# The relationship between the loss of *GSTM1* and *GSTT1* as risk factors for Diabetes mellitus type 2 diabetes of Thi Qar province

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## Summary

A total of 96 blood samples were collected from the Center for Diabetes and Endocrinology in Thi Qar Governorate. The sample consisted of 50 healthy blood samples, including (university teachers, students and others). All samples were aged between 20-65 years. The blood was kept in container tubes on an anticoagulant (EDTA) and kept at a temperature of -20 ° C until it was used to extract DNA from the two groups. GSTM1, GSTT1, which responsible for the Detoxification and Albumin were amplified using PCR polymerization technology.

This study was designed to study the role of Glutathione-S-transferase (Mo-1), theta-1 (glutathione-S-transferase) genes and their contribution to the risk of Type 2 diabetes in Thi Qar Governorate. (39) males and (57) females. A study of some risk factors that increase the percentage of infections that included a number of factors (such as housing, smoking, age, sex, family history, and stress) The prevalence of the disease is higher among urban (55.33%) than in rural areas. The risk of infection among females increases by 59.37% compared to males. patients with a family history had a higher risk compared to those without a family history (77.08%), while the proportion of patients with hypertension was 39.58% compared with the healthy group, while the group of smokers was 28.12% compared to the healthy group. It was found that the highest proportion of patients was among patients aged more than (50) years and was 36.45%) compared to the group of healthy.

The results of the statistical analysis showed that GSTM1 loss increased (OR2.27) compared with the healthy group, while the risk of GSTT1 was increased (OR1.51) 1.51 when compared to the healthy group, while there was no significant difference in the loss of both genes compared to The healthy group.

## Introduction

Diabetes Mellitus (DM) is the high blood sugar (hyperglycemia), which results from an imbalance in the production of insulin (Zia *et al*.,2012;Shaikh *et, al,* 2012)Which affects the metabolism of proteins, fats and carbohydrates and affect the balance of water, and continuous disorders may lead to functional changes in the cells of the body, especially blood vessels, causing the emergence of complications of diabetes affecting the eyes and kidneys and nervous system and may lead to death (Frier, *et al*.,1999).

There are other clinical symptoms, such as thirst, increased urination, ketone acid, high blood cholesterol, increased triglyceride and general weakness (Smith & Beckett, 2000), diabetes is classified into two types, type 1 diabetes Formerly known as insulin-dependent diabetes (IDDM) (Dahan *et al*., 2009). It occurs by destroying cells by T-cell pancreas, which leads to insulin deficiency and usually occurs at the beginning of childhood (Daneman, 2009). The first type is about 5-10% of diabetics (Taplin, *et al.,* 2008).

Type 2 diabetes is known as non-insulin-dependent diabetes or adult diabetes (NIDDM),( Moore *et al*., 2003). About 85-90% of diabetics (Adeghat *et al*., 2006) are characterized by an increase in triglycerides, low HDL cholesterol and high levels of low-density lipoproteins (VLDL-s) (Elnasri *et al.* Thernod, *et al*., 2009;) Type 2 diabetes (T2DM) become a pandemic in the 21st century, an autosomal metabolic disease involving complex interactions between multiple genes, pathways and environmental factors, has been characterized by insufficient levels of insulin production and the irregular balance of glucose (Gupta *et al*., 2008). The identification and description of the T2DM gene

scene become "important" for the development of targeted therapies and preventive measures (Jamil *et al*., 2014).

## Aim of study

Identify the relationship between loos of (*GSTM1*,*GSTT1*) gene and risk factors such as sex, smoking, etc., and their contribution to the occurrence of the type 2 diabetes .

## Materials and Methods

### Samples Collection

A total of 146 blood samples were collected by 96 samples from the Diabetes and Endocrine Center in Th Qar Governorate for people with type 2 diabetes. And 50 blood samples of healthy people (teaching, students, and people with free work). Two to three ml of venous blood was taken from the healthy and sick groups. Blood samples were placed in container tubes on EDTA Anticoagulant and preserved at temperature 20-°C A form of information about the patient and healthy groups including (age, smoking, area of residence, incidence of stress, and family history).

### DNA Extraction

DNA extraction from patient and healthy samples included several steps based on the leaflet attached to Kit DNA Extraction manufactured by Geneaid((Korean origin) :

## Polymerase Chain Reaction (PCR)

PCR technique was used to amplify GSTM1 and GSTT1 genes according to the method of (Rand *et al.*, 1996). The following materials were used. The sequence of the primers shown in Table 1 was used. The reaction method was performed with a 20 µl reaction mixture as shown in Table 2, based on the leaflet attached to Bioneer's Green Master Mix. After completing all the additives, mix the samples with Vortex, then transfer the samples to Thermo cycle and fill the device according to the program shown in Table (1).

**Table (1) primers of Genes**

| Primers | | Primer Sequences | Lengthbp | Tm | TA | Refrence |
|---|---|---|---|---|---|---|
| *GSTM1* | F | 5-GAACTCCCTGAAAAGCTA AAG C-3 | 22 | 64 C | 59 C | AI-Badran, A. I. & AI Mayah, M. K. (2014). |
| | R | 5-GTTGGG CTCAAA TAT ACG GTG G- 3 | 22 | 64 C | 59 C | |
| *GSTT1* | F | 5-TTC CTT ACT GGT CCT CAC ATC TC-3 | 23 | 64 C | 59 C | AI-Badran, A. I. & AI-Mayah, M. K. (2014). |
| | R | 5-TCA CCG GAT CAT GGC CAG CA -3 | 20 | 64 C | 59 C | |

Tm: Melting temperature Ta: Annealing Temperature

F: Forward R: Reverse

The working method was performed with a 20 µL reaction mixture as shown in the following table (2):

**Table (2) represents the chemicals for the reaction mixture and its sizes.**

| Chemicals | Volume | |
|---|---|---|
| Master Mix | 5 uL | |
| Primer Forward | 1uL | One gene |
| Primer Reverse | 1uL | One gene |
| DNA | 5uL | |
| D.W | 8uL | |

| Total | 20uL |
|---|---|

After completing all the additives, the samples were mixed with Vortex for half a minute. The samples were then placed in a thermal cycler was operated according to the following program:

**Table (3) PCR condition for amplification of genes.**

| No . of Steps | Stage | Temperature | Time | No . of Cycle |
|---|---|---|---|---|
| 1 | Denaturation 1 | 94 C | 3 min | 1 Cycle |
| 2 | Denaturation 2 | 94 C | 1 min | 30 |
| 3 | Annealing | 59 C | 1 min | |
| 4 | Extension 1 | 72 C | 1 min | |
| 5 | Final Extension 2 | 72 C | 5 min | 1 Cycle |

## Detection of products of (PCR)

The method of electrical relay(elecrophorsis) itself to detect (DNA) But with the use of DNA Marker, concentration of agarose (%1.2) And dissolve it in 60 mL of TBE precipitate to become the final concentration (2%) After detection by UV, the results were recorded as follows:

The appearance of the band at the 215 bp means the presence of the GSTM1 gene and the appearance of the band at the 350 bp means that the albumin gene is used as an internal control. While the presence of a band at the 480 bp means the presence of the GSTT1 gene after comparison with DNA Marker (Arand, *et al.*, 1996)

## Results

Loss of GSTM1gene was found to be 46.86% in patients samples while 28% was in healthy samples. In statistical analysis, GSTM1 deletion and

risk of type 2 diabetes were associated with approximately 2 and a half patients Compared to the healthy group of the gene (OR=2.27; 95%CI= 4.73-1.08) While the risk of loss of GSTT1 1.51 in patients compared to control group (OR = 1.51; 95% CI = 3.36-0.68). While GSTM1 and GSTT1 were not at risk of type 2 diabetes when compared to the comparison group was (12%) and a significant difference (OR = 1.10; 95% CI = 3.43-0.35) as shown in the following table:

**Table (4) shows the Genetic patterns of the control groups and patients .**

| **95% CI*** | **OR** | **Patients %N.96** | **control %N.50** | Genetic patterns |
|---|---|---|---|---|
| ——— | 1.0 | (%53.125) 51 | (%72) 36 | ***GSTM1(+)**** |
| 4.73 – 1.08 | 2.27 | (%46.87) 45 | (%28) 14 | ***GSTM1(-)**** |
| ——— | 1.0 | (%70.83) 68 | (%78) 39 | ***GSTT1(+)**** |
| 3.36 – 0.68 | 1.51 | (%29.16) 29 | (%22) 11 | ***GSTT1(-)**** |
| ——— | 1.0 | (%43.75) 42 | (%62) 31 | ***GSTM1,GSTT1(+)*** |
| 3.43 – 0.35 | 1.10 | (%9.37) 9 | (%12) 6 | ***GSTM1,GSTT1(-)*** |

* (+) The presence of the gene *95% CI Confidence Interval

* (-) Loss of the gene * OR Odds Ration

## Effect of gender difference *GSTM1* and *GSTT1* in the occurrence of type 2 diabetes.

### A- Male:

The results of the current study showed that the risk of type 2 diabetes increased by 3.20 when GSTM1 was lost in male patients and significantly higher compared with healthy group (OR.3.20; 95% CI = 8.94-1.15). There was no significant difference in the lost of GSTT1

or both genes loss in male patients. As shown in the following table (5) .

**Table (5) Genotypes of *GSTM1* and *GSTT1* in control samples and patients by sex (males).**

| 95%CI` | OR | Patient %N=39 | Control %N= 30 | Genetic patterns |
|---|---|---|---|---|
| ———— | 1.0 | (%46.15) 18 | (%73.33) 22 | ***GSTM1(+)*** |
| 8.94 – 1.15 | 3.20 | (%53.84) 21 | (%26.66) 8 | ***GSTM1(-)*** |
| ———— | 1.0 | (%71.79) 28 | (%76.66) 23 | ***GSTT1(+)*** |
| 3.86 – 0.43 | 1.29 | (%28.20) 11 | (%23.33) 7 | ***GSTT1(-)*** |
| ———— | 1.0 | (%33.33) 13 | (%56.66) 17 | ***GSTM1,GSTT1(+)*** |
| 5.16 - 0.18 | 0.98 | (%7.69) 3 | (%13.3) 4 | ***GSTM1,GSTT1(-)*** |

* (+) The presence of the gene *95% CI Confidence Interval

* (-) Loss of the gene * OR Odds Ration

## B-Famale

The results of the current study showed that the incidence of infection in female patients increased by (1.70) when GSTT1 was lost (OR = 1.70; 95% CI = 5.84-0.49) compared with the healthy group and the risk was (1.69) when GSTT1 was lost (OR=1.69 ; 95%CI =5.05-0.57) while the risk of infection increases by approximately one and a half when the genes are lost together compared to the healthy group (OR

=.49; 1 = 8.11- 0.62) difference as shown in the following table (6) : 95%CI

**Table (6) Genetic patterns of *GSTM1* and *GSTT1* in control samples and patients by sex (females)**

| 95%CI` | OR | Patient %N=57 | Control %N=20 | Genetic patterns |
|---|---|---|---|---|
| ———— | 1.0 | (%57.89) 33 | (%70) 14 | ***GSTM1(+)*** |
| 5.05 – 0.57 | 1.69 | (%42.10) 24 | (%30) 6 | ***GSTM1(-)*** |
| ———— | 1.0 | (%70.17) 40 | (%80) 16 | ***GSTT1(+)*** |
| 5.84 – 0.49 | 1.70 | (%29.82) 17 | (%20) 4 | ***GSTT1(-)*** |
| ———— | 1.0 | (%50.87) 29 | (%70) 14 | ***GSTM1,GSTT1(+)*** |
| 8.11 – 0.62 | 1.49 | (%10.52) 6 | (%10) 2 | ***GSTM1,GSTT1(-)*** |

* (+) The presence of the gene *95% CI Confidence Interval

* (-) Loss of the gene * OR Odds Ration

## C. Comparison of Genotypes of the two patient groups according to age group:

The results of the study, when comparing the genotypes of patients by age group, showed no significant differences in patients aged less than or older than 50 years when the gene GSTT1 was lost (OR = 0.85; 95% CI, 2.07-0.35). (2.20; 95% CI = 9.98-0.48), while there is significant difference in GSTM1 loss (1.88) compared to healthy group (OR = 1.88. ; 95% CI = 4.28-0.82). As in the following table (7):

**Table (7) shows the comparison between genetic models of *GSTM1* and *GSTT1* in patient samples by age groups less and older than 50 years.**

| 95%CI` | OR | Patient group older than 50 year | Patient group less than 50 year | Genetic pattern |
|---|---|---|---|---|
| ———— | 1.0 | (%46.29) 25 | (%61.90) 26 | ***GSTM1(+)*** |
| 4.28 – 0.82 | 1.88 | (%53.70) 29 | (%38.09) 16 | ***GSTM1(-)*** |
| ———— | 1.0 | (%72.22) 39 | (%69.04) 29 | ***GSTT1(+)*** |
| 2.07 – 0.35 | 0.85 | (%27.77) 15 | (%30.95) 13 | ***GSTT1(-)*** |
| ———— | 1.0 | (%37.03) 20 | (%52.38) 22 | ***GSTM1,GSTT1(+)*** |
| 9.98 – 0.48 | 2.20 | (%11.11) 6 | (%7.14) 3 | ***GSTM1,GSTT1(-)*** |

* (+) The presence of the gene *95% CI Confidence Interval

* (-) Loss of the gene * OR Odds Ration

## Molecular genetics study

DNA samples from the control group and patients were detected by electrophoresis on 0.8% agarose gel following the( Sambrook *et al*. 1989) shown in Figure 1

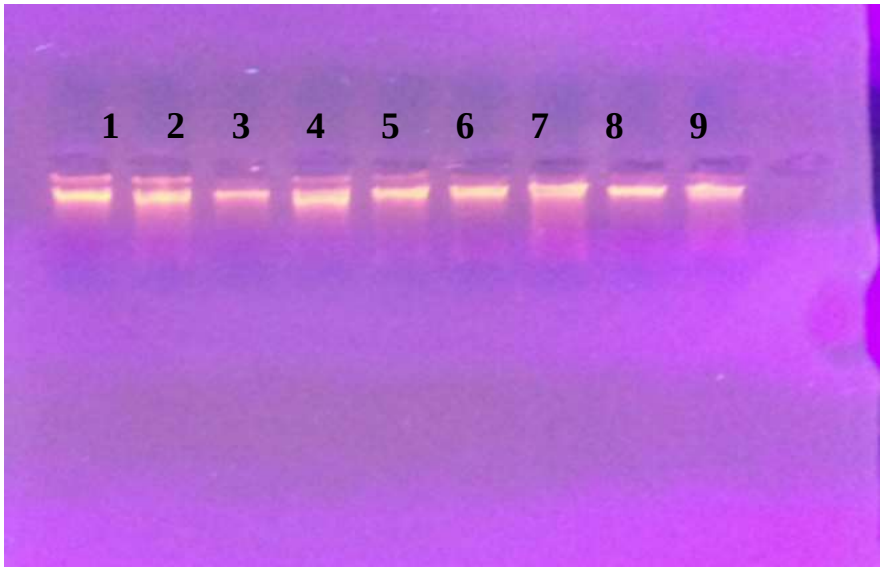


**Figure 1 Electrophoresis of DNA on the agarose gel at a concentration of 0.8%**

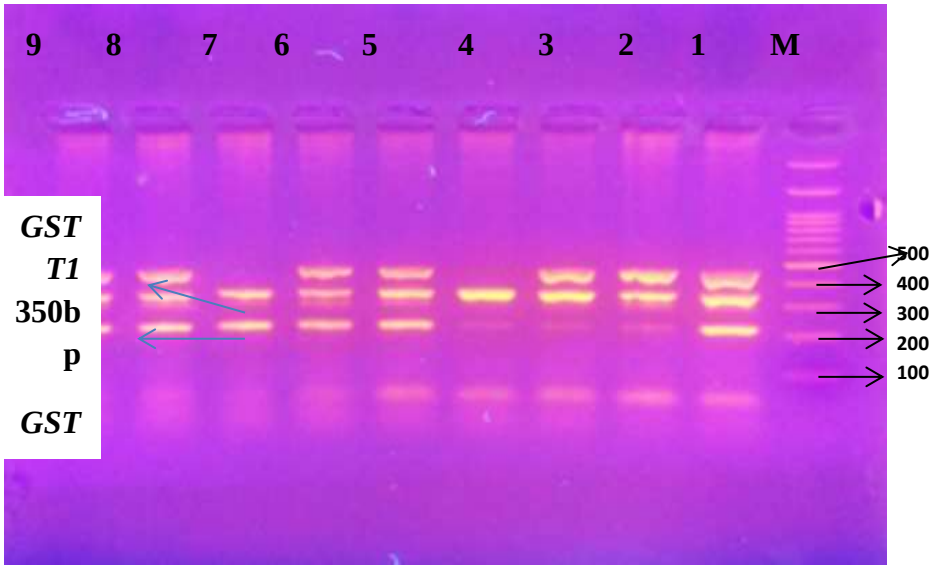


**Figure 2 Electrophoresis for PCR products on the agarose gel at a concentration of 2%.**

M: Standard DNA (1000-3000bp)
Lane 2,3 missing *GSTM1* gene
Lane 4 missing *GSTM1*, *GSTT1* genes
Lane 7 missing *GSTM1* gene
Lane5,6,8,9 normal (contains three genes)

## Discussion

Diabetes is one of the most common diseases in almost all countries. The number of people with diabetes is increasing due to population growth, urbanization, aging, obesity and low physical activity. Oxidation has been shown to play a major role in causing type 2 diabetes. The reason for that B-cell are low in antioxidant agents such as glutathione peroxide's and catalase ( Amer , 2011). GSTs have multiple forms distributed among different body tissues and play a key role in detoxification and tissue damage prevention( Sheehan *et al* . 2001) There are many complex mechanisms in the human body to protect the body against environmental factors, including inappropriate food, smoking, ultraviolet radiation and free radicals that result from irregular oxidation. People differ slightly in the production of anti-toxin, which are susceptible to various diseases, such as Diabetes Glutathione is important as neutralizing free radicals (Guengerich,. 1992 Grant *et al* ,. 2006).

The results of the present study showed a table (1). The percentage of patients in urban areas was 58.33%, while those living in rural areas reached 41.66%. There was no significant difference between the urban and rural areas and the percentage of (P.0.616). This is because urban dwellers are less active than rural dwellers, and rural dwellers are less

check to specialized centers and less to eat fast food (Al-Rajhi *et a.*, 2008) .

The results of the current study showed that there were significant differences in gender, with the highest percentage of females (59.37%) and males (40.62%) that's agreed with results of (Amer *et al.*, 2011) it's showed (55%) females (45%) males, As well as the study by (AI-Badran & AI-Mayah, 2014) where the percentage of females with type 2 diabetes was 62.4%, while males were 37.6%.study of (Nowier *et al*., 2009), recorded the percentage of females is (65.52%) and males (34.48%).

This study differed from a study conducted by (Mosser *et al*., 2012), which showed that there was no significant difference between male and female infection rate (P = 0.88). It also differed from the study conducted by (Porojan *et al.*, 2015), which showed that there was no significant difference between the sexes, as the value of( P = 1.2) was also different from the study conducted by(Pinheiro,. *et al* ,. 2013) Which showed that there was no effect of sex difference among patients with diabetes where value. (P = 0.59). The main factors that make females higher than males are higher oxidative stress in males than females. High levels of estrogen in females protect them from aging by Regulate the expression of genes related to oxidation (Vina *et al*., 2011).

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