

GSTT1 AND GSTM1 GENE POLYMORPHISMS IN SARCOIDOSIS

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ABSTRACT. *Introduction:* Sarcoidosis is a granulomatous disease of unknown cause, which affects all systems, especially the lungs and the lymphatic system. Genetic and environmental factors are held accountable for the etiology. Based on the general opinion, sarcoidosis develops after exposure to a specific environmental agent by genetically susceptible individuals. The present study aimed to evaluate the disease susceptibility of the GSTT1 and GSTM1 gene polymorphisms in the patients with sarcoidosis. *Method:* The present study included 78 patients; 38 patients with histopathologically verified sarcoidosis and 40 control subjects. Multiplex PCR method was used to determine the GSTT1 and GSTM1 gene polymorphisms. The genotype was determined based on the bands formed in the agarose gel electrophoresis. The statistical analysis was done using the chi-square test. *Results:* The positive/negative genotype rates were 79%/21% and 53%/47%, respectively in the case group for the GSTT1 and GSTM1 gene polymorphisms, whereas the positive/negative genotype rates were 77%/23% and 55%/45% in the control group. There was no statistically significant difference in the positive and negative genotypes compared with the case group and the control group for the GSTT1 and GSTM1 gene polymorphisms ($p > 0.05$). *Discussion:* The results from the present study suggest that there is not any association with the control group for the disease susceptibility of the GSTT1 and GSTM1 gene polymorphisms in patients with sarcoidosis, and this result should be supported by large-scale studies because of the limited number of cases in the present study. (*Sarcoidosis Vasc Diffuse Lung Dis* 2016; 33: 253-257)

KEY WORDS: sarcoidosis, polymorphism, GSTT1, GSTM1

INTRODUCTION

Sarcoidosis is a granulomatous disease of unknown etiology, which is chronic, multi-systemic and may affect every organ and tissue (1). The final diagnosis should be established by pathologically demonstrating the non-caseating granulomas in the

samples from the biopsy along with the clinical and radiological findings, and by excluding other infectious diseases, autoimmune disorders (Wegener's granulomatosis, etc...), neoplasms, drug reactions, hypersensitivity pneumonitis, and the diseases caused by occupational and environmental exposure that may lead to such lesions (2). The pulmonary and the hilar node involvement is more common; however, the skin, the spleen, the liver, the nervous system, the locomotor system, and the eyes may also be involved (3). The pulmonary involvement is above 90% (4), whereas the extrapulmonary involvement varies between 10% and 40% (5).

Glutathione (gamma glutamyl cysteinyl glycine) is a tripeptide consisting of glutamic acid,

Received: 22 January 2015

Accepted after revision: 13 August 2015

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cysteine and glycine (6). It is involved in the DNA and protein syntheses, the regulation of the enzyme activities, the cellular functions such as intracellular and extracellular transports, as well as the cell defense as an antioxidant. The enzymes catalyzing the reactions of glutathione with xenobiotics are called "glutathione-S-transferases", i.e. "GST" (7). It has been shown that GSTM1 is polymorphic in humans and it does not exist in 35-60% of the people. This enzyme does not exist in 50-60% of the white population; whereas it exists in 28% of the Northern American black population, and in lower percentages such as 22% in Nigerians. Likewise, GSTT1 is also polymorphic and does not exist in 10-65% of the human populations. GSTT1 activity is not present in 17% of the American white population, 39% of the Nigerians and 3.2% of the British living in India. The absence of the GSTM1 and GSTT1 activity (null genotype) results from the homozygous deletion of these genes (8, 9). Oxidative stress is believed to contribute to the development of several diseases such as diabetes, atherosclerosis, and cancer (10). GST is one of the defense mechanisms against the damage caused by the oxidative stress. The polymorphism in the GST gene impairs the defense against the oxidative stress and allows for diabetes development. There are many broad studies conducted on the GST gene polymorphism in several diseases, such as cancer susceptibility and chemotherapy response, in particular (11, 12).

METHODS

The present study was designed as a prospective controlled study. The study was initiated in the Uludağ University Faculty of Medicine, Department of Thoracic Diseases after obtaining the approval of the Uludağ University Faculty of Medicine Medical

Research Ethics Committee. The study included 38 sarcoidosis patients who were histopathologically diagnosed with sarcoidosis and followed-up in Uludağ University Faculty of Medicine, Department of Thoracic Diseases outpatient clinic between September 2010 and December 2010, and 40 healthy control subjects. The entire study and control group consisted of individuals from the Turkish race. The patients included in the study group were receiving treatment eligible for the sarcoidosis stage. All individuals who agreed to participate in the study provided written informed consent.

DNA Isolation

Peripheral venous blood samples of 2 cc were taken from the patient and control groups into the EDTA tubes for gene analyses. The DNA isolation was performed in Uludağ University Faculty of Medicine, Department of Medical Genetics. Zeydanlı M.D. performed the DNA isolation kit procedure and the samples were kept at -20°C until analysis.

Polymerase Chain Reaction Protocol

The PCR method is based on the adhesion of the respective primers to the respective primer segment at optimum temperature after the two strands of the genomic DNA becomes a single strand under heat and the addition of four deoxynucleotide triphosphates (adenine, guanine, cytosine, thymine) to the new chain in an environment catalyzed by the enzyme DNA Taq polymerase. The present study employed the multiplex PCR method to determine the GSTM1 and GSTT1 polymorphisms in the isolated DNA. The PCR mixture was prepared for this purpose. PCR was performed using the primers presented in Table 1 for each gene polymorphism.

The PCR procedure was conducted in the PCR machine by using the following temperatures and

Table 1. Sequence of the primers used for the GSTT1 and GSTM1 gene polymorphisms

	Primer (forward)	Primer (reverse)	The Resulting Product (Base Pair)
GSTM1	5'-TTCCTTACTGGTCCTCACATCTC-3'	5'-TCACCGGATCATGGCCAGCA-3'	219
GSTT1	5'-GAACTCCCTGAAAAGCTAAAGC-3'	5'-GTTGGGCTCAAATATACGGTGG-3'	459
Albumin (control)	5'-GCCCTCTGCTAACAAGTCCTAC-3'	5'-GCCCTAAAAGAAAATCCCCAATC-3'	350

times as a PCR cycle program for the GSTT1 and GSTM1 gene polymorphisms.

Lid temperature, (specific to the device type) 103°C, 1- Initial denaturation 94°C, 5 minutes; 2- Denaturation 94°C, 1 minute; 3- "Annealing" 57°C, 1 minute; 4- "Extension" 72°C, 1 minute; 5- Final "Extension" 72°C, 10 minutes (34 cycles with steps 2, 3, and 4, respectively)

Gel Electrophoresis Protocol

Agarose Gel Electrophoresis is one of the standard methods used to separate and identify the DNA and PCR products. In the present study, 2% agarose gel electrophoresis was used to identify the products amplified by PCR. The gel of 2% was prepared by mixing 5 ml of 10xTris-Boric Acid-EDTA (TBE) solution with 45 ml of dH₂O. Then, 1 g of agarose was added into the mixture. The solution was heated in the microwave at the "medium-high" setting until dissolved. Then, 5 µl of ethidium bromide was added into the melted gel and mixed. The gel was poured into the electrophoresis apparatus and allowed to cool. The electrophoresis tank was filled with 1xTBE and made ready for the gel-running procedure. The PCR products were processed with bromophenol blue and loaded into the agarose gel. The products were run at 90-100 V for 15 minutes. For the GSTM1 and GSTT1 studies, the individuals with appearing bands were considered positive and the individuals without appearing bands were considered deletion (null).

Determining Genotypes

It was expected to obtain products with 219 bp for GSTM1, 459 bp for GSTT1, and 350 bp for albumin (control) at the end of the PCR reactions in GSTM1 and GSTT1.

Statistical Analysis

All data were analyzed using SPSS 13.2 statistical package. All data were expressed in mean ± standard deviation (SD). The genotypes were compared using chi-square test. $p < 0.05$ was considered statistically significant.

RESULTS

The positive/negative genotype rates were 79%/21% and 53%/47%, respectively in the case group for the GSTT1 and GSTM1 gene polymorphisms, whereas the positive/negative genotype rates were 77%/23% and 55%/45% in the control group. There was no statistically significant difference in the positive and negative genotypes compared to the case group and the control group for the GSTT1 and GSTM1 gene polymorphisms ($p > 0.05$).

The mean age of sarcoidosis patients and control cases were 46.84 ± 9.95 and 45.92 ± 10.21 , respectively. There was no difference in terms of age and gender between two groups. While the ratio of present genotypes in GSTT1 and GSTM1 gene polymorphisms was determined as 79% and 53% in the sarcoidosis patients group, the same ratio was determined 77% and 55% in the control cases group respectively (Table 2). There was no statistically significant difference in terms of present and null genotypes in the GSTT1 and GSTM1 polymorphisms between sarcoidosis patients group and control cases groups ($p > 0.05$) (Fig.1).

DISCUSSION

Sarcoidosis is a chronic inflammatory disease, characterized by granulomatous inflammation,

Table 2. GST M1 and GST T1 polymorphism rates among the patient group and control group

Genes	Genotype	Sarcoidosis patient group (n 38) %	Control patient group (n 40) %	p-value
GSTT1	Null	8 (21%)	9 (23%)	0.877 1 (Reference)
	Present	30 (79%)	31 (77%)	
GSTM1	Null	18 (47%)	18 (45%)	0.833 1 (Reference)
	Present	20 (53%)	22 (55%)	

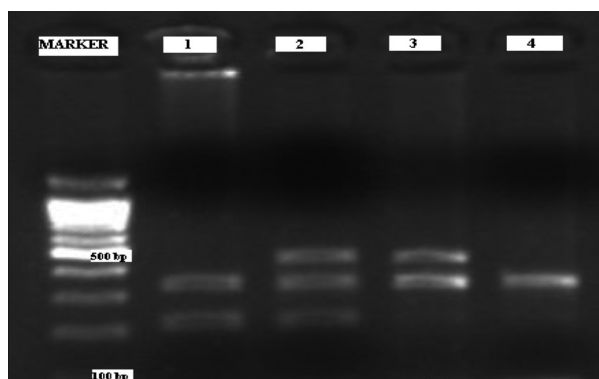


Fig. 1. A representative multiplex PCR analysis of GST polymorphisms. Albumin (350 bp), GSTT1 (459 bp), and GSTM1 (219 bp) genes. PCR products resolved by agarose gel electrophoresis. Line MARKER is a 100 bp DNA Ladder. Line 1 is the GSTM1 present genotype and GSTT1 is the null genotype, line 2 is GSTT1 and GSTM1 present genotype, line 3 is the GSTT1 present genotype, and GSTM1 null genotype, and line 4 is both GSTT1 and GSTM1 null genotype.

prominently involving the respiratory system. The etiology of this disease has not yet been elucidated and the contribution of genetics is not yet completely understood. The immunologic phenomena that characterize sarcoid inflammation involve diffuse infiltration of parenchymal organs with CD4+ T lymphocytes of the T type, formation of granulomas and distortion of normal organ micro-architecture, which can eventually disturb normal function of the involved organ (13).

The etiological basis of sarcoidosis has not yet been elucidated. Possible hypotheses implicate various infectious, environmental and genetic factors, but none has been consistently associated with this disease. We performed a comprehensive literature search for sarcoidosis candidate genes; this revealed a number of possible genes and their respective variants that could be implicated in the etiopathogenesis of sarcoidosis, but had not been considered in previous genetic association studies (14).

We have reported that there is no association between GSTT1 and GSTM1 gene polymorphism and risk of sarcoidosis in Turkish people based on our prospective study.

GSTP1 codes for an enzyme of the glutathione S-transferase class, involved in detoxification reactions. The GSTP1 enzyme has been previously examined for its roles in the etiology of respiratory diseases, in the regulating of the extent of oxidative damage of tissues and in inactivation of reactive oxygen spe-

cies (ROS) (15). ROS have also been implicated in the regulation of inflammatory processes, and their role in various inflammatory disorders has been established (16). We chose to study the GSTP1 gene, as sarcoidosis is characterized by increased oxidative damage and increased levels of GSTP1 enzyme (17). Maver et al. investigated the GSTP1 polymorphism in 180 Slovenian patients with sarcoidosis; however, they did not find any significant difference (14).

When the previous studies were reviewed, we did not find any study investigating the correlation of the GSTT1 and GSTM1 gene polymorphisms with sarcoidosis. However, the GSTT1 and GSTM1 polymorphisms were investigated by studies examining lung cancer, asthma, and chronic obstructive pulmonary disease (COPD). Karam et al. conducted a study with 126 children with asthma and found the GSTM1 null polymorphism significantly higher. At the end of this study, they concluded that the GST gene polymorphism has a very important role in the pathogenesis and etiology (18). Pan et al. maintained that the GSTT1 and GSTM1 polymorphisms were associated with the risk of lung cancer in a gender-specific manner in the Chinese race (19). Zuntar et al. identified the GSTP1 polymorphism in their study with a COPD group; however, they did not find any significant difference in the GSTT1 and GSTM1 gene polymorphisms (20).

In conclusion, while our study did not find a significant correlation between the GSTT1 and GSTM1 polymorphism and sarcoidosis in the Turkish population, we believe that a larger sample size and further elucidation of other single nucleotide polymorphisms are required to reach a definitive conclusion.

ACKNOWLEDGEMENTS

We would like to thank the staff in the Department of Pulmonology and Department of Genetics at Uludag University, Faculty of Medicine Hospital.

Contributors Conception:

EU, DY, TY, and FC; study design: all; recruitment: DY and FC; analysis and interpretation: DY, F, and MK; data collection: DY; wrote the manuscript: FC; revised the manuscript: FC.

Funding:

Uludag University Medical Faculty Hospital Charity.

REFERENCES

1. Wu JJ, Schiff KR. Sarcoidosis. *Am Fam Physician* 2004; 70: 312-22.
2. Jundson MA. Sarcoidosis: clinical presentation, diagnosis and approach to treatment. *Am J Med Sci* 2008; 335: 26-33.
3. Takahashi R, Shibuya Y, Shijubo N, Asaishi K, Abe S. Mammary involvement in a patient with sarcoidosis. *Intern Med* 2001; 40: 769-71.
4. Wu JJ, Schiff K. Sarcoidosis. *Am Fam Physician* 2004; 70: 312-22.
5. Harris KP, Faliakou EC, Exon DJ, Nasiri N, Gui GP. Isolated sarcoidosis of the breast. *J R Soc Med* 2000; 93: 196-7.
6. General Principles In: Sipes IG, Mcquuen, Ganddfi AJ, Bond JA (eds). *Comprehensive Toxicology*, Vol. 13 Elsevier Science, New York 1997.
7. Hayes JD, Pulford DJ. The Glutathione S-Transferase Super-gene Family: Regulation of GST and the Contribution of the Iso-enzymes to Cancer Chemoprotection and Drug Resistance. *Crit Rev Biochem Mol Biol* 1995; 30: 445-600.
8. Whalen R, Boyer TD. Human Glutathione S-Transferases. *Sem Liver Dis* 1998; 18: 4.
9. Chen CL, Liu Q, Pui CH, et al. Higher Frequency of Glutathione S-Transferase Deletions in Black Children With Acute Lymphoblastic Leukemia. *Blood* 1997; 89: 1701-7.
10. Lyons TJ. Oxidized low density lipoproteins: A role in the pathogenesis of atherosclerosis in diabetes? *Diabetes Med* 1991; 8: 411-9.
11. Ryberg D, Skaug V, Hewer A. Genotype of glutathione S-transferase M1 and P1 and their significance for lung DNA adduct levels and cancer risk. *Carcinogenesis* 1997; 18: 1285-9.
12. Watters JW, McLeod HL. Recent advances in the pharmacogenetics of cancer chemotherapy. *Curr Opin Mol Ther* 2002; 4: 565-71.
13. Baughman RP, Lower EE, du Bois RM. Sarcoidosis. *Lancet* 2003; 361: 1111-8.
14. Maver A, Medica I, Salobir B, Tercelj M, Peterlin B. Lack of association of immune-response-gene polymorphisms with susceptibility to sarcoidosis in Slovenian patients *Genet Mol Res* 2010; 9 (1): 58-68
15. Montano MM, Deng H, Liu M, et al. Transcriptional regulation by the estrogen receptor of antioxidative stress enzymes and its functional implications. *Oncogene* 2004; 23: 2442-53.
16. Rahman I. Oxidative stress, chromatin remodeling and gene transcription in inflammation and chronic lung diseases. *J Biochem Mol Biol* 2003; 36: 95-109.
17. Sabounchi-Schutt F, Mikko M, Eklund A, et al. Serum protein pattern in sarcoidosis analysed by a proteomics approach. *Sarcoidosis Vasc Diffuse Lung Dis* 2004; 21: 182-90.
18. Karam AR, Pasha HF, El-Shal SA, Rahman HMA, Gad DM. Impact of glutathione-S-transferase gene polymorphisms on enzyme activity, lung function and bronchial asthma susceptibility in Egyptian children. *Gene* 2012; 497: 314-9.
19. Pan C, Zhu G, Yan Z, Zhou Y, Liu Z. Glutathione S-Transferase T1 and M1 Polymorphisms Are Associated with Lung Cancer Risk in a Gender-Specific Manner. *Oncol Res Treat* 2014; 37: 164-9.
20. Zuntar I, Petlevski R, Dodig S, Popovic-Grle S. GSTP1, GSTM1 and GSTT1 genetic polymorphisms and total serum GST concentration in stable male COPD. *Acta Pharm* 2014; 64: 117-29.