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Lack of association between the glutathione-s-transferase genes (*GSTT1* and *GSTM1*) and nasal polyposis*

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SUMMARY

Objectives: *To evaluate the glutation-S-transferase (GST) polymorphisms (GSTM1 and GSTT1) in nasal polyposis (NP).*

Methods: *The study population consisted of 102 unrelated healthy individuals and 98 patients with NP (67 without asthma, 31 with asthma). Genotyping of the polymorphism in the GSTM1 and GSTT1 genes was performed using the multiplex polymerase chain reaction (PCR)-based method.*

Results: *GSTM1 and GSTT1 null-genotypes were found in 46.1% and 23.5% of the controls, and in 43.9% and 33.7% of the NP patients, respectively. These differences were not significant (for GSTM1 null odds ratio (OR) = 0.92; 95% confidence interval (CI) = 0.52-1.6 and for GSTT1, OR = 1.65; 95% CI = 0.89-3.07). Although no significant difference for combined GSTM1 and GSTT1 null genotypes between control (8.8%) and NP patients (17.3%) was found, there was a 2.16-fold increased proportion in the NP with the combined GSTM1-null and GSTT1-null genotype (OR = 2.16; 95% CI = 0.91-5.13).*

Conclusion: *These results suggest that there is lack of association between GSTM1 and GSTT1 polymorphisms and NP. The GSTM1 or GSTT1 polymorphisms had also no relevant developing effect on NP patients without or with asthma.*

Key words: glutation-S-transferase genes, polymorphism, nasal polyposis, genotype

INTRODUCTION

Nasal polyposis (NP) is a benign lesion associated with the inflammation of the nasal mucosa and the sinuses. To date, several theories such as genetic susceptibility, infection, anatomic abnormalities, and local immunological imbalances have been postulated to play a role in its pathogenesis. However, its exact pathogenesis is still unknown. Genetic etiology is suspected in the background of the formation of NP as well, on the basis of familial aggregation. Some investigators have suggested that a genetic basis might be present in these patients. Increasing epidemiologic, immunohistochemical, genetic and molecular evidence suggests that heterogeneity might also exist in this disease. NP is probably a multifactorial and polygenic disease [1-3]. Recently strong evidence has been demonstrated in relation to oxidative stress in the pathogenesis of NP, and antioxidants may have a preventive role in free-radical-mediated tissue damage in NP [4], whereas no data are available regarding a glutation-S-transferase (*GST*) gene polymorphism in NP.

The *GST* superfamily represents a major group of detoxification enzymes. There are four main classes of *GST* isoenzymes in humans (α, θ, μ and π), with partially overlapping substrate specificities [5]. The *GSTM1* enzyme belongs to the μ class and the *GSTT1* enzyme to the θ class. Genes coding for *GSTM1* and *GSTT1* proteins can have null genotypes in humans due to the deletions of both paternal and maternal alleles, resulting in lack of active protein [6,7]. Inherited homozygous deletions of the *GSTT1* or *GSTM1* genes lead to an absence of enzymatic activity. The *GST* genes are polymorphic, and have polymorphism frequencies that are related to ethnicity. Approximately 50% of the white population are homozygous for deletions in *GSTM1*, and approximately 15-30% are homozygous for deletions in *GSTT1* [8,9].

The *GSTM1* and *GSTT1* null genotypes were reported to be associated with increased risk factors that predispose to some inflammatory disease [10,11]. Therefore, we investigated the association between *GSTM1* and *GSTT1* null genotype polymorphisms and NP.

MATERIALS AND METHODS

Patients

The study was carried out in a hospital-based case-control study conducted at the Meram Medical School, University of Selcuk.

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Ninety-eight patients with NP (67 without asthma, 31 with asthma) were chosen from the files and 102 controls were used. The patients and controls were from the same geographic region and of the same ethnic origin. Control subjects were selected from among healthy people with no history of cardiovascular disease, cancer, chronic degenerative neurological disease, chronic obstructive pulmonary disease, hepatitis, diabetes, hypertension, atopy, autoimmune diseases, allergies in general or alcohol abuse.

The patients and control subjects provided extensive histories, and physical examination, anterior rhinoscopy, nasal endoscopy, paranasal sinus computed tomography (CT), complete blood count, routine biochemical tests and skin prick tests were performed. The patients with asthma had been diagnosed and were followed by the Department of Chest Disease. All asthmatic patients were receiving regular inhaled corticosteroids, and β_2- adrenoreceptor stimulants, for use as required. The demographics of the study population are shown in Table 1. The study was approved by the human ethics committee of University of Selcuk, and informed consent was obtained from all participants.

Table 1. Characteristics of the study population.

Characteristics	Patients (n, %)	Controls (n, %)
	98 (100)	102 (100)
Age (years)	36.37+14.17	47.15+14.49
Gender		
Male	47(47.95%)	57(55.9%)
Female	51(52.04%)	45(44.1%)
Patient's group		
Without asthma	67(68.36%)	
With asthma	31(31.63%)	

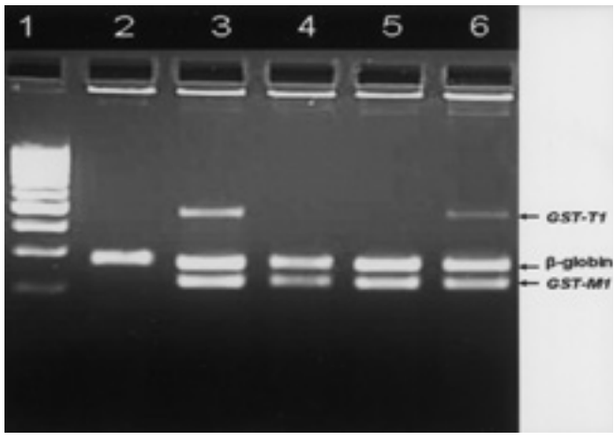

Figure 1. Multiplex PCR patterns for *GSTM1* and *GSTT1* genes. The β-globin gene was used as an internal positive control. Lane 1 is the size marker (100 bp), lanes 3 and 6 represent present genotypes for both *GSTM1* and *GSTT1* genes, and lane 2 represents null genotypes for both *GSTM1* and *GSTT1* genes. Lanes 4 and 5 represent present genotypes for the *GSTM1* gene and null genotypes for the *GSTT1* gene. (PCR=Polymerase Chain Reaction, *GSTM1*=glutation-S-transferase M1, *GSTT1*=glutation-S-transferase T1).

DNA extraction and genotyping of GSTT1 and GSTM1

Venous blood (4 ml) was collected by venipuncture into sterile siliconized Vacutainer tubes containing 2ml EDTA from both patients and control subjects. Immediately after collection, whole blood was stored at +4°C until use. Genomic DNA for polymerase chain reaction (PCR) analysis was isolated using standard procedures. *GSTM1* and *GSTT1* genotypes were determined by multiplex PCR using three sets of primers to amplify a 215-bp sequence of the *GSTM1* gene [12], a 268-bp sequence of the β-globin gene [12], and a 480-bp segment of the *GSTT1* gene [13]. The PCR reactions were carried out in 50 µl containing 10 µl of crude DNA extract, 10x PCR buffer (50 mM KCl, 50 mM Tris-Cl, and 1.25 mM MgCl2), 5 mM deoxynucleoside triphosphate mixture, 10 mM of each primer, 1 unit of Taq DNA polymerase (Fermentas), and sterile water. After a 3-min incubation at 94°C, a first round of 10 cycles was performed (30s at 94°C, 1 min at 59°C, and 1 min at 72°C), followed by a second round of 25 cycles (30 s at 94°C, 30 s at 57°C, and 30 s at 72°C, with a 3-s/cycle increase in extension time). The primers used were (*a*) 5'-TTCCTTACTGGTCCTCACATCTC-3' and 5'-TCACCG-GATCATGGCCAGCA-3' for *GSTT1*; (b) 5'-GAACTCCCT-GAAAAGCTAAAGC-3' and 5'-GTTGGGCTCAAATAT-ACGGTGG-3' for *GSTM1*; and (*c*) 5'-CAACTTCATCCACGTTCACC-3' and 5'-GAAGAGC-CAAGGACAGGTAC-3' for β-globin as an internal control. Presence or absence of the β-globin gene band was used to determine failed PCR. The products of the multiplex PCR (215 bp for *GSTM1*, 480 bp for *GSTT1*, and 268 bp for β-globin) were separated by electrophoresis through a 2.5% agarose gel, stained with ethidium bromide and visualized under UV light (Figure 1).

Statistical Analysis

The associations between the genotype frequencies of *GSTM1* and *GSTT1* and the control, and patients groups were assessed by Odds Ratios (OR) and Confidence Intervals (95% CI). Also genotype of *GSTM1* and *GSTT1* distributions were compared between groups using the χ^2 test. All statistical analysis was performed using the statistical software SSPS 11.5.

RESULTS

The frequencies of *GSTM1* and *GSTT1* genotypes in the control and the patients with NP are shown in Table 2. The frequency of *GSTM1*-null genotypes in the control group (46.1%) did not differ significantly from that in the NP group (43.9%), (OR=0.92; 95% CI= 0.52-1.6). The frequency of *GSTT1*-null individuals was 23.5% in the control, and 33.7% in the NP group. However, this difference did not reach statistical significance (OR=1.65; 95% CI=0.89-3.07).

The frequencies of *GSTM1* and *GSTT1* genotypes in the patients with NP (without and with asthma) are shown in Table 3. The frequency of *GSTM1*-null genotypes in the without asthma and with asthma group was 46.3%, and 38.7%, respectively. There was no significant difference between both groups for the *GSTM1*-null genotype frequency (OR=0.73; 95% CI=0.31-1.75).

Table 2. Association between *GSTM1* and *GSTT1* genotypes and nasal polyposis.

	Patients (n=98) n (%)	Control (n=102) n (%)	OR	95% CI	p-values
GSTM1					
Present	55 (56.1)	55 (53.9)	1 (reference)	-	
Null	43 (43.9)	47 (46.1)	0.92	0,52-1,6	0,754
GSTT1					
Present	65 (66.3)	78 (76.5)	1 (reference)	-	
Null	33 (33.7)	24 (23.5)	1.65	0,89-3,07	0,112

OR=odds ratio; CI=confidence interval. The data were analyzed by the χ^2 test.

Table 3. Association between *GSTM1* and *GSTT1* genotypes and nasal polyposis subgroups.

	Without asthma (n=67) n (%)	With asthma (n=31) n (%)	OR	95% CI	p values
GSTM1					
Present	36 (53.7)	19 (61.3)	1 (reference)	-	
Null	31 (46.3)	12 (38.7)	0,73	0,31-1,75	0,630
GSTT1					
Present	45 (67.2)	20 (64.5)	1 (reference)	-	
Null	22 (32.8)	11 (35.5)	1,12	0,46-2,75	0,978

OR=odds ratio; CI=confidence interval. The data were analyzed by the χ^2 test.

Table 4. Summarized data on the combined distribution of *GSTM1* and *GSTT1* presence and null genotype alleles in controls and in the patient's group.

GSTM1	GSTT1	Patients (n=98) n (%)	p-values*	Controls (n=102) n (%)	p-values**	OR	95% CI
Present	Present	Without asthma=26 (38.8))	0,942			1,14	0,48-2,71
		With asthma=13 (41.9)					
		Total NP=39 (39.8)		40 (39.2)	0.933	1,02	058-1,80
Null	Present	Without asthma 19 (28.4))	0,722			0,74	0,27-1,99
		With asthma 7 (22.6)					
		Total NP=26 (26.5)		38 (37.3)	0,104	0,60	0,33-1,11
Present	Null	Without asthma 10 (14.9))	0,797			1,37	0,45-4,18
		With asthma 6 (19.4)					
		Total NP=16 (16.3)		15 (14.7)	0,752	1,13	0,52-2,43
Null	Null	Without asthma 12 (17.9))	0,829			0,88	0,28-2,76
		With asthma 5 (16.1)					
		Total NP=17 (17.3)		9 (8.8)	0,073	2,16	0,91-5,13

OR=odds ratio; CI=confidence interval. The data were analyzed by the χ^2 test.

* *p values between without asthma and with asthma groups.*

** *p values between Total NP (Nasal polyposis) and control groups.*

The frequency of *GSTT1*-null genotypes in the without asthma and with asthma group was 32.8%, and 35.5%, respectively. There was also no significant difference between these groups for the *GSTT1*-null genotype frequency (OR=1.12; 95% CI=0.46-2.75).

The combined distribution of present and null of *GSTM1* and *GSTT1* alleles in the NP patients and in the control group are shown in Table 4. According to the presence of normal or mutant *GSTM1* and *GSTT1* alleles, all individuals were divided into four groups: (a) those with the *GSTM1* and *GSTT1* both present genotypes; (b) those with the *GSTM1*-null and *GSTT1*-present genotypes; (c) those with the *GSTM1*-present and *GSTT1*-null genotypes; (d) those with the *GSTM1* and *GSTT1* both null genotypes.

The frequency of combined *GSTM1*-present and *GSTT1*-present genotypes in the NP and the control group were 39.8%, and 39.2%, respectively. There was no significant difference between the NP and control group for this respect (OR=1.02; 95% CI=0.58-1.80). The data concerning both present genotypes in the subgroup of NP (without and with asthma) were 38.8% and 41.9%, respectively. There was no significant difference between these groups (p=0.94). There was a 2.16-fold increased proportion in the NP with the combined *GSTM1*-null and *GSTT1*-null genotype (OR=2.16; 95% CI, 0.91-5.13) when compared to the control group, but this difference did not reach statistical significance. In addition, there was also no significant difference between without asthma (17.9%) and with asthma (16.1%) groups for both null genotypes frequency (p=0.82).

DISSUSSION

Although multiple factors may be involved in the development of polyps, the etiology and pathogenesis of NP remain poorly understood. Investigations about the pathogenesis of NP have focused primarily on mucosal edema, which has been attributed to mast cell degranulation, vascular congestion, and an altered ion transport mechanism of the nasal epithelium [14-16]. It has been reported that epithelial damage might be essential for the initiation of polyp formation in the mucosa [17]. Wladislavosky-Waserman et al. [18] demonstrated that epithelial damage frequently occurred in NP. However, they did not report on the causative factors of epithelial damage in NP. Recent studies showed structural abnormalities in NP, especially epithelial cell injury caused by high concentration of inflammatory cells such as eosinophils and products of these cells [15,19]. The number of inflammatory cells in NP was higher than in normal tissue. Eosinophils, neutrophils, macrophages and lymphocytes were found in high concentrations in NP tissue and may lead to extensive production of oxidants [15,19].

Exposure to oxidants, whether endogenous or exogenous, can initiate free-radical-mediated reactions and lead to oxidative stress. Antioxidant systems help to defend the body against the occurred free radicals and oxidative stress but might become overwhelmed during periods of chronic oxidative stress. There is a crucial balance between protection against free radicals and their generation [20]. A previous study demonstrated that patients with NP had significantly lower blood and tissue antioxidant levels compared with the control group [4]. It was also reported that oxidants for patients with NP in both tissue and blood levels were significantly higher than in the control group [19].

Oxidative stress, with the formation of reactive oxygen species (ROS), is a key component of inflammation. A certain physiologic level of ROS is crucial for the proper regulation of cell functions such as intracellular signaling, transcription activation, cell proliferation, inflammation, and apoptosis [21]. Although host antioxidant defenses should detoxify ROS, individuals differ in their ability to deal with an oxidant burden, and such differences are, in part, genetically determined [22]. The number of genes involved in these complex genetic disorders has not been fully determined. Inability to detoxify ROS is likely to perpetuate the inflammatory process, make alterations in the epithelium by way of the mechanisms mentioned above, and this leads to mucosal edema in the early stage and epithelial damage in the advanced stage. The *GST* family of genes is critical in the protection of cells from ROS because they utilize as substrates a wide variety of products of oxidative stress. *GSTM1* and *GSTT1* demonstrate activity toward phospholipid hydroperoxide. These *GSTs* may also influence the synthesis of eicosanoids via modulation of ROS levels. Furthermore, the ROS-derived products are essential in the mobilization of arachidonic acid, with subsequent production of pro-inflammatory eicosanoids [22].

Most studies in the literature deal with asthma and association with *GST* polymorphisms [11,23,24], but no data are available concerning *GST* polymorphisms in patients with NP. Several studies have shown that *GSTM1* and *GSTT1* polymorphisms were associated with increased risks for asthma [11,23]. In contrast, another study has reported that there was no association between *GST* and asthma severity and *GST* null alleles were not proven to be important in asthma [24]. These frequencies of *GST* polymorphisms may be related to ethnicity, geographic location, socioeconomic status and environmental factors.

Although it has been stated that patients with NP had significantly lower blood and tissue antioxidant levels compared with the control group [4], no data are available about *GST* gene polymorphisms and whether a relation between the *GST* gene and NP pathogenesis. In this study, we investigated the *GSTT1* and *GSTM1* gene polymorphism in patients with NP and healthy individuals. We also compared different groups (without asthma and with asthma) in patients with NP. In this respect, there were no differences between the NP without asthma or with asthma groups and control subjects. We did not observe an association between the *GSTM1* and *GSTT1* polymorphisms and NP. The combined *GSTM1* null and *GSTT1* null genotype was 2.16-times more common in NP patients than in control subject, but this difference was not significant (OR=2.16; 95% CI, 0.91-5.13).

In conclusion, no association was found between *GSTM1* or *GSTT1* polymorphisms and NP. Additional studies are needed to assess the association between the polymorphisms in the *GST* genes and NP, and to extend it to other ethnic groups.

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