Original Research Article

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

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Abstract

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).

Keywords
Vitiligo, polymorphism, glutathione S-transferase, Polymerase Chain Reaction.

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
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 PKSDS (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 CCA 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...
vitriligo 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% Cl = 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% Cl=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 onest 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 is indicates to the role of genetic factors in the pathogenesis of vitiligo.

We observed Kobner 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 ± SD)            | 32±15.2      | 31.8±11.2    |          |
| Onset age (year, mean±SD)                | 20.8 ± 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

| GSTM1 Genotypes | Control (N=90) | Patients (N=100) | OR | CI 95%     |
|-----------------|----------------|------------------|----|-----------|
| GSTM1 Present (+) | 46 (%51.11) | 40 (%40) | 1.0 | —         |
| GSTM1 Null (-) | 44 (%48.49) | 60 (%60) | 1.56 | 0.88 – 2.79 |
| Total | 90 (100%) | 100 (100%) | — | —         |

| GSTT1 Genotypes | Control (NO=90) | Patients (NO=100) | OR | CI 95%     |
|-----------------|-----------------|------------------|----|-----------|
| GSTT1 Present (+) | 72 (%80) | 72 (%72) | 1.0 | —         |
| GSTT1 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%     |
|-------------------|-----------------|------------------|----|-----------|
| GSTM1(+) , GSTT1(+) | 36 (40%) | 30 (30%) | 1.0 | —         |
| GSTM1(-) , GSTT1(-) | 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.

References

Abd Rabou, F., Elserogy, H., Gheida, S., EL-Ashmawy, A. 2011. Glutathione S-Transferase Gene Polymorphisms (GSTM1 and GSTT1) in Vitiligo Patients. Life Sci. J., 8(4): 785-792.

Akrem, J., Baroudi, A., Aichi, T., Houch, F. and Hamdaoui, M.H. 2008. Profile of vitiligo in the south of Tunisia. Int. J. Dermatol., 47: 670-674.

Ali, R., Shasul Ahsan, M., Azad, M., Ashik Ullah, M.D., Bari, W., et al. 2010. Immunoglobuline levels of vitiligo patients. Pak. J. Pharm. Sci., Vol.23, No.1, pp.97-102.

Alkhateeb, A., Fain, P.R., Thody, A., Bennett, D.C. and Spritz, R.A. 2003. Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their
relatives. Pigment. Cell. Res., 16: 208-214.
Alzolibani, A. 2009. Genetic epidemiology and heritability of vitiligo in the Qassim region of Saudi Arabia. Acta Dermatovenerol Alp Pannonica Adriat. 18(3): 119-125.
Arycan, O., Koç, K., Ersoy, L. 2008. Clinical characteristics in 113 Turkish vitiligo patients. Acta Dermatoven, APA Vol 17, No 3. 129-132.
Bassiouny, D.A. and Khorsheed, M.M. 2013. Glutathione S-transferase M1 and T1 genetic polymorphisms in Egypt with nonsegmental vitiligo. Dermatol., 38(2): 160-163.
Boisseau-Garsaud, A., Garsaud, P., Cales-Quist, D., Helenon, R., Queneherve, C., Clair, R. 2000. Epidemiology of vitiligo in the French West Indies (Isle of Martinique). Int. J. Dermatol., 39(1): 18-20.
Gopal, K.V., Rama Rao, G.R., Kumar, Y.H., Appa Rao, M.V., Vasudev, P., Srikant. 2007. Vitiligo: a part of a systemic autoimmune process. Indian J. Dermatol. Venereol. Leprol., 73: 162e5.
Guarner, F., Asmundo, A., Sapienza, D., Cannavo, S. 2011. Glutathione S-transferase M1/T1 gene polymorphisms and vitiligo in a Mediterranean population. Pigment Cell Res., 24: 731-733.
Handa, S. and Kaur. I. 1999. Vitiligo: clinical findings in 1436 patients. J. Dermatol., 26: 653-657.
Handa, S. and Dogra, S. 2003. Epidemiology of childhood vitiligo: a study of 625 patients from North India. Pediatr. Dermatol., 20: 207-221.
Hann, S.K., Nordlund, J. 2000. Vitiligo. Blackwell Science, Oxford, UK.
Hazneci, E., Karabulut, A.B., Ozturk, C., et al. 2005. A comparative study of superoxide dismutase, catalase, and glutathione peroxidase activities and nitrate level in vitiligo patients. Int J Dermatol., 44: 636-40.
Huggins, R.H., Janusz, C.A., Schwartz, R.A. 2006. Vitiligo: A sign of systemic disease. Indian J. Dermatol. Venereol. Leprol., 72: 68-71.
Liu, J.B., Li, M., Yang, S., Gui, J.P., Wang, H.Y., Du, W.H., Zhao, X.Y., Ren, Y.Q., Zhu, Y.G., Zhang, X.J. 2005. Clinical profiles of vitiligo in China: an analysis of 3742 patients. Clin. Exp. Dermatol., 30: 327–31.
Liu, L., Li, C., Gao, J., Li, K., Gao, L., Gao, T. 2009. Genetic polymorphisms of glutathione S-transferase and risk of vitiligo in the Chinase population. J. Invest Dermatol., 129: 2646-2652.
Nebert, D.W., Vasiliou, V. 2004. Analysis of the glutathione S-transferase (GST) gene family. Hum. Genomics, 1: 460–4.
Raju, B.P., Sundar, P.K., Nagaraju, U., Bhat, V., Raveendr, L., KeshavaLu, L. 2011. Characteristics of childhood vitiligo in Bangalore with special reference to associated ocular abnormalities. J. Indian Soc. Tele dermatol., 4: 1–10.
Ramaiah, A., Puri, N. and Mojamdar, M. 1989. Etiology of vitilgo. A new hypothesis. Acta. Dermatol. Venereol., 69: 323-326.
Sambrook, K.J., Fritsh, E.F. and Maniatis, T. 1989. Molecular cloning laboratory manual, 2nd ed., Cold Spring Harbor Llaboratory Press. U.S.A.
Shankar, D.S., Shashikala, K., Madala, R. 2012. Clinical patterns of vitiligo and its associated co morbidities: A prospective controlled cross-sectional study in South India. Indian Dermatol. Online, 3(2): 114–118.
Singh, M., Singh, G., Kanwar, A.J., Belhaj, M.S. 1985. Clinical pattern of vitiligo in Libya. Int. J. Dermatol., 24: 233–5.
Singh, S., Singh, U., Pandey, S.S. 2011.
Study of total antioxidants status in Indian vitiligo patients. *Egyptian Dermatol. Online J.*, Vol. 7 No 1: 2., 1-7.

Uhm, Y.K., Yoon, S.H., Kang, I.J., *et al.* 2007. Association of glutathione S-transferase gene polymorphisms (GSTM1 and GSTT1) of vitiligo in Korean population. *Life Sci. J.*, 81: 223-227.

Usha, S. and Pandey, S. 2011. Epidemiological profile of vitiligo in Northern India. *J. Appl. Pharma. Sci.*, 01(10): 211-214.

Yun, B.R., El-sohemy, A., Cornelis, M.C., *et al.* 2005. Glutathione S-transferase M1, T1, and P1 genotypes and rheumatoid arthritis. *J. Rheumatol.*, 32: 992-7.

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