**Research article**

# Association between GSTM1, GSTT1 gene polymorphisms and asthma in adult patients from Tikrit population of Iraq

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## ABSTRACT

**Introduction and Aim:** Asthma is known as a polygenic and multifactorial disease. The underlying debate about the role of genetics in the development of asthma is still unclear. The objectives of this research are to examine whether the GSTM1 and GSTT1 gene polymorphisms are associated with asthma susceptibility.

**Materials and Methods:** A total of 70 patients with asthma and 20 healthy individuals were investigated in this study. Genotyping was carried out by using PCR protocol for analysis of GSTM1 and GSTT1 null/positive genotypes.

**Results:** Patients with asthma (34.285%) demonstrated a greater prevalence of the GSTM1, GSTT1 (-) genotype than the healthy subject (10%, P-value 0.012). A positive correlation was found between GSTT1, GSTM1 (+) genotype and healthy individuals (40%) compared with asthmatic patients (24.285%).

**Conclusion:** The results of this research support the idea that GSTM1, GSTT1 (-) genotype may play critical roles in asthmatic inflammatory response. Further experimental investigations are needed to estimate the role of GSTM1 and GSTT1 polymorphisms in asthma.

**Keywords:** Asthma; GSTM1; GSTT1; polymorphism; genetic.

## INTRODUCTION

Asthma is a common disease, estimated to affect around 8-10% of the human population. Among the approximate 339 million patients suffering from the disease globally, it is significantly more common among young boys (<14 years) and in adult women, (1). It is estimated that by 2025 there could be a further increase of 100 million patients with asthma worldwide (2). Asthma is a chronic disease that involves airway inflammation and is linked to bronchial hyper responsiveness due to exposure to allergic antigens (3). It shows as episodic or persistent symptoms of wheezing, uncontrolled coughing, dyspnea (shortness of breath) and deficiency of air (4, 5). Although genetic influence is clearly apparent, genes and environmental interaction may be explained the international variations in incidence and prevalence of asthma and allergy (6).

Biologically, the interaction between air pollutants components and different known antioxidant genes means that the impact (or regulation) of these genes is modified (either directly or indirectly) by the presence of the pollutant, and vice versa (7). Enzymatic and non-enzymatic antioxidants play a major role in reducing oxidative stress and dietary antioxidants (e.g., vitamins C and E) and glutathione, a major protective antioxidant in the lungs that also plays a key role in the control of pro-inflammatory responses (8). The glutathione S-transferases (GST) are ubiquitous enzymes encoded by large gene families, which are essential for the antioxidative defenses of various organs in the human body including the respiratory system (9). GSTs is known for their potency to catalyze the conjugation of reduced glutathione (GSH) to a large number of hazardous/toxic molecules, including therapeutic drugs, xenobiotics and carcinogens (10). Thus, asthma is considered as a common oxidative stress-related disease.

Two common polymorphisms of glutathione S-transferase M1 (encode by GSTM1 gene) and Glutathione S-transferase theta-1 (encode by GSTT1 gene) have been associated with asthma risk (11, 12, 13). The GSTM1 locus has been identified on chromosome 1p13.3 (14), consisting of 8 exons stretching to a region of 21,244 bases, transcript length of 1,161 bps and translation length of 218 residues (Ensembl GRCh37 release 78) (15). The GSTT1 gene has been identified in chromosome 22q11.2 (16), consisting of five exons and extending to 8.64 kb ranging from 24384774 to 24376131 (NCBI 37, August 2010) (17).

The genetics distribution of GSTM1 and GSTT1 null allele frequencies vary according to geographical region and ethnic groups (12). Studies over the past two decades have provided important information on the presence of null genotypes of GST M1 and GSTT1 enzymes and their important roles in asthma pathogenesis among the various human population, such as, Turkey (18), Tunisian (11), and Egyptian (19) populations. Therefore, the objective of this research was to determine whether an association between GSTM1 and GSTT1 deletion and asthma

pathogenesis exists in samples from Tikrit population, Iraq.

## MATERIALS AND METHODS

### The reason for choosing GSTM1 and GSTT1 in the current study

Among all the enzymes of GST family, two main deletion polymorphisms in theta1 and Mu1 (GSTT1 and GSTM1) have revealed clinical importance. Deletions loci results in the loss of enzyme, particularly in subject with null genotypes. Both the genes have been frequently reported as absent (null) in most of the pathologies, including asthma.

### Study population and sample collection

A total of 90 Tikriti people not related to each other were included in the current research. The asthmatic group included 70 patients and the healthy control group included 20 persons. The study design also included a structured interview and questionnaire for recording data. An ethical review of this study was approved by the Faculty of Science, University of Tikrit, Iraq. Approximately 3 mL of whole venous blood was collected and input into the EDTA tube for genetic analysis.

### DNA extraction and genotyping of GSTM1 and GSTT1

Genomic DNA was extracted from 200 µl of peripheral blood samples using the protocol described previously (20), and extracted DNA stored at -20 °C to -80 °C until use. DNA integrity was evaluated by agarose gel electrophoresis (1%). DNA concentration and purity were measured in a Nanodrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). GSTM1 and GSTT1 locus using the polymerase chain reaction (PCR) protocol to detect null alleles variants, as described previously (21). The specific sequence of primer that targeted GSTM1 is as follows forward primer: 5'-GAACTCCCTGAAAAGCTAAAGC-3; reverse primer; 5'-GTTGGGCTCAAATATA CGGTGG-3' for the amplified specific fragment of 215 bp fragment. The primers used in amplification of the GSTT1 (480 bp) fragment was forward primer: 5-TTCCTTACTGGTCCTCACATCTC-3; reverse primer 5'-TCACCGGATCATGGCCAGCA-3'. Also, CYP1A1 forward primer: 5'-GAACTGCCACTTCAGCTGTCT-3' and reverse primer 5'-CAGCTGCATTTGGAAGTGCTC-3' were used to produce an internal fragment (312-bp) of the gene. The PCR amplification program included an initial denaturation for 4 minutes at 95 °C, then 35 cycles of 1 min at 94 °C, 1 min at 58 °C, 1 min at 72 °C, and the final extension step was then done at 72 °C for 10 mins. The products of PCR (DNA fragments) were separated by electrophoresis on 2% agarose gels, stained with Red safe (iNtRON Biotechnology USA) allows visualizing fragments of DNA by ultraviolet light for the analyses of genotypes.

### Statistical analysis

Statistical analyses were performed using SPSS version 20 computer software. Comparison between asthmatic patient and healthy subject were performed using student's t-test with P-values <0.05 as the significance for the patients and healthy group.

## RESULTS

GSTs play an important role in protecting the cell against oxidative stress (os) and toxic elements. Thus, oxidative stress plays a critical role in asthmatic pathogenesis (22). We genotyped 90 unrelated individuals' adults, 70 of whom were asthmatic patients, and 20 unrelated healthy controls.

Fig.1 shows the result of PCR products after agarose gel electrophoresis. The PCR amplified fragments are 215 bp for GSTM1, 480 bp for GSTT1 and 312 bp for CYP1A1. All DNA bands in each sample were sufficiently clear to obtain a reliable genotype

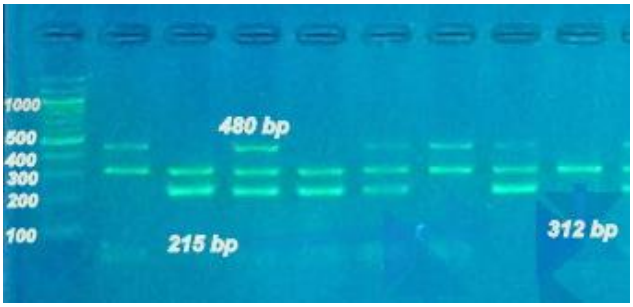


**Fig. 1:** The results obtained from PCR product of GSTM1 and GSTT1 locus

As seen from Fig. 1 the third, fifth, and sixth lanes, respectively, were electrophoretic patterns of PCR-amplified GSTM1 and GSTT1 genes. Reference gene band (312 bp) was identified in all samples.

A 215 bp band illustrating DNA samples with the GSTM1 (+) genotype was observed in the second, third, fourth, fifth and seventh lanes, while the first, sixth and eighth lanes, implies the GSTM1 (−) genotype. The first, third, fifth, sixth and seventh lanes indicated the presence of the 480 bp bands in samples with GSTT1 (+) genotype, while the second, fourth and eighth lanes, representing the null GSTT1 (−) genotype. Table 1 and Figure 2 illustrates the distribution of the observed allele and genotype frequencies for GSTM1 and GSTT1. Among the 90 individual volunteers.

**Table 1:** Allele and genotype frequencies for GSTM1 and GSTT1

| Alleles / Genotypes | Patients (%) n=90 | | Controls (%) n=20 | | P-value |
|---|---|---|---|---|---|
| | Observed No. | Expected No. | Observed No. | Expected No. | |
| GSTM1(+) * | 10 (%14.285) | 10.9 | 4 (20%) | 3.1 | *0.012 |
| GSTM1(-) * | 60 (%85.714) | | 16 (80%) | | |
| GSTT1(+) | 19 (27.142%) | 14.8 | 6 (30%) | 6.2 | |
| GSTT1(-) | 51 (72.857%) | | 14 (70%) | | |
| GSTM1, GSTT1 (+) | 17 (24.285%) | 18.7 | 8 (40%) | 8.2 | |
| GSTM1, GSTT1 (-) | 24 (34.285%) | 25.7 | 2 (10%) | 2.5 | |

*Significant differences less than the probability level P≤0.05

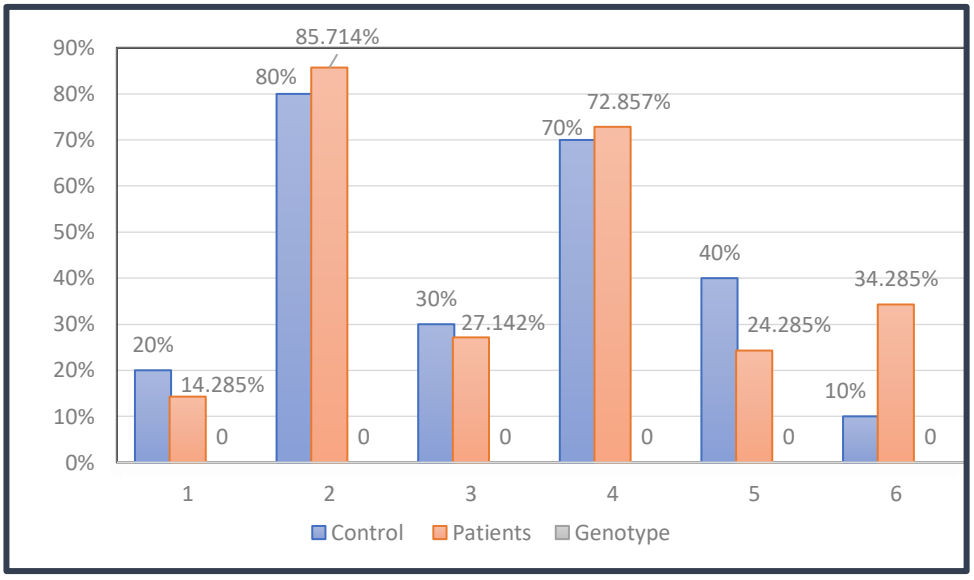


**Fig. 2**: The percentage of healthy (control group) and patient individuals based on their alleles and genotype frequencies (Blue color = control, orange color = patients, 1. GSTM1(+), 2. GSTM1(-), 3. GSTT1(+), 4. GSTT1(-), 5. GSTT1, GSTM1 (+), 6. GSTT1, GSTM1 (-)

The allelic frequencies of the GSTM1 (+) was 14.285% in the asthmatic patients and 20% in the healthy group. Whereas the allele frequencies of the GSTM1 (-) alleles were 85.714% in the asthmatic patients and 80% in the healthy group. Allelic frequencies of the GSTT1(+) were more frequent in the control groups than in asthmatic patients (30% vs 27.142%). While GSTT1(-) being the common genotype among asthmatic patients with accounting for 72.857%.

Heterozygous, GSTM1, GSTT1 (+) genotype was identified in 24.285% of cases (17 subjects) and 40% of the control healthy group (8 subjects), the GSTM1, GSTT1 (-) genotype was existent in 24 cases (34.285%) and in only 2 of the healthy group (10%). The presence of the GSTT1, GSTM1 (-) genotype confers an increased risk of asthma Susceptibility (*0.012).

## DISCUSSION

Glutathione is an antioxidant molecule that protecting cellular constituents and tissue from the effect of free radicals. In GSTM1 and GSTT1 locus, gene polymorphisms were classified as wild-type and deletion-type polymorphisms. Human GSTM1 and GSTT1 loci are polymorphic and the null genotypes cause loss of enzyme function. Many epidemiological studies investigating the likely effects of genetic polymorphism in both GSTM1 null and/ or GSTT1 null genotypes showed the cause in the development of diseases related to oxidative stress, and asthma susceptibility (13, 19). Our research is first to determine the relationship between GSTM1 and GSTT1 gene polymorphisms and asthma in the Tikrit population.

It is interesting to note that GSTM1(-) genotype was more frequent among asthmatics compared to the

non-asthmatic individuals. GSTM1(-) genotype is caused because of a 1500 bp deletion (23). GSTM1 catalyzes the detoxification of genotoxic compounds involving hydrocarbon epoxides and oxidative stress products such as DNA hydroperoxide (24). The GSTM1(-) allele deletion and homozygous deletion for both alleles lead to absence of protein production. These findings suggest that GSTM1 null polymorphism may be linked to the risk of asthma in Tikrit people, but future studies on the current topic are therefore recommended.

GSTP1 gene has also been reiterated in the genetic epidemiology field as a factor in asthma patients' studies. Another important finding was that the absence of GSTT1 genotype was common in subjects with asthmatic disease. Roughly all members of the GST family reveal genetic polymorphism, which can result in a complete lack or diminishing in enzyme activity (25). Thus, individuals with GSTM1 null genotype had further susceptible to increased oxidative stress. Therefore our data show that GSTT1 null may be identified as a candidate gene for asthma in the Tikrit population, but also further studies, which take these variables into account, will need to be undertaken.

The homozygous genotype GSTT1, GSTM1 (-) was found in 34.285% of asthmatic patients and 10% of healthy people, these results show a significant increase between both groups (P = 0.012). While the GSTT1, GSTM1 (+) genotype was found in 24.285% of asthmatic and 40% of controls. Therefore, the results of this research indicate that combined GSTT1, GSTM1 (-) genotypes raise the susceptibility to asthma in the Tikrit population.

This also accords with our earlier observations, which showed GSTT1, GSTM1 (-) homozygous genotypes to be associated with asthma pathogenesis. This study supports evidence from previous observations (9,13), that detected the distribution of GSTT1, GSTM1 (-) genotype was different between asthmatic and non-asthmatic individuals, thus maybe playing a vital role in the susceptibility of asthma. On the other hand, these results differ from (24), which demonstrated the homozygous GSTM1 (-) genotype to be a low-penetrance susceptibility genetics factor for bronchial asthma and an determinant for the early onset of the disease. Also, our results are contrary to a study from Rome, Central Italy who observed a negative association between asthmatic patients and GST polymorphisms (12). These differences can be explained as pathogenesis of asthma is complex with multiple interacting genes. Most researchers are of the opinion that the pathogenesis of asthma is a type caused by multiple interacting genes, which is the result of interaction among numerous genes and environmental influences factors (25). Recently, one interesting finding showed the gene network of asthma pathogenesis involving 755 genes/proteins and 62,603 interactions (26).

## CONCLUSION

The most obvious finding emerging from this study is that GSTT1, GSTM1 (-) genotypes are significantly associated with the risk of asthma in samples from the Tikrit population. Although, no association was shown for GSTM1 (-) or GSTT1 (-) alone with adult asthma. The findings of this investigation complement those of earlier studies in other human population. A further study could assess the effects of other genes related to asthmatic pathogenesis.

## CONFLICT OF INTEREST

Authors declare that there is no conflict of interest for this study.

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