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Study of Genetic Variations in Detoxification Genes GSTT1 and GSTM1 in Thalassemia Patients in Dhi Qar Province

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ABSTRACT
Thalassemia is the most well-known hereditary blood disorder that causes this symptom Anemia is caused by defective genes, after which you feel the absence of globin proteins in the body. Thalassemia results from genetic defects that hinder the normal production of hemoglobin. Hemoglobin is the basic protein found in red blood cells and is responsible for binding and carrying red blood cells for oxygen to be transported. From the lungs to various tissues of the body, normal hemoglobin in adults consists of two pairs of chains. One pair of chains is called and the other pair is called. The synthesis of these proteins must be coordinated to ensure equal levels in red blood cells. GSTs (GSTT1, GSTM1) genes are known detoxification factors. Genetic variation in these genes leads to the absence of functional activity of the enzyme or to a decrease or increase in metabolic activity. The aim of this study is to determine the effect of the absence of the detoxification genes GSTT1 and GSTM1 on the incidence of thalassemia in Dhi Qar Governorate. The total blood samples were 150 samples. 100 samples were from those with thalassemia, as they were collected from the Dhi Qar Center for Hereditary Blood Diseases, and 50 samples were from healthy people for the purpose of comparison, collected from various places. From the governorate, 1 ml of blood was used for the purpose of extracting DNA and then amplifying it with a multiplex PCR device and detecting genes using ultraviolet rays. As for the remaining blood, it was used for the purpose of conducting comprehensive blood tests.

Keywords: GSTT1, GSTM1, Thalassemia Patients

INTRODUCTION

Thalassemia is an autosomal recessive disorder that causes anemia Chronic disease due to a decrease in the synthesis of hemoglobin B or its lack of production. (Jiang et al., 2021; Williams, 2012; Weatherall, 2006; Kassab *et al*., 2003), which includes a variety of molecular defects after thalassemia among mono-diseases The origin is due to a single inheritance that is most common throughout the world (Taher and Musallam *et al*., 2021). It affects about 4.4 out of every 10,000 births worldwide, and it affects both males and females, as it is a disease that is not affected by sex (Smith, 2022). Normal hemoglobin in adults consists of two pairs of globin chains, which are α chain pair and β chain pair. The synthesis of these proteins is coordinated to ensure equal production levels in cells that making up red blood cells (Ribeil et al., 2013). Thalassemia is classified into alpha, and beta ased on mutations in the globin genes that lead to a qualitative change or deficiency in the production of globin α and β chains of adult hemoglobin (Kattamis *et al* 2022). Depending on the severity of the clinical symptoms, each of them is classified into thalassemia major, intermedia, and minor. Patients with thalassemia major suffer from severe anemia that appears early in life, requiring lifelong blood transfusions and iron chelation therapy. As for thalassemia intermedia, patients with it suffer from varying symptoms from mild to moderate or severe anemia and do not require continuous blood transfusions. As for thalassemia minor, those affected suffer from mild anemia without symptoms and do not need blood transfusions (Cappellini et al., 2008). Thalassemia major occurs when a child inherits two defective globin genes, one from each parent, while minor occurs when a child inherits one defective globin gene from only one parent (Cao et al., 2010; Danjou et al., 2011) β thalassemia is considered the most common type of thalassemia (Ayyildiz et al., 2020). It is caused by the failure of one or both chains to produce normal globin β, while the globin 1 gene will It continues to produce normal globin α. These mutations result in a defect in the production of A and B globin chains, which ultimately leads to the accumulation of free globin chains that produce highly toxic aggregates (Khandros *et al*., 2012). Thalassemia in its acute clinical form was first recognized by Dr. Thomas Cooley in 1925, who described a syndrome of anemia, bone deformities, and splenomegaly among offspring the Italian “Cooley’s anemia” which

was named after him (Nigam *et al*., 2017). Wipple and Bradford renamed this disease "thalassemia" (Cooley, 1946). But its mild form is called "La Malattia di Rietti-Greppi-Micheli" was identified independently in the United States and is now known as (Thalassemia major) and (Thalassemia intermediate). The prevalence of beta thalassemia varies geographically, with higher frequencies in certain regions. There is a high prevalence of beta thalassemia in the Mediterranean region (e.g., Italy, Greece, Cyprus, the Middle East, such as Iraq, Iran, and Saudi Arabia), and parts of Asia (e.g., India, Pakistan, and Bangladesh) (Kountouris et al., 2014). The frequency of the alpha thalassemia gene is higher than that of beta thalassemia in the regions of Southeast Asia, as the frequency of the alpha thalassemia gene reached 25% (Weatherall, 2010; Vichinsky, 2012). Due to genetic factors and historical migration patterns, therefore historically these regions have higher rates of thalassemia, which is Now a global concern, it has been found that 5% of the world's population has a globin mutation, and 1.7% carry alpha or beta thalassemia (Angastiniotis et al., 1986; Rund et al., 2005). Consanguineous marriage is among the common risk factors for thalassemia. Where the offspring are expected to receive Inbreeding has identical genetic copies from both parents. (Bittles, 2001; Hamamy et al., 2011).

MATERIALS AND METHODS

Blood samples (5 mL) were collected, with patient samples obtained from the Dhi Qar Center for Hereditary Blood Diseases, while control samples were collected from various locations within the governorate. The collection period extended from January 1 to April 30, 2024.

1. DNA Extraction

Genomic DNA was extracted following the method described by (Dairawan, 2020). A 200 µL aliquot of whole blood was transferred into microcentrifuge tubes, and the extraction steps were carried out, including cell lysis, DNA binding, and multiple washing steps until purified DNA was obtained.

2. DNA Detection Using Electrophoresis

DNA integrity was assessed via agarose gel electrophoresis (2% agarose gel) using 1X TBE buffer and Bromophenol Blue dye. The electrophoresis was performed at 70V for 25–30 minutes until the Bromophenol Blue dye migrated toward the positive electrode. Upon completion, the gel was examined under UV transillumination using a UV Transilluminator to visualize DNA bands.

3. Multiplex Polymerase Chain Reaction (MPCR)

The MPCR technique was performed following the method of (Dermawan ,2021). using a 50 µL reaction mixture to amplify the GSTT1 and GSTM1 genes. The reaction mixture contained previously extracted DNA, Master Mix, double-distilled water, and three pairs of primers for each of the GSTT1 and GSTM1 genes, with Albumin as an internal control. Additionally, Safety Dye was included, and the reaction was conducted in a Thermal Cycler.

4. Detection of MPCR Products Using Electrophoresis

The MPCR products were analyzed using 1.5% agarose gel electrophoresis to confirm the presence of amplified DNA fragments. After 25–30 minutes of electrophoresis, the gel was exposed to UV light, and the results were documented using a digital camera.

Statistical Analysis

The statistical analysis was conducted using the (OR) and (X^2) tests with a significance level of $P<0.05$ and a 95% confidence interval (CI) to study the effect of the deletion of GSTT1 and GSTM1 genes on the susceptibility to thalassemia. This was done to compare the patient samples with the healthy controls.

RESULTS

Genotypes of Patient and Healthy Samples

Table presents the distribution of genetic deletions for *GSTT1* and *GSTM1* between the patient and healthy groups, and indicates whether the differences between the two groups are statistically significant. The *GSTT1* gene deletion was observed in 11% of the patient group and 10% of the healthy group, with an odds ratio (OR) of 0.89 and a 95% confidence interval (CI) ranging from 0.29 to 2.74. The P-value of 0.85 suggests that this difference is not statistically significant. As for the *GSTM1* gene deletion, it was found in 28% of the patient group and 34% of the healthy group, with an OR of 1.32 and a 95% CI between 0.63 and 2.75. The P-value of 0.45 indicates no significant association between the *GSTM1* gene deletion and the presence of thalassemia. Regarding the double genetic deletion of both *GSTT1* and *GSTM1*, it was observed in 4.477% of the patients and 8.823% of the healthy group, with an OR of 2.06 and a wide 95% CI ranging from 0.39 to 10.82. Although the OR is elevated, the P-value of 0.85 indicates no significant difference. In general, the results from this table suggest that the deletions of *GSTT1* and *GSTM1*, either individually or combined, do not show a statistically significant difference between thalassemia patients and healthy individuals, indicating that these genetic deletions are not strongly associated with the occurrence of thalassemia in this population sample.

Table 1: Presence or Absence of Genotypes in Patient and Healthy Groups for Both Genders

Genotype	Percentage of Patients	Percentage of Healthy individuals	*OR	*95 % CL	P- Value
GSTT1 (+)*	89 (% 89)	45 (% 90)	1	———	
GSTT1 (-)*	11 (% 11)	5 (% 10)	0.89	0.29-2.74	0.85
GSTM1 (+)	72 (% 72)	33 (% 66)	1	———	
GSTM1 (-)	28 (% 28)	17 (% 34)	1.32	0.63-2.75	0.45
GSTT1 , GSTM1 (+)	64 (%95.52)	31 (%91.17)	1	———	
GSTT1 , GSTM1 (-)	3 (%4.477)	3 (%8.823)	2.06	0.39-10.82	0.85

* OR Odd Ratios (+)* Gene Presence * 95 % CI Confidence Interval *(-) Gene Absence

Comparison of *GSTT1* and *GSTM1* Genotypes in the Patient Group Based on Family History

Table presents the distribution of genetic deletions for *GSTT1* and *GSTM1* among thalassemia patients based on the presence or absence of a family history of the disease and evaluates the statistical significance of these genetic patterns. The *GSTT1* gene deletion was observed in 9% of patients with a family history and 3% of patients without a family history. The odds ratio (OR) was 1.89, with a 95% confidence interval (CI) ranging from 0.22 to 3.56, and a P-value of 0.86, indicating that the presence of the *GSTT1* gene deletion is not statistically significant between the two groups. As for the *GSTM1* gene deletion, it was found in 22% of patients with a family history and 6% of patients without a family history. The OR was 0.70, with a 95% CI between 0.25 and 2.00, and a P-value of 0.51, suggesting no significant association between the *GSTM1* gene deletion and family history of thalassemia. The double genetic deletion of both *GSTT1* and *GSTM1* was observed in 4.255% of patients with a family history and 9.523% of patients without a family history. The OR was 2.36, with a 95% CI between 0.31 and 18.04, and a P-value of 0.40, indicating no significant difference. In general, the results from this table suggest that the deletions of *GSTT1* and *GSTM1*, either individually or combined, do not have a significant association with the family history.

Table 2: Genotypes of GSTT1 and GSTM1 Genes in Patient Samples Based on Family History

Genotype	Present	Not Present	*OR	*95 % CL	P- Value
GSTT1 (+)*	64 (%64)	24 (%24)	1	———	
GSTT1 (-)*	9 (%9)	3 (%3)	1.89	0.22 – 3.56	0.86
GSTM1 (+)	52 (%52)	20 (%20)	1	———	
GSTM1 (-)	22 (%22)	6 (%6)	0.70	0.25 – 2.00	0.51
GSTT1 , GSTM1 (+)	45 (%95.74)	19 (%90.47)	1	———	
GSTT1 , GSTM1 (-)	2 (%4.255)	2 (%9.523)	2.36	0.31 – 18.04	0.40

* OR Odd Ratios (+)*Gene Presence * 95 % CI Confidence Interval (-)*Gene Absence

DISCUSSION

Despite these variations in disease contexts, the findings of this study as shown in table (1) suggest that GST deletions may not be critical factors in thalassemia susceptibility. This conclusion aligns with the work of (Weich *et al*., 2016), who found no significant effect of these deletions on chronic myeloid leukemia (CML) risk or treatment response. Association Between *GSTT1* and *GSTM1* Deletions and Family History of Thalassemia. The results presented in Table 2 indicate that the deletion of *GSTT1* and *GSTM1* genes, whether individually or in combination, is not significantly associated with a family history of thalassemia. The *GSTT1* deletion was found in 9% of patients with a family history of thalassemia compared to 3% of those without a family history, yielding an odds ratio (OR) of 1.89 and a p-value of 0.86, indicating no statistically significant difference between the two groups. This finding is consistent with studies conducted by (Liu *et al*., 2022), which reported no significant association between *GSTT1* deletions and genetic predisposition to diseases such as diabetes and cancer. Similarly, (Malik *et al*,. 2016) found no significant correlation between *GSTT1* deletions and family history of various cancers, reinforcing the lack of genetic predisposition associated with this deletion in the studied population. Regarding the *GSTM1* deletion, it was present in 22% of patients with a family history and 6% of those without a family history. However, the calculated OR of 0.70 and p-value of 0.51 indicate no significant relationship. This observation aligns with the research of (Townsend *et al*., 2017), who found no significant influence of family history on *GSTM1* polymorphism prevalence in non-cancerous diseases. Additionally, (Eslami and Sahebkar, 2014) reported that family history did not affect the association between *GSTM1* deletions and chronic disease risks, further supporting the findings of this study. The double deletion of

both *GSTT1* and *GSTM1* genes was observed in 4.255% of patients with a family history of thalassemia and 9.523% of those without a family history. Although the OR was calculated at 2.36, the wide confidence interval (0.31–18.04) and non-significant p-value (0.40) indicate that this result lacks statistical robustness. This aligns with findings by (Hruska *et al*., 2017), who reported similar trends in GST double deletions, where higher OR values were observed, but statistical significance was often lacking due to small sample sizes and wide confidence intervals.

CONCLUSION

Overall, our findings suggest that *GSTT1* and *GSTM1* deletions do not appear to play significant role in thalassemia susceptibility or its inheritance patterns. While these genetic variations have been linked to certain malignancies, their influence on non-cancerous hematological disorders like thalassemia remains limited. Further large-scale studies incorporating diverse populations are required to better elucidate the potential genetic and environmental interactions influencing thalassemia.

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