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ORIGINAL ARTICLE

The association between glutathione S-transferase P1 polymorphisms and asthma in Egyptians

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KEYWORDS

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Abstract *Background:* Asthma is an inflammatory airways disease caused by an interaction between susceptibility genes and a diverse group of environmental factors. The GSTP1 Ile105Val polymorphism has been associated with asthma in several studies.
Objective: To examine the hypothesis that polymorphism in the GSTP1 locus is associated with asthma and related phenotypes in a population of subjects stratified by airway obstruction as well as atopic status (IgE level and history).
Methods: Fifty patients with bronchial asthma and fifty normal control subjects were enrolled in this study and were subjected to asthma questionnaire, spirometric studies, conventional polymerase chain reaction (PCR) with enzyme digestion to determine GSTP1 genotype, serum immunoglobulin E (IgE) measurement and eosinophilic count.

Abbreviations: FEV1, forced expiratory volume at the end of the first second; FVC, forced vital capacity; SVC, slow vital capacity; IgE, immunoglobulin E.

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Results: The genotype distributions for GSTP1 gene polymorphism in bronchial asthma subjects and controls showed no significant difference. Both patients and controls were found to be in Hardy-Weinberg equilibrium. Presence of the Val/Val genotype did not confer a decreased risk for developing bronchial asthma (odd ratio (OR) = 0.34, 95% confidence interval (CI) = 0.06–1.91, $P = 0.0331$). There was no significant relationship between GSTP1 genotypes and the severity of asthma. In addition, the frequency of GSTP1 genotypes distribution achieved no significance when assessed according to degree of airway obstruction or control of asthma. No associations between the GSTP1 genotypes and atopic status or IgE level were identified.

Conclusion: The present study does not support a substantial role of GSTP1 gene polymorphism in the development of asthma. However, large studies with accurate measurement of the environmental exposure are needed in order to reach adequate power to detect gene-environment interactions and other genes involved in the antioxidant pathway.

1. Introduction

Bronchial asthma is a complex chronic inflammatory disorder of the airways characterized by reversible airflow obstruction, airway hyperresponsiveness, and activation of inflammatory cells and mediators in the airways. It is a complex multifactorial disease with airway oxidative stress being a cardinal feature and an important pathway in asthma pathogenesis.[1–3] Advances in asthma management are likely to depend on a better understanding of how genetic factors influence susceptibility to, outcome in[3,4] and pharmacogenomics[5] of this disease.

Although asthma is polygenic, present knowledge suggests that candidate genes include those that determine the key clinical phenotypes of bronchial hyperresponsiveness and atopy. Bronchial hyperresponsiveness could be considered as an exaggerated response to bronchoconstrictor stimuli. It reflects the presence of bronchial inflammation and usually its severity parallels that of asthma symptoms, although some individuals with bronchial hyperresponsiveness remain asymptomatic.[6]

While host atopy is a recognized risk factor for airway inflammation, atopy alone cannot cause asthma.[6] Indeed, although 30–50% of the population is atopic, only 5–7% will develop asthma.[1]

The presence of inflammation in the airway is an important biochemical feature of asthma. Oxidative stress, with the formation of reactive oxygen species (ROS), is a key component of inflammation.[1,7] 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.[7] Inability to detoxify reactive oxygen species should perpetuate the inflammatory process, activate bronchoconstrictor mechanisms, and precipitate asthma symptoms.

Glutathione-S-transferase (GST) enzymes, which play an important role in antioxidant defenses, may therefore influence asthma risk. GST are recognized as a supergene family of enzymes critical in cell protection from the toxic products of reactive oxygen species (ROS)-mediated reactions, and modulation of eicosanoid synthesis.[6]

Many candidate genes are implicated in the pathogenesis of bronchial asthma.[8–10] Glutathione S-transferase (GST) is such a gene due to its role in protection against oxidative stress.[11] The GST enzyme (E.C.2.5.1.18) superfamily consists of alpha, kappa, mu, omega, pi, sigma, theta, and zeta isoforms in humans;[11] enzymes encoded by members of the mu, theta, and pi class GST gene families are critical in the protection of cells from reactive oxygen species.[7,12] GSTP1 – the predominant GST expressed in human lung – is a candidate because it catalyzes the detoxification of byproducts of lipid and DNA oxidation.[13,14] Genetic polymorphisms in GSTP1 have been implicated as risk factors for asthma. Four alleles of this gene have been identified: GSTP1*A (Ile105-Ala114), GSTP1*B (Val105-Ala114), GSTP1*C (Val105-Val114), and GSTP1*D (Ile105-Val114).[14,15]

A common polymorphism results in a substitution of valine (Val) for isoleucine (Ile) at codon 105, which forms part of the active site for binding of hydrophobic electrophiles, and affects substrate-specific catalytic activity. Homozygotes for the Val allele have been shown to be at reduced risk of asthma in some studies.[16]

In this context our aim was to study the genetic polymorphisms in GSTP1 in Egyptian patients suffering from bronchial asthma and comparing them with control subjects. We have examined the hypothesis that polymorphism in the GSTP1 locus is associated with asthma and related phenotypes. We have determined the prevalence of these genotypes in a population of subjects stratified by atopic status (IgE level and history) as well as by airway obstruction.

2. Methods

This case-control study included fifty patients with bronchial asthma and fifty normal subjects as control for the genotyping. All subjects were lifelong nonsmokers and had not suffered a viral infection within at least the 6 weeks preceding the study. The study protocol was approved by the local ethics committee, and all subjects provided written informed consent.

All Patients with bronchial asthma were selected according to GINA guidelines.[17] The questionnaire, used in the present study, was derived from the Asthma Therapy Assessment Questionnaire (ATAQ),[18] asthma-related quality-of-life score,[19] and the Asthma Control Questionnaire (ACQ).[20]

2.1. Spirometric study

Spirometric measurements were made by using Chestgraph HI-701 (version 1).

The ATS/ERS standards[21,22] were followed for spirometric measurements and reversibility test. The standards for choosing FEV1, VC, FEV1/VC and reversibility are:

(1) The best FEV1 and VC, not necessarily from the same tracing.
(2) FEV1 is referred to VC rather than just FVC. Ratio of FEV1 to VC is capable of accurately identifying more obstructive patterns than its ratio to FVC, because FVC is more dependent on flow.

The patients were either asthmatic and atopic or asthmatic but nonatopic. Individuals were diagnosed as nonatopic on the basis of having no past history of allergic symptoms and normal IgE levels. Atopic individuals were defined by: (1) a personal history of allergies, seasonal rhinitis, eczema, or allergic conjunctivitis and (2) total serum IgE level > 100 IU/ml. Patients were diagnosed as having asthma by: (1) a history of wheezing, cough, dyspnea, and/or chest tightness; (2) spirometric demonstration of airflow obstruction reversible with a β-agonist bronchodilator.[21] Patients were classified into mild, moderate and severe, and controlled and uncontrolled according to GINA guidelines.[17] Subjects with any other past or current disorder, respiratory or nonrespiratory, were excluded.

2.2. Genotyping for Ile105Val GSTP1 polymorphism[2]

Genomic DNA was extracted from blood lymphocytes using Wizard Genomic DNA purification kit (Promega) by alcohol precipitation method and following instructions of the manufacturer.

The GSTP1 c.313A → G resulting in Ile105Val polymorphism was determined using PCR based restriction fragment length polymorphism described by Harries et al.[23] The oligonucleotides used to amplify the target DNA of a 176 bp fragment are as follows F: ACCCCAGGGCTCTATGGGAA and R: TGAGGGCACAAGAAGCCCCT. Genomic DNA (100 ng) was used as a DNA template in a total of 50 µl reaction containing 1× PCR master mix of master mix GoTaq® Master Mix (Promega, Inc, Madison, USA) and 25 picomoles of each primer. The reaction condition was as follows: initial denaturation step at 97 °C for 5 min followed by 35 repetitive cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 60 s and extension at 72 °C for 60 s. Final extension at 72 °C for 5 min was also included. The PCR products were digested in 25 µl for 1 h at 37 °C with 2U Alw261 (Fermentas, Life Science, EU). The digested products were then resolved in 3% agarose gel stained with ethidium bromide. The presence of a-176 pb fragment indicated the wild-type genotype (Ile/Ile), whereas the 85 and 91 pb fragments indicated the homozygous polymorphic genotype (Val/Val). Heterozygote was recorded in presence of all three fragments as shown in Fig 1. Negative and positive controls were included in all reactions.

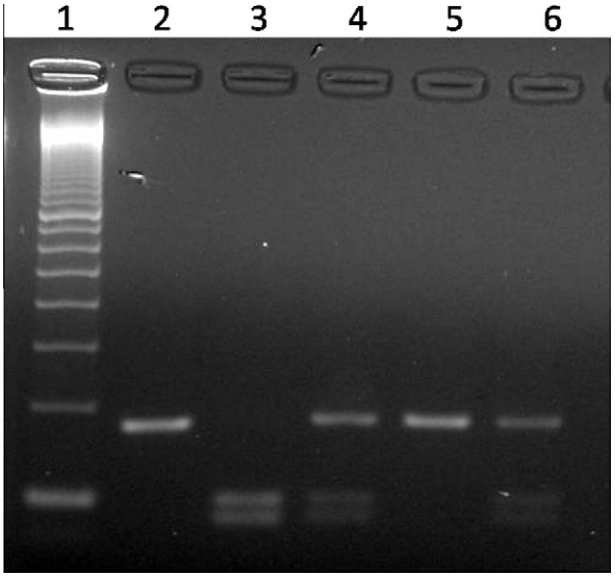

Figure 1 Captured gel image of GSTP1genotype polymorphism using Aw261 RFLP. The PCR and digested products were resolved in 3% agaorse gel stained with ethidium bromide and visualized using UV transilluminator. Lane 1: DNA marker, Lane 2: Undigested PCR product (176 bp), Lane 3: Val/Val genotype (homozygous digestion by Aw261 resulting in 85 and 91 bp bands), Lanes 4 and 6: Ile/Val genotype (heterozygous digestion by Aw261 resulting in 176 bp band of wild allele and 85 and 91 bp bands of variant allele), Lane 5: (Ile/Ile) wild genotype and absence of digestion by Aw261 restriction endonuclease.

2.3. Serum IgE estimation

IgE was analyzed by nephelometery, in which polysterene particles coated with monoclonal antibodies to IGE are aggregated when mixed with sample containing human IgE. Method standardization was performed against the IFCC/PCR/CAP reference preparation. The samples were automatically diluted 1:20 to obtain a measuring range from 25.0 to 975.8 IU/mL. Reference interval in adults is < 100 IU/mL.[24]

2.4. Eosinophilic count

Total and absolute eosinophil count were estimated using automated count on Sysmex counter and Romanowsky stain.[25]

2.5. Statistical analysis

The raw data were coded and transformed into coding sheets. The results were checked. Then, the data were entered into SPSS system files (SPSS package version 18) using personal computer. Output drafts were checked against the revised coded data for typing and spelling mistakes. Finally, analysis and interpretation of data were conducted.

The following statistical measures were used:

- Descriptive statistics including frequency, distribution, mean, and standard deviation were used to describe different characteristics.
- Kolmogorov–Smirnov test was used to examine the normality of data distribution.
- Univariate analyses including: *t*-test, ANOVA test, and Kruskal Wallis test were used to test the significance of results of quantitative variables. Moreover, Pearson Chi-Square test were used to test for significance among qualitative variables.
- Genotypes frequency of GSTP1 gene polymorphism in bronchial asthma subjects were tested for being expressed in Hardy-Weinberg equilibrium using Chi-Square test.
- Odds ratio and 95% confidence interval were calculated to estimate risk caused by GSTP1 gene polymorphism and different studied parameters among cases with bronchial ashma.
- The significance of the results was at the 5% level of significance.

Table 1 The personal characteristics of the studied cases and controls.

Personal characteristics	Cases (n = 50)	Controls (n = 10)	Significance (P)
Sex			
Males	28%	50%	0.172
Females	72%	50%	
Age (years)			
Range	18–63	19–63	0.573
Mean ± SD	38.4 ± 12	40.8 ± 14.4	
BMI (kg/m^2)			
Range	17.0–50.2	17.9–37.9	0.770
Mean ± SD	28.8 ± 6.7	29.5 ± 6.6	

X^2: Pearson chi-square test.

Table 2 The laboratory and pulmonary function results among studied cases and controls.

Laboratory/pulmonary function results	Cases (n = 50)	Controls (n = 10)	Significance (P)
WBC count/ml (×1000)			
Min–Max	4.5–9.7	4.7–8.4	t = 0.46 (0.65)
Mean ± SD	6.8 ± 1.4	6.6 ± 1.3	
Esinophils count/ml			
Min–Max	135–840	130–694	t = 0.234 (0.023)*
Mean ± SD	448.2 ± 170.5	311.5 ± 159.9	
Esinophils%			
Min–Max	3–10	2–9	t = 2.61 (0.012)*
Mean ± SD	6.5 ± 1.9	4.7 ± 2.0	
FEV1 (L)			
Min–Max	0.6–4.4	2.1–4.7	t = 2.18 (0.033)*
Mean ± SD	2.8 ± 0.9	2.9 ± 0.9	
FEV1%			
Min–Max	27.6–116.9	88.8–102.9	t = 7.024 (<0.0001)*
Mean ± SD	71.5 ± 20.4	93.5 ± 3.9	
FVC (L)			
Min–Max	1.4–5.7	2.6–5.6	t = 0.94 (0.351)
Mean ± SD	3.5 ± 1.1	3.9 ± 1.0	
SVC (L)			
Min–Max	1.4–5.7	2.6–5.6	t = 0.842 (0.403)
Mean ± SD	3.6 ± 1.1	3.9 ± 1.0	
FEV1/SVC			
Min–Max	37.3–81.4	71.1–83.7	t = 6.538 (<0.0001)*
Mean ± SD	61.5 ± 14.2	77.6 ± 4.4	

* Significant at P = 0.05.

Table 3 Distribution of GSTP1 Genotypes among patients with bronchial asthma and control subjects.

GSTP1 genotypes	Cases (n = 50)	Control (n = 50)	P	Odds ratio (95%CI)
Ile/Ile	22 (44.0%)	15 (30.0%)	X^2 = 2.62 (0.269)	
Val/Val	3 (6.0%)	6 (12.0%)		0.34 (0.06–1.91)
Ile/Va	25 (50.0%)	29 (58.0%)		0.59 (0.23–1.49)

ORs were calculated against Ile/Ile.

3. Results

The personal characteristics of the studied cases and controls are summarized in Table 1. The cases and controls were matched as regards age, sex and body mass index (BMI).

Table 2 shows the laboratory and pulmonary function results among studied cases and controls.

Eosinophilic count and percent, FEV1, FEV1% predicted, and FEV1/SVC showed a statistically significant difference between cases and controls whereas FVC and SVC

Table 4 Distribution of GSTP1 Allele frequencies among patients with bronchial asthma and control subjects.

	No.	GSTP1 genotypes			Allele frequencies	
		Ile/Ile	Val/Val	Ile/Val	Ile	Val
Cases	50	22 (44.0%)	3 (6.0%)	25 (50.0%)	0.69	0.31
Controls	50	15 (30.0%)	6 (12.0%)	29 (58.0%)	0.59	0.41

$X^2 = 2.17$, $P = 0.141$.

did not show a significant difference. We stratified the fifty patients with bronchial asthma into ordered categories as regards airway dysfunction. First, patients with mild asthma (17 patients = 34%) to be compared with those with moderate and severe asthma (33 patients = 66%) (Table 6). Second, those having FEV1 $\geqslant$ 80% predicted (16 patients = 32%) and those with FEV1 < 80% predicted (34 patients = 68%) (Table 7). Third, as regards control of asthma, we classified the patients into patients with controlled (10 patients = 20%), partially controlled (5 patients = 10%) and uncontrolled (35 patients = 70%) asthma (Table 8).

Table 5 Laboratory and pulmonary function results of the studied cases according to GSTP1 genotypes.

Laboratory/pulmonary function results	GSTP1 genotype			Significance (P)
	Ile/Ile (n = 22)	Val/Val (n = 3)	Ile/Val (n = 25)	
WBCs count/ml (×1000)				
Min–Max	4.5–9.65	4.8–6.7	4.6–9.3	$^{K}X^2 = 5.75$ (0.056)
Mean ± SD	7.4 ± 1.5	5.9 ± 0.9	6.5 ± 1.4	
Esinophils count/ml				
Min–Max	135–840	283–643	139–749	$^{K}X^2 = 2.199$ (0.333)
Mean ± SD	482.4 ± 170.9	514.7 ± 201.0	410.0 ± 165.2	
Esinophils%				
Min–Max	3–9	6–10	3–9	$^{K}X^2 = 3.26$ (0.196)
Mean ± SD	6.5 ± 1.8	8.7 ± 2.3	6.2 ± 1.9	
Ig E (IU/ml)				
Min–Max	16.4–3070	262–375	16.4–4250	$^{K}X^2 = 0.187$ (0.911)
Mean ± SD	672.1 ± 896.3	329.7 ± 59.7	848.2 ± 1279.2	
FEV1 (L)				
Min–Max	0.92–4.43	1.4–2.3	0.62–4.12	$^{K}X^2 = 0.934$ (0.627)
Mean ± SD	2.4 ± 1.0	1.9 ± 0.5	2.2 ± 0.9	
FEV1%				
Min–Max	43.3–115.1	45.9–75.7	27.6–116.9	$^{K}X^2 = 2.03$ (0.362)
Mean ± SD	76.2 ± 22.3	62.1 ± 15.1	68.4 ± 18.8	
FVC (L)				
Min–Max	1.8–5.7	3.0–3.5	1.4–5.4	$^{K}X^2 = 1.209$ (0.546)
Mean ± SD	3.7 ± 1.3	3.3 ± 0.3	3.4 ± 1.0	
SVC (L)				
Min–Max	1.88–5.71	3.0–3.6	1.4–5.4	$^{K}X^2 = 0.904$ (0.636)
Mean ± SD	3.7 ± 1.3	3.3 ± 0.3	3.4 ± 1.0	
FEV1/SVC				
Min–Max	43.2–81.4	45.7–67.4	37.3–77.9	$^{K}X^2 = 0.925$ (0.630)
Mean ± SD	63.5 ± 10.9	57.1 ± 10.8	62.8 ± 12.0	
Change of FEV1 (L)				
Min–Max	0.02–0.73	0.39–0.41	0.07–0.57	$^{K}X^2 = 2.651$ (0.266)
Mean ± SD	0.32 ± 0.2	0.4 ± 0.01	0.3 ± 0.1	
Change of FEV1%				
Min–Max	0.01–34.1	17.6–28.1	0.02–36.5	$^{K}X^2 = 2.641$ (0.267)
Mean ± SD	15.6 ± 9.6	21.8 ± 5.5	14.9 ± 9.5	
Change of FVC%				
Min–Max	0.02–17.6	2.3–14.6	0–44.8	$^{K}X^2 = 0.604$ (0.739)
Mean ± SD	6.8 ± 5.8	7.3 ± 6.4	6.9 ± 9.1	

KX^2: Kruskal Wallis test.

3.1. PCR results

Using Alw261 restriction enzyme analysis for the GSTP1 polymorphism three genotypes were found: Ile/Ile, Ile/Val, and Val/Val. Samples showing a band of 176 bp band are homozygous for the Ile allele (restriction site absent), samples revealing 85 and 91 pb fragments bp bands are homozygous for the Val allele (restriction site present) and samples showing all three fragments are heterozygous (Tables 3 and 4, Fig. 1).

3.2. Genotype distributions for GSTP1 gene polymorphism in bronchial asthma subjects and controls (Table 3 and 4)

The genotype distributions for GSTP1 gene polymorphism in bronchial asthma subjects and controls showed no significant difference ($P = 0.269$) where the homozygous Ile/Ile genotype was present in 22 cases (44%) and 15 of the control group (30%),the Val/Val genotype was observed in only 3 cases (6%) and 6 of the control group (12%), while the heterozygous Ile/Val was found in 50% of cases (25 subjects) and 58% of the controls (29 subjects) (Table 3). The allele frequencies of the Val and Ile alleles in asthmatic patients (Table 4) were 0.31 and 0.69 respectively whereas in controls we reported frequencies of 0.41 and 0.59 for Val and Ile alleles respectively with no significant difference between both groups ($P = 0.141$). Both patients and controls were found to be in Hardy–Weinberg equilibrium ($P = 0.233$ and 0.159 respectively). Presence of the Val/Val genotype did not confer a decreased risk for developing bronchial asthma (OR = 0.34, 95% CI = 0.06–1.91, $P = 0.0331$). Moreover, no association was found between GSTP1 gene polymorphism and degree of asthma severity ($P = 0.269$) (Table 6).

Table 5 shows laboratory and pulmonary function tests results of the studied patients with bronchial asthma according to GSTP1 genotyping. There were no significant differences between patients with different GSTP1 genotypes as regards WBCs count, eosinophilic count, eosinophilic percentage and IgE levels. Also there were no significant differences between patients with different GSTP1 genotypes as regards variables related to the degree of airway obstruction

Table 6 Association of GSTP1 genotypes with degree of severity of asthma.

GSTP1 genotypes	Cases		Control	P1	P2
	Mild asthma	Moderate/severe asthma			
Ile/Ile	10 (58.8%)	12 (36.4%)	15 (30.0%)	$X^2 = 3.23$ (0.198)	$X^2 = 2.62$ (0.269)
Val/Val	0 (0.0%)	3 (9.1%)	6 (12.0%)		
Ile/Val	7 (41.2%)	18 (54.5%)	29 (58.0%)		

P1: significance between mild and moderate/severe asthma.
P2: significance between cases and controls.

Table 7 Association of GSTP1 genotypes with degree of airway obstruction.

GSTP1 genotypes	FEV1% less than 80	FEV1% 80 or more	*P*	Odds ratio (95%CI)
Ile/Ile	12 (36.4%)	10 (58.8%)	$X^2 = 3.23$ ($P = 0.198$)	1.0
Val/Val	3 (9.1%)	0 (0.0%)		NA
Ile/Val	18 (54.5%)	7 (41.2%)		2.14 (0.55–8.62)

NA: cannot be calculated.

Table 8 Association of GSTP1 genotypes with age of onset, level of control and degree of severity of asthma.

Medical history	GSTP1 genotype					
	Ile/Ile ($n = 22$)		Val/Val ($n = 3$)		Ile/Val ($n = 25$)	
	No.	%	No.	%	No.	%
Age of onset of asthma						
Childhood	12	54.5	3	100.0	11	44.0
Adulthood	10	45.5	0	0.0	14	56.0
Control of disease						
Uncontrolled	14	63.6	3	100.0	18	72.0
Partially controlled	2	9.1	0	0.0	3	12.0
Controlled	6	27.3	0	0.0	4	16.0
Severity of asthma						
Mild	10	45.5	0	0.0	7	28.0
Moderate	4	18.1	2	66.7	11	44.0
Severe	8	36.4	1	33.3	7	28.0

Table 9 IgE level according to Genotype distributions for GSTP1 gene polymorphism in patients with bronchial asthma.

GSTP1 genotypes	Ig E		*P*	Odds ratio (95%CI)
	Less than 100	More than 100		
Ile/Ile	7 (31.8%)	15 (68.2%)	X^2 = 1.90 (0.386)	1.0
Val/Val	0 (0.0%)	3 (100.0%)		0.0 (0.0–6.6)
Ile/Val	5 (20.0%)	20 (80.0%)		0.54 (0.12–2.41)

Table 10 Atopic history of the studied patients with bronchial asthma according to GSTP1 genotyping.

Variables	GSTP1 genotype						Significance (*P*)
	Ile/Ile (*n* = 22)		Val/Val (*n* = 3)		Ile/Val (*n* = 25)		
	No.	%	No.	%	No.	%	
History of atopy							
Absent	13	59.1	1	33.3	15	60.0	X^2 = 0.88 (0.67)
Present	9	40.9	2	66.7	10	40.0	

Significance: chi-square test.

or response to bronchodilators including FEV1, FEV1% of predicted, FEV1/SVC, change of absolute FEV1 and FEV1% of predicted after administration of inhaled bronchodilator.

3.3. Association of GSTP1 genotypes with severity of airway obstruction

The frequencies of GSTP1genotypes in the studied patients with bronchial asthma achieved Hardy–Weinberg equilibrium. We stratified the fifty patients with bronchial asthma into ordered categories as regards airway dysfunction. First, patients with mild asthma (17 patients = 34%) to be compared with those with moderate and severe asthma (33 patients = 66%) (Table 6). Second, those having FEV1 $\geqslant$ 80% predicted (16 patients = 32%) and those with FEV1 < 80% predicted (34 patients = 68%) (Table 7). Third, as regards control of asthma, we classified the patients into patients with controlled (10 patients = 20%), partially controlled (5 patients = 10%) and uncontrolled (35 patients = 70%) asthma (Table 8). The data were inspected according to the stratification to determine whether GSTP1 genotypes were associated with severity of airway dysfunction. Table 6 shows the GSTP1 genotype frequencies according to the severity of asthma. Chi - square test analysis showed absence of significant relationship between GSTP1 genotypes and the severity of Asthma. In addition, the frequency of GSTP1 genotypes distribution achieved no significance when assessed according to degree of airway obstruction or control of asthma (Tables 7 and 8). The relative frequencies of the GSTP1 Ile105 and GSTP1 Val105 alleles in the groups stratified by severity of airway obstruction were not found to be significantly different.

3.4. Association of GSTP1 genotypes with atopy

As regards atopy we repeated stratification of patients: First, those with IgE < 100 (12 patients = 24% patients) and those with IgE > 100 (38 patients = 76%). Second, those with history of atopy (21 patients = 42%) and those without history of atopy (29 patients = 58%). Table 9 shows GSTP1 genotype frequencies in relation to IgE level. Frequencies for GSTP1 genotypes in subjects with IgE levels < 100 IU/ml and > 100 IU/ml were not significantly different (P = 0.44). Table 10 shows association of GSTP1 genotypes with atopic history. No associations between the GSTP1 genotypes and atopic status were identified.

4. Discussion

Asthma is a complex multifactorial disease with an obvious genetic predisposition, immunological aberration, and involvement of environmental factors. Polymorphisms of the GST genes are known risk factors for some environmentally-related diseases. Several population studies have linked genetic variation in human GSTP1 with enhanced susceptibility to asthma and the severity of symptoms.[26–28]

In 2000 Fryer et al.[14] identified associations between GSTP1 genotype and both bronchial hyperresponsiveness and atopy. Ile105/Val105 genotype was linked with a significantly reduced risk of airway reactivity/obstruction that was intermediate to that with the GSTP1 Ile105/Ile105 and GSTP1 Val105/Val105 genotypes. They suggested that GST genes are candidates for having a role in bronchial hyperresponsiveness because the enzymes they encode modulate ROS levels.[29] Fryer et al.[14] hypothesized that individual ability to detoxify ROS and their products, determined by polymorphism in genes such as those for GST, contributes to the development of bronchial hyperresponsiveness and asthma. They reported that the presence of *GSTP1Val/Val* genotype conferred a sixfold lower risk of asthma than did GSTP1Ile/Ile and that the frequency of *GSTP1Val/Val* genotype correlated negatively with severity of airway dysfunction.

In 2001 Hemmingsen et al.[13] suggested that GSTP1 (Val 105- Ala 113) and possibly GSTP1 (Val105-Val114) are protective against asthma and related phenotypes.

In 2000 Spiteri et al.[6] reported that the association between the GSTP1 genotype and severity of airway dysfunction

remained significant after correction for potential confounding factors (age, sex, skin prick tests, and IgE levels). They suggested that GSTP1 genotypes have potential implications for individual susceptibility to the damaging effects of ROS.

In 2004 Aynacioglu et al.[27] have also reported that the frequency of GSTP1 Val homozygote was significantly lower in the group of patients with asthma than in the control individuals (3.8% *versus* 12.1%, $P = .01$).

In 2006 Romieu et al.[16] found that asthmatic children with glutathione S-transferase P1 Valine/Valine genotypes appear more susceptible to developing respiratory symptoms related to ozone exposure.

In 2007 Hanene et al.[30] in their works on Tunisian children, suggested the presence of associations of *GSTM1*, T1, and P1 with childhood asthma and atopy. As for the *GSTP1*, they found significant differences between cases and control regarding the genotype frequencies of the GSTP1Ile105Val polymorphisms. They found that asthmatic children have low frequency of GSTP1Val allele compared with healthy children ($P = .002$).

In 2008 Imboden et al.[31] suggested that GSTP1 Ile105Val genotype strongly determines the progression of BHR to physician-diagnosed asthma in the general population.

In 2009 Babusikova[11] suggested that increased oxidative stress and *GST-T1* genetic polymorphism are associated with children asthma and atopy and, therefore, may contribute to the pathogenesis of asthma.

In 2010 Piacentini et al.[2] studied the gene-environment interactions in a multicentre Italian field study and reported that GSTA1, GSTM1, GSTO2 and GSTT1 genotypes found in the group of asthmatic patients seem to differ from the frequencies of those found in the control group.

In 2008 Jiansheng Zhou et al.[32] performing their work in Mouse Model of Asthma demonstrated that the ability of GSTP1 to attenuate allergic responses directly correlated with the levels of GSTP1 expression in the mouse lung. They defined GSTP1 as an important modulator of allergic airways disease.

In the present study, the homozygous genotype (Ile/Ile) was present in 44% of the patients and in 30% of the controls, the Val/Val genotype was observed in only 6% of patients and 12% of controls while the heterozygous genotype Ile/Val was found in 50% of patients and 58% of controls with no significant difference between both groups ($P = 0.331$) (Table 1).

The present study took place between 2007 and 2011. In 2010 Minelli et al.[8] performed their meta-analysis using a total of 14 published and 3 primary studies with 3363 affected and 14 442 non-affected. Although these studies suggested a possible protective effect of the Val allele, but heterogeneity was extreme. Few studies of them evaluated wheezing and BHR and most reported no associations, although weak evidence was found for positive associations of GSTM1 null and GSTP1 Val allele with wheezing and a negative association of GSTP1 Val allele with BHR. Minelli et al.[8] findings did not support a substantial role of GST genes alone in the development of asthma.

Mak et al.[33] investigated the association of GST gene polymorphisms and its enzyme activity with the risk of asthma in Hong Kong Chinese adults. They reported that the distribution of various genotypes or alleles of the GSTT1, GSTM1 and GSTP1 was not significantly different between patients with asthma and healthy controls.

Presence of the Val/Val genotype in our study did not confer a decreased risk for developing bronchial asthma (OR = 0.34, 95% CI = 0.06–1.91, $P = 0.0331$); also, no association was found between GSTP1 gene polymorphism and degree of asthma severity. Minelli et al.[8] also reported a non significant risk in his Meta-analyses on GSTP1 Ile105Val polymorphism in a total of 14 published and 3 primary studies with 3363 affected and 14 442 non-affected individuals, where OR equaled 0.79 (CI = 0.57–1.08) for Val/Val vs Ile/Ile.[8]

Several studies were not in agreement with our results suggesting that subjects with the GSTP1 Val/Val genotype have reduced risk of asthma compared to Ile/Ile subjects.[6,16,34–36] Romieu et al.[16] also stated that children with GSTP1 Ile/Ile genotype were more likely to have moderate-to-severe asthma ($P = 0.009$) than children with GSTP1 Ile/Val or Val/Val genotypes.

Many factors could account for the observed discrepancies among studies with the racial and environmental differences among the populations being highly significant contributors.

Piacentini et al.[2] analyzed the possible association between polymorphism in several cytosolic GST genes including GSTP1, air pollution and asthma development. Among all the polymorphisms studied, the frequencies of GSTA1, GSTM1, GSTO2 and GSTT1 genotypes but not GSTP1 genotypes found in the group of asthmatic patients seem to differ from the frequencies of those found in the control group. They concluded that the final result of their research should hopefully lead to a better understanding of gene-environment interactions, so allowing earlier prediction and diagnosis of asthma disease and providing an efficient means of prevention.

In the present study both patients and controls were found to be in Hardy Weinberg equilibrium ($P = 0.233$ and 0.159 respectively). This was in agreement with Fryer et al.[14] who reported that allele frequencies achieved Hardy–Weinberg equilibrium. The Hardy–Weinberg equilibrium states that both allele and genotype frequencies in a population remain constant – that is, they are in equilibrium – from generation to generation unless specific disturbing influences are introduced. Those disturbing influences include non-random mating, mutations, selection, limited population size, ''overlapping generations'', random genetic drift, gene flow and meiotic drive. Genetic equilibrium is an ideal state that provides a baseline against which to measure change.[37]

4.1. Association of GST genotypes with atopy

In the present study atopy and eosinophilia were significantly associated with asthma.

Asthma and allergy represent complex phenotypes. Strong evidence for genomic factors predisposing subjects to asthma/allergy is available. However, methods to utilize this information to identify high risk groups are variable.[38]

We stratified our patients into asthmatic and atopic (42%), and asthmatic but not atopic (58%). We did not identify significant associations between GSTP1 and atopy in our study population. Also, in our study there was no significant difference in subjects carrying different GSTP1 genotypes as regards levels of IgE (whether $>$ or <100 IU/ml) $P = 0.44$. The sample size of fifty patients with asthma limited our power to detect statistically significant associations. This is not in agreement with Fryer et al.[14] who reported that subjects with IgE levels

> 100 IU/ml demonstrated significantly reduced GSTP1 Val105/Val105 frequencies as compared with those with IgE levels < 100 IU/ml.

In contrary to our findings, Tamer et al.[36] found that subjects with the GSTP1 homozygous Val/Val genotype had a 3.55 fold increased risk of having atopic asthma compared to nonatopic asthma (OR = 3.55; 95% CI, 1.10–12.56). These results suggested that the GSTT1 and GSTM1 null genotypes and the GSTP1 Val/Val polymorphism may play important roles in asthma pathogenesis. It is possible that intermediate electrophilic metabolites, arising in the first phase of detoxification, are not metabolized by GST enzymes in asthmatic patients and are not excreted. These intermediate metabolites may damage cells and generate oxidative stress, and so contribute to the pathogenesis of asthma.[36]

A variety of review papers describe genes associated with allergy/asthma.[38–41] Ober et al.[40] list genes associated with asthma or atopy in more than 10 studies. Joubert et al.[9] suggested that GSTP1's role in xenobiotic metabolism and antioxidation is consistent with asthma etiology. They suggested that variation in this gene may result in differing metabolism of environmental toxins across individuals. They speculated that individuals with multiple risk alleles across GSTP1 genes are more susceptible to harmful effects of environmental toxins, and that this sensitivity may contribute to the development of asthma or asthma exacerbation.

Spiteri et al.[6] showed that the frequency of the GSTP1 Val/Val genotype was reduced in atopic subjects compared with nonatopic control. They hypothesized that susceptibility to persistent airway inflammation in atopic individuals is characterized by an inherited deficiency in the effectiveness of detoxification of inhaled irritants and products of oxidative stress such as reactive oxygen species (ROS).

4.2. Eiosinophilic count

In the present study there were statistically significant difference between asthmatic patients and control as regards eosinophilic count and eosinophilic percentage. However there was no association between them and GSTP1.

An imbalance between the oxidative forces and the antioxidant defense systems favoring an oxidative injury has been implicated in the pathogenesis of asthma.[42] Oxidative injury leads to increased lipid peroxidation, increased airway reactivity and secretions, production of chemoattractant molecules, and increased vascular permeability,[7,43] which collectively lead to an augmentation of the existing inflammation that is a hallmark of asthma. There is ample evidence supporting the presence of a systemic oxidative injury in asthma. An increased production of reactive oxygen species was shown for eosinophils and macrophages obtained from the peripheral blood of patients with asthma.[44,45]

It was found that the increased oxidative burden in asthma was the result both of increased oxidative stress as evidenced by increased malondialdehyde; and of decreased antioxidant capacity as evidenced by the lowered reduced glutathione.[46] GSTP1 works by catalyzing the detoxification of base propenals that arise from DNA oxidation forming reduced glutathione i.e. it is responsible for the antioxidant defense system.[29] This may explain absence of correlation between eosinophilic count and GSTP1 as each of them works on the different limb of the oxidants/antioxidant system.

4.3. Association of GSTP1 with airway obstruction and severity of asthma

In the present study, as expected, there were significant differences in FEV1 values between the asthmatic and control groups. We examined the relationship between GSTP1 genotypes and ordered categories representing various methods to classify airway dysfunction. Patients with mild asthma were compared to those with moderate and severe asthma. Also we repeated stratification of patients into two groups: those having FEV1 $\geqslant$ 80% predicted and those with FEV1 < 80% predicted. Finally as regards control of the asthma, we classified the patients into patients with controlled, partially controlled and uncontrolled asthma. We did not find any association between GSTP1 genotypes and either form of degree of airway dysfunction in asthma.

Although the val105 val genotype of the GSTP1 enzyme was found to be a significant determinant of the oxidative burden in the systemic circulation,[8] Dut et al.[3] reported that it had no effect on the airways. Dut et al.[3] examined the variables that had a potential to influence the oxidative stress such as age, sex, age of onset, skin test positivity, IgE levels, eosinophil counts, smoke exposure, pet ownership, family history of atopic diseases, asthma diagnosis, asthma severity, and polymorphisms at GSTM1, GSTT1, and GSTP1 genes. They concluded that the only factor that determines the oxidative stress is the presence of the airways disease, i.e. asthma. This is consistent with our finding as regards absence of correlation between polymorphism at GSTP1 gene and airway dysfunction which is related to oxidative stress and inflammation. In this respect, it was found to be some difference between the systemic circulation and the airways. Dut et al. used exhaled breath condensate as a noninvasive method to investigate the oxidative burden in airways. They showed that in addition to the systemic level, there is a very strong oxidative burden at the local level, i.e., in the airways of asthmatic children.[3] The increased oxidative burden in the airways has two components: increased oxidative stress as evidenced by increased malondialdehyde and decreased antioxidant capacity as evidenced by the lowered glutathione.[3] Kelly et al. in a study involving 20 asthmatics and 20 controls, had found that oxidized glutathione content in bronchoalveolar lavage was higher in asthma patients than in controls whereas reduced glutathione content was similar.[47] As reduced glutathione is the factor that is affected by GSTP1, this explains absence of GSTP1 polymorphism effect on airway dysfunction. Any therapeutic approach targeting the oxidative burden in asthma should definitely take this into account because abolishing the oxidative insult in both the systemic and local compartments may be necessary to achieve a full therapeutic effect.

In agreement to our findings, Minelli et al.[8] performed a systematic review and meta-analysis including unpublished data from the large Avon Longitudinal Study of Parents and Children (ALSPAC). They reported lack of evidence of an important role of GST genes in the development of asthma, wheezing and bronchial hyperresponsiveness which is in agreement with negative findings on lung function in children for all three GST genes from the ALSPAC, although an association between GSTM1 and GSTP1 genes and lung function in childhood has been previously suggested.[48]

In their systematic review, Minelli et al.[8] found that few studies evaluated the effects of GSTP1 on wheezing and bronchial hyperresponsiveness, and the findings were in opposite

directions for the two outcomes. The Val allele was protective for bronchial hyperresponsiveness, in agreement with the results for asthma, but associated with increased risk of wheezing. These effects were suggested by two relatively small studies, but the large ALSPAC cohort did not provide convincing evidence of any association with either outcome. Moreover, the results from the ALSPAC, which represented the largest sample in the meta-analysis, were negative. On the other hand, GST genes could interact with genes coding for other detoxifying enzymes which induced in response to oxidative stress. Supporting this hypothesis is some evidence of interaction between GSTM1 null genotype and NQO1 Pro187Ser polymorphism on asthma.[34]

This systematic review[8] only focused on asthma risk, and did not consider the possible association of GST genes with asthma severity in patients affected by the disease. A finding from the Southampton study was the association of the GSTT1 null allele with an increased severity score in patients with asthma. There is evidence suggesting that GST genes, in particular GSTM1 and GSTP1, might also interact with air pollution and tobacco smoke exposures in exacerbating respiratory symptoms and decreasing lung function in asthmatic individuals.[49,50]

In contrast to our findings, Fryer et al.[14] found that compared with GSTP1 Ile105/Ile105, GSTP1 Val105/Val105 was associated with a decreasing severity of bronchial hyperresponsiveness. The association between GSTP1 Val105/Val105 and airway obstruction/reactivity remained significant after correction for atopic status, age, and gender. Compared with GSTP1 Ile105/Ile105, the heterozygote genotype GSTP1 Ile105/Val105 was linked with a significantly reduced risk of airway reactivity/obstruction that was intermediate to that with the GSTP1 Ile105/Ile105 and GSTP1 Val105/Val105 genotypes. These findings are compatible with their view that the association of GSTP1 genotypes with clinical asthma phenotypes is predominantly with bronchial hyperresponsiveness. An underlying cause for absence of association between airway dysfunction and GSTP1 genotypes in our study may be the relatively small number of patients with asthma.

Spiteri et al.[6] found that trend analysis showed a significant decrease of GSTP1 Val/Val (with parallel increase of GSTP1 Ile/Ile) genotype frequency with increasing severity of airflow obstruction/bronchial hyperresponsiveness.

5. Conclusion

The present study does not support a substantial role of GSTP1 gene polymorphism in the development of asthma. Also, it is not related to the severity of asthma, degree of airway obstruction and/or atopy. However, large studies with accurate measurement of the environmental exposure are needed in order to reach adequate power to detect gene–environment interactions and other genes involved in the antioxidant pathway.

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