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Glutathione S-Transferase (*GSTM1*, *GSTT1*) Genes Polymorphisms Associated with Vitiligo Disease in Thi Qar Province/South of Iraq

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ABSTRACT

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Vitiligo is a common skin disease caused by a deficiency in the production of melanocyte. It is a non-contagious disease that affects the general public, without distinction of race, sex. The aim of this study was to find the clinical features and evaluate the association between *GSTT1*, *GSTM1* null genotypes and the development of vitiligo in Dhi Qar province in southern Iraq. This study included 100 vitiligo patients and 90 controls (mean age 32 ± 15.2 , 31.8 ± 11.2 respectively). 80% of patients were males while rest 20% were females. Vitiligo does not show any significant association with smoking. 35% patients are associated with the positive family history. 12% cases reported physical trauma as the physical factor. 10% patients were associated with autoimmune disease. Polymerase Chain Reaction PCR was used for the analysis of the study null genotypes. We found that the *GSTM1*-null might play an important role in risk for vitiligo ($OR=1.56$; 95%CI =0.88-2.79), and the *GSTT1*-null genotype was significantly associated with the susceptibility to vitiligo ($OR=1.55$; 95 % CI= 0.79-3.06). In combined analysis *GSTT1*, *GSTM1* null genotypes showed significant associations with vitiligo susceptibility ($OR=2.70$; 95%, CI=1.03-7.08).

Introduction

Vitiligo is an acquired depigmentary disorder characterised by the appearance of white patches resulting from the loss of functional melanocytes from the skin. The prevalence of vitiligo is 0.1–2.0% in various populations worldwide irrespective of race and gender (Hann *et al.*, 2000). The etiology of vitiligo is still unknown and several

theories has been proposed, involving, autoimmune, neurogenic factors, oxidative stress (Gopal *et al.*, 2007). Accumulation of free radicals in the epidermal layer of influenced skin have been suspected to be involved in the pathophysiology of vitiligo. There is a failure of antioxidative system in vitiligo melanocytes with resultant free radical mediated destruction in melanocyte

(Hazneci *et al.*, 2005). The glutathione S-transferase (GSTs) superfamily of genes consist of the largely expressed phase II isoenzymes involved in defense against oxidative stress. They can catalyze the detoxification of reactive-oxygen species and conjugation of glutathione to endogenous and exogenous electrophile substrates, thus detoxifying a variety of electrophilic compounds generated by damage to cells caused by reactive oxygen species (Nebert *et al.*, 2004). The GSTS gene family consists of six subfamilies: Alpha (GSTA), Mu (GSTM), Omega (GSTO), Pi (GSTP), Theta (GSTT), and Zeta (GSTZ) (4). The GSTs gene polymorphisms (GSTM1 and GSTT1) were thought that they play a role in the susceptibility of several diseases such as asthma and rheumatoid arthritis (Yun *et al.*, 2005). They were suggested to have a role in susceptibility of vitiligo (Abd Rabou *et al.*, 2011; Uhm *et al.*, 2007).

Subjects and Methods

This study included 100 patients with vitiligo (80 males and 20 females). Their ages ranged from 1-72 years, attending the consultative clinic of dermatology of Al-Hussein Teaching Hospital in the province of Dhi Qar during the period from April 2012 to February 2013. The control group consist of 90 healthy subjects included 63 males and 27 females (1-65 years).

Blood Samples

About 2.5 ml venous blood samples were collected into an EDTA vacutainer tubes used for genomic DNA extraction.

DNA Extraction

Genomic DNA was extracted from peripheral blood leukocytes, using PK\SDS (Sambrook *et al.*, 1989).

DNA Amplification

DNA amplification was done by multiplex polymerase chain reaction to investigate the presence or absence of the GSTM1, GSTT1 genes in the genomic DNA, and albumin gene as an internal control. The primers of GSTM1 gene were as follow: 5\'-GAG GAA CTC CCT GAA AAG CTA AAG-3\' (forward) and 5\'- CTC AAA TAT ACG GTG GAG GTC AAG-3\' (reverse). The GSTT1 gene was amplified with the following primers: 5\'-TTC CTT ACT GGT CCT CAC ATC TC-3\' (forward) and 5\'-TCA CCG GAT CAT GGC CAG CA-3\' (reverse). The primers of albumin gene are as follows: 5\'- GCC CTC TGC TAA CAA GTC CTA C -3\' (forward) and 5\'- GCC CTA AAA AGA AAA TCG CCA ATC -3\' (reverse).

PCR was carried out in a total volume of 25 µl with 5 µl of DNA, 1 µl of each of GSTM1, GSTT1 and albumin primer, 5 µl master mix, 9 µl distal water. The amplification conditions were initial denaturation at 95°C for 5 minutes followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 58°C for 1 minute, extension at 72°C for 1 minute, and final extension at 72°C for 10 minutes.

Statistical Analysis

Statistical analysis of the this study was conducted, using the mean, standard deviation, chi-square test and odd ratio test with 95% confidence intervals (95% CI) by SPSS V.17.

Results and Discussion

Clinical Results

In present study, clinical features were demonstrated in table- 1. The mean age of

vitiligo patients and controls group was 32 ± 15.2 and 31.8 ± 11.2 respectively. On the other hand the mean onset was 20.8 ± 13.8 of patients. Out of 100 patient 80% were male while 20% female. 33 % patients were smoker while 67% were non smoker. 35% patients had family history of vitiligo while the rest 65 % had no family history. 12 % of cases development vitiligo as a results of trauma (Koebner phenomenon) and 10 % of cases are associated with autoimmune disease.

Laboratory Results

Table (2) shows the association between GSTM1 and GSTT1 deletion and vitiligo. Amplified products (GSTM1: 216 bp; GSTT1: 480 bp; Albumin; 350 bp) were then analyzed electrophoretically on an ethidium bromide-stained 2% agarose gel [Fig. 1]. In this study, the frequencies of GSTM1 null genotype and GSTT1 null genotype in vitiligo patients were significantly compared with the controls (OR= 1.56, 95% CI=0.88--2.79), (OR = 1.55, 95% CI = 0.79 – 3.06), respectively. In combination analysis with both genes, the results suggested significant association of vitiligo risk with both GSTM1 \ GSTT1 null genotypes (OR=2.70, 95% CI=1.03 – 7.08).

The mean age of vitiligo patients and controls group was 32 ± 15.2 and 31.8 ± 11.2 respectively with statistical significant between two groups (P=0.047). Vitiligo was found to be more common among the age group of 20-29 years of age. On the other hand the mean onset was 20.8 ± 13 . Further, the incidence of vitiligo was 76 % cases below 30 year of age as compared to a low incidence of 24% in individuals over 30 years of age, which is in agreement with Ali *et al.*, (2010) who found the onset of vitiligo was generally at less than

30 years in age. Also Liu *et al.*, (2010) report that 73% of their patients were under the age of 30. Likewise, Singh *et al.*, (Singh *et al.*, 1985) report that 75% of the patients in their series were between 10 and 39 years old. This finding means more and more younger people are getting affected with this disorder.

Although vitiligo affects both sexes equally (Alkhateeb *et al.*, 2003), most of the studies show a female preponderance (Akrem *et al.*, 2008). A male preponderance observed in our study. This is in agreement with Handa and Kaur, (Handa *et al.*, 1999) who found a predominance of males in their patients. The observed male preponderance can be explained by their exposure to occupational pollutants during work.

Vitiligo does not show any significant association with smoking (P=0.10). This is in agreement with Ali *et al.*, (2010) and Usha, S. and Pandey (2011) who found no statistical significant between vitiligo and smoking.

A positive family history was reported in 35 % cases in our study, which is agreement with the findings of Boisseau-Garsaud (2000) who found 30% of vitiligo patient have positive family history, which is higher than other studies that reported 17.8%, 22%, 20% (13,15,17) respectively. Otherwise our study is lower than Alzolibani (2009) who found 56.8 % of vitiligo patient have positive family history. This indicates to the role of genetic factors in the pathogenesis of vitiligo.

We observed Koebner phenomenon in 12 % of patients. This was similar to that of Handa and Dogra (11.3%). This prevalence is lower than 33.33% and 24.6% reported by Ali *et al.*, (2010) and Raju *et al.*, (2011) respectively.

Table.1 Clinical characteristics of 100 vitiligo patients and 90 healthy controls

Clinical features	Patient N=100	Controls N= 90	P. value
Average age (year, mean \pm SD)	32 \pm 15.2	31.8 \pm 11.2	
Onset age (year, mean \pm SD)	20.8 \pm 13.8	--	
Gender			
Male	80 (80%)	63 (70%)	0.11
Female	20 (20%)	27 (30%)	
Total	100 (100%)	90 (100%)	
Smoking			
Smoker	33 (33%)	20 (22.22%)	0.10
No smoker	67 (67%)	70 (77.78%)	
Total	100 (100%)	90 (100%)	
family history			
Positive family history	35 (35%)	--	--
Negative family history	65 (65%)	--	--
Total	100 (100%)		
Koebner phenomenon			
Yes	12 (12%)	--	--
No	88 (88%)	--	--
Total	100 (100%)		
Associated disease			
Diabetes mellitus	5(5%)	--	--
Thyroid	3(3%)	--	--
Rheumatoid arthritis	1(1%)	--	--
Alopecia areata	1(1%)	--	--
None	90 (90%)		
Total	100 (100%)		

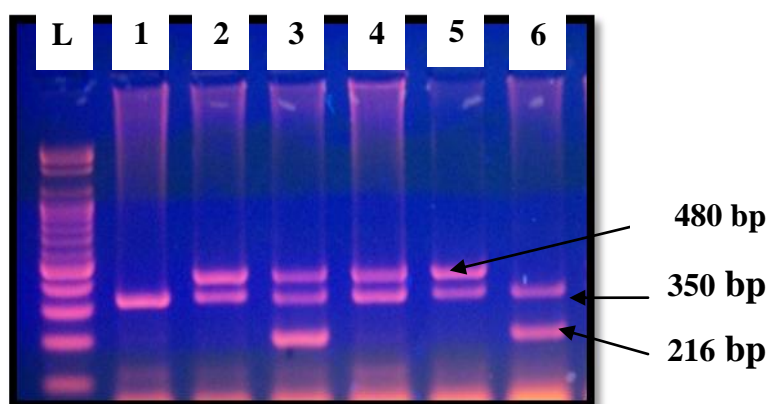
P.value < 0.05 significant*

Table.2 Frequencies of the genotypes of *GSTM1* and *GSTT1* among the patients and controls and the associations with risk of vitiligo

<i>GSTM1</i> Genotypes	Control (N=90)	Patients (N=100)	OR	CI 95%
<i>GSTM1</i> Present (+)	46 (%51.11)	40 (%40)	1.0	——
<i>GSTM1</i> Null (-)	44 (%48.49)	60 (%60)	1.56	0.88 – 2.79
Total	90 (100%)	100 (100%)		
<i>GSTT1</i> Genotypes	Control (NO=90)	Patients (NO=100)	OR	CI 95%
<i>GSTT1</i> Present (+)	72 (%80)	72 (%72)	1.0	——
<i>GSTT1</i> Null (-)	18 (%20)	28 (%28)	1.55	0.79 – 3.06
Total	90 (100%)	100 (100%)		
Combined genotypes	Control (NO=90)	Patients (NO=100)	OR	CI 95%
<i>GSTM1</i> (+), <i>GSTT1</i> (+)	36 (40%)	30 (30%)	1.0	——
<i>GSTM1</i> (-), <i>GSTT1</i> (-)	8 (8.89%)	18 (18%)	2.70	1.03 – 7.08
Others	46 (51.11)	52 (52%)		
Total	90 (100%)	100 (100%)		

95% CI, Confidence Interval; OR, Odds ratio.

Fig.1 Polymerase chain reaction products were analyzed on 2% agarose gel.



L : DNA Ladder (1500 pb); **Lane 1**: *GSTM1* & *GSTT1* null genotype; **Lane 2,4,5**: *GSTM1* null genotype; **Lane 3** : Normal (contain both genes); **Lane 6** : *GSTT1* null genotype

This may be explained as due to release of antigens of injured melanocytes into the blood and production of antibodies against them resulting in further loss of melanocytes (Ramaiah *et al.*, 1989).

In our study autoimmune diseases were seen in 10% cases. The association with diabetes mellitus was 5% followed by thyroid disease in 3% and 1% for each rheumatoid arthritis and alopecia areata patients. Huggins *et al.*, (2006) reported 1-7% diabetes mellitus, Usha and Pandey, reported 1%,2% rheumatoid arthritis and alopecia areata respectively. Arycan *et al.*, (2008) reported 4.4% thyroid disease.

In this study, we found that the GSTM1 and GSTT1 null genotype showed a significant association with vitiligo disorder. These results agreed with Liu *et al.*, (2009) who suggested that the GSTT1-null genotype and GSTM1-null genotype association with susceptibility to vitiligo. Also the current study agreed with Uhm *et al.*, and Bassiouny and Khorshied (2013) as they showed significant associations between vitiligo and GSTM1-null genotype. But our results were different from Uhm *et al.*, and Bassiouny and Khorshied (2013) as they showed a non-significant associations between the disease and GSTT1-null genotype. On other hand Abd Rabou *et al.*, and Guarner *et al.*, (2011) showed a non-significant associations between the disease and GSTT1-null genotype, and also GSTM1-null genotype, in contrast to the current study, which showed associations.

In combined analysis, both GSTM1-null and GSTT1-null genotypes showed highly significant association with vitiligo. These results agreed with the study by Liu *et al.*, and Abd Rabou *et al.*, (2009) as they showed the same significant association. Also the present results agreed with the study by Uhm *et al.*, Bassiouny and Khorshied and Guarner *et al.*, and (2009, 2013) that showed a significant

association of the disease with GSTM1 null/GSTT1 null type.

The GSTM1 and GSTT1 null genotype leading to impairment in the antioxidant system in vitiligo, this is in turn leading to excess free radical which causes destruction of melanocytes or dysregulation of melanogenesis and activates an autoimmune response (2011).

In conclusion, vitiligo is depigmenting disorder resulting from the loss of melanocyte in the skin. The pathogenesis of vitiligo is proposed to be associated with many factors as environmental and genetic factors. Vitiligo was found to be more common in the adulthood. Vitiligo was found to be associated trauma, autoimmune disease. The GSTM1 and GSTT1 null genotype play an important role in the pathogenesis of vitiligo. Null genotype of both genes increase a risk of the disease, because of the deficiency of the antioxidant system caused by the lack of GSTM1 and GSTT1 cannot be easily recovered.

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