conventional significance threshold of 0.05, suggesting that the observed distributions are likely due to chance rather than a meaningful biological relationship. However, it is important to note the limitations of the Chi-Square test in these cases, as a large proportion of cells (83.3% for RF and 77.8% for ESR) had expected counts less than 5, which may affect the reliability of the results. Overall, the analysis does not support a significant correlation between the examined genotype and the levels of RF or ESR in this sample.

Discussion

Glutathione peroxidase 1 (GPX1) is a major intracellular antioxidant enzyme involved in the pathogenesis of RA through its critical role in controlling oxidative stress within inflamed synovial tissues (13). In RA, excessive production of ROS by activated immune cells promotes synovial hyperplasia, cartilage destruction, and bone erosion, while GPX1 normally limits this damage by reducing hydrogen peroxide and lipid peroxides and modulating redox-sensitive inflammatory pathways such as NF-κB and MAPK (14). It has been suggested that genetic variability in the GPX1 may modulate disease susceptibility and severity (15). One study suggests the importance of GPX1 C/T polymorphism (rs1800668) in development of RA in Pakistani population (16). Importantly, it was reported that the functional Pro198Leu (rs1050450) variant, can influence enzyme activity and antioxidant capacity (17). The Leu allele has been associated with reduced GPX1 activity and impaired detoxification of ROS, potentially predisposing individuals to heightened oxidative stress and sustained inflammatory responses. However, despite these studies, the association between Pro198Leu (rs1050450) variant of GPX1 and RA remains inconclusive and the present study was designed to clarify this relationship.

In the present study we investigated the association between the Pro198Leu polymorphism of the antioxidant gene GPX1 and the risk of developing RA in an Iranian population. Our main finding was that the genotype in this polymorphism including C and T alleles were not significantly associated with an increased risk of RA. The Hardy–Weinberg equilibrium was showing a balanced allele distribution where the p value was not significant. These data imply that GPX1 might not be a major contributor to the pathogenesis of RA.

Several limitations should be considered when interpreting the findings of this GPX1 polymorphism study in RA. The relatively small sample size may have limited the statistical power to detect modest genetic effects, particularly for low-frequency alleles, increasing the risk of type II error. Insufficient power may also hinder reliable subgroup analyses based on disease severity, sex, or clinical phenotypes. In addition, the study focused on selected GPX1 polymorphisms rather than comprehensive gene-wide or haplotype analyses, which may overlook the combined effects of multiple variants. Potential population stratification and lack of replication in independent cohorts further limit the generalizability of the results. Finally, the absence of functional assays and measurements of GPX1 activity or oxidative stress biomarkers restricts the ability to directly link genetic variation to biological consequences, underscoring the need for larger, well-powered studies integrating genetic, functional, and clinical data.

Conflict of Interest

The authors declared that they have no conflict of interest.

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