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Glutathione S-transferase (T1 & M1) Null Genotypes as Risk Determinants in Sudanese for Developing Type Two Diabetes Mellitus

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Abstract: Glutathione S-transferases (GSTT1 and GSTM1) are globular proteins responsible for the detoxification of xenobiotics and the prevention of oxidative damage. Functional polymorphisms of the antioxidant glutathione and enzymes have a role in the pathogenicity of T2DM and its complications. The present study is aimed to assess association between GSTM1 and GSTT1 genetic polymorphisms with T2DM in Sudanese people. This case-control study was done in Khartoum. A total of 181 Sudanese (115 T2DM patients and 66 controls) included in this study. Genomic DNA was extracted and processed by Multiplex PCR. The present genotype and null genotypes of GSTT1 and GSTM1 were identified. The biochemical parameters have been estimated in plasma samples. Glycated hemoglobin was also estimated in the whole blood samples. SPSS version 20 was used for the analysis of genotypes distribution, mean values, P-values and Odd Ratio with 95% confidence intervals. Sudanese patients with T2DM showed an increased frequency of the GSTT1 null genotype when compared to the non-diabetic controls (48.7% versus 28.8%). The diabetic patients showed a lower frequency of GSTT1 genotype compared to their controls (51.3% versus 71.2%). The study revealed a high frequency of the GSTM1 null genotype in diabetic Sudanese compared to their controls (40.9% versus 36.4%); while type-2 diabetes mellitus patients showed a lower frequency of GSTM1 present genotype compared to non-diabetic Sudanese (59.1% versus 63.6%). The GSTM1 and GSTTI genes polymorphism can be a good marker for early identification of Sudanese people at risk of T2DM and its complications.

Keywords: Glutathione S-transferase (GSTT1), Glutathione S-transferase (GSTM1), Type 2-Diabetes Mellitus, Sudan

Introduction

Oxidative stress seems to play a significant role in many human diseases. The Glutathione S-transferases (GSTT1 and GSTM1) are globular proteins responsible for the detoxification of xenobiotics and the prevention of oxidative stress (Wang *et al*., 2006; Amer *et al*., 2011). Glutathione S-transferases (GSTs), play an essential role in protecting against products of oxidative damage and xenobiotic compounds (Palma-Cano *et al*., 2017; Wang *et al*., 2006). The oxidative stress may increase with the polymorphism of GSTM1/GSTT1 genes (Almoshabek *et al*., 2016; Mustafa *et al*., 2010). Oxidative stress plays an important role in the pathogenesis of T2DM (Stephens *et al*., 2009). The polymorphisms of the antioxidant enzymes have been involved in the pathogenesis of T2DM. The low levels of antioxidant enzymes or the non-functionality result in

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excessive reactive metabolites that initiate stress-related pathways thereby leading to insulin resistance and T2DM (Monisha and Vats, 2013). GTTs have an essential role in the detoxification of oxidative stress products, which are one of many risks factors that may be associated with several types of disease affect processes such as T2DM (Sombié *et al*., 2020). T2DM is growing in a quick manner whole over the world (Raza *et al*, 2014a; 2014b). Type 2 diabetes is also one of the multifactorial disorders with heterogeneous disturbances of metabolism, the genetics and environmental factors playing important role in its causes (Dadbinpour *et al*., 2013; Kerner and Brückel, 2014). The cause is either impaired insulin action, insulin secretion or both (Kerner and Brückel., 2014). Diabetes now more than 350 million people worldwide, it is increasing rapidly because of secondary factors, such as obesity, hypertension and lack of physical activity (Cilenšek *et al*., 2012). In diabetes, due to the defects in cellular metabolism the free radicals increase (Dadbinpour *et al*., 2013). These radicals react with other vital cellular molecules causing diabetes side effects (Dadbinpour *et al*., 2013). Sudan is one of the African countries, in which diabetes mellitus is a growing health problem in all socio-economic classes. The national history of the disease is associated with poor glycemic control and a high prevalence of complications (Elrayah *et al*., 2005). The prevalence of T2DM in Sudan, as in many other low-income countries, is increasing to epidemic proportions, T2DM in Sudan is common among the adult population of northern Sudan (Abdelgadir *et al*., 2002). The prevalence of T2DM was found to be at range (4-10.8%) in Northern State and Khartoum State (Elbagir *et al*., 1996; Osman *et al*., 2013).

The long term affects and complications of diabetes include progressive development of retinopathy, nephropathy and neuropathy with microvascular and macrovascular disorders. Macrovascular disorders such as atherosclerosis are recognized as major causes of mortality in the diabetic population (Alvin *et al*., 2001). Although the exact cause of Cardiovascular Diseases (CVD) including atherosclerosis remains unsolved, the pro-oxidative and pro-inflammatory vascular environments are fundamental contributors to the initiation and progression of cardiovascular diseases (Thomas *et al*., 2008).

This study aimed to find if there any association between genetic polymorphisms of (GSTT1 and GSTM1) in Sudanese with T2DM.

Materials and Methods

Patients Selection and Data Collection

In this hospital-based case-control research, which done in Khartoum state and Ribat University Hospital; which is a tertiary care hospital. The period of the study was from September 2015 to May 2018. The study includes 115 Sudanese patients with T2DM (57 females and 58 males) and 66 healthy volunteers as control (29 females 37 and males). The research was approved by the Ethics Research Committee (The National Ribat University). The objectives of the study were explained at the beginning to all individuals under study and written consent was obtained from each participant in the study. The clinical information was collected through questionnaires, by a physician and by the aid of the clinical records.

Biochemical Estimations

Fasting plasma glucose was measured by the glucose oxidase-peroxidase method. The serum creatinine level was measured by a kinetic Jaffe method. Serum cholesterol was assessed by the cholesterol oxidase-peroxidase method. Serum triglycerides were measured by the glycerol phosphate oxidase-peroxidase amidopyrine method. High-Density Lipoprotein (HDL) cholesterol and Low-Density Lipoprotein (LDL) (Immunoinhibition) were assessed by Mindray BS-380, a fully automated chemical analyzer. Glycated hemoglobin and highly sensitive C- reactive protein were estimated in full automated chemistry analyzer (COBAS Integra 400 plus).

DNA Extraction

Peripheral blood (2.5-3.0 mL) was collected from the patients in vacutainers containing EDTA as an anticoagulant. Genomic DNA was extracted in whole blood using the Generation DNA purification capture column kit (Analytica Jena, Berlin Germany) then stored at -20°C.

Polymerase Chain Reaction (PCR)

Primers for GSTT1 and GSTM1 gene were from Microgen Company. GSTT1 primers (GSTT1-Forward: (GAACTCCCTGAAAAGCTAAAGC) and GSTT1-Reverse: GTTGGGCTCAAATATACGGTGG) and GSTM1primers (GSTM1-Forward: TTCCTCACTGGTCCTCACATCTC and GSTM1-Reverse: TCACCGGATCATGGCCAGCA). Albumin gene primers as internal control, (Albumin-Forward: GCCCTCTGCTAACAAGTCCTAC) (Albumin-Reverse: GCCCTAAAAAG AAAATCGCC AATC) Primers were prepared by using 10 µL of primer added to 90µL of sterile de-ionized water. Forward and reverse primers were prepared in separate Eppendorf tubes.

The mixture was prepared by adding 0.25 µL of forward primer, 0.25 µL of reverse primer of each gene and 17 µL sterile water to PCR Premix tube and finally 2 µL of DNA. 20 µL total volume. The experiment consists of DNA in 0.5 mL (PCR Premix tube, intron Biotechnology). Using multiplex PCR for all subjects.

The amplification was performed using initial denaturation at the temperature 95°C for 3 min, then followed by 30 cycles at the same temperature (95°C) for 30 sec. Then at 60°C for 30 sec, followed by 72°C for 30 sec and the final extension of 72°C for 7 min. The amplified results were identified using electrophoresis in a 1.5% agarose gel then stained with 0.5 mg/mL ethidium bromide. The lengths of the product were 480, 215 and 350 bp for the GSTT1, GSTM1 and Albumin locus, respectively. The null genotypes and present genotype of GSTM1 and GSTT1 genes were identified.

Results

The study revealed that; the mean age in the T2DM subjects was (53.60±10.08 years) and in non-diabetic (51.53±8.12 years), respectively. The biochemical and clinical profiles of the patients and the controls were shown in Table 1. The mean Body Mass Index (BMI), Fasting Blood Sugar (FBS), HbA1C, total serum cholesterol, LDL cholesterol and triglycerides in the diabetic patients were significantly higher than in the control volunteers (P = 0.000), but HDL cholesterol was significantly decreased in diabetic patients (P = 0.000). Highly sensitive C Reactive Protein (hs-CRP) was significantly increased in diabetic patients compared to their control subjects (P = 0.007). Table 2 shows the comparison of the biochemical parameters according to genotypes; the null genotype of Glutathione S-Transferase enzyme (GSTT1) in diabetes mellitus revealed a significant increase in glucose, hs–CRP, total cholesterol and LDL cholesterol (P value: 0.004, 0.018, 0.022 & 0.002, respectively) and insignificantly increase in triglycerides and HbA1C, but HDL cholesterol was significantly decreased (P = 0.021) as shown in (Table2), while the null genotype of glutathione S-transferase enzyme (GSTM1) in diabetes mellitus revealed a significant difference in LDL cholesterol (P = 0.004), (Table 3).

A significant difference in GSTM1& GSTT1 genotypes distribution in Sudanese patients with T2DM showed a higher frequency of the GSTT1 null genotype compared to their controls (48.7% versus 28.8%) as presented in (Table 4). The diabetic group showed a lower frequency of GSTT1 present genotype compared to their controls (51.3% versus 71.2%), (OR 2.348, P value 0.012) which reflected a significant association of GSTT1-null genotype with T2DM (Table 4). The study also revealed a higher frequency of the GSTM1 null genotype when compared to their controls (40.9% versus 36.4%) with (OR: 1.210 and P: value 0.636), as shown in (Table 5).

Table 1: The clinical and biochemical parameters of the Sudanese with type 2 diabetes mellitus patients and their controls

Parameters Gender	T2DM (n = 115) 58 (males); 57 (females)	Controls (n = 66) 37 (males); 29 (females)	p Values
Age (years)	53.60±10.8	51.53±8.12	0.163
BMI (kg/m^2)	27.76±3.82	22.939±1.31	0.000***
FBS (mg/dL)	178.13±77.59	97.74±8.53	0.000***
Hb A1C (%)	8.47±2.062	4.33±0.868	0.000***
Cholesterol (mg/dL)	208.29±58.878	167.74±20.256	0.000***
Triglyceride (mg/dL)	140.96±60.38	98.95±27.85	0.000***
LDL (mg/Dl)	108.97±37.98	82.52±11.24	0.000***
HDL (mg/dL)	44.09±9.097	49.02±6.96	0.000***
Highly sensitive C reactive protein (mg//L)	17.18±52.12	3.70±1.75	0.007**

For finding P values; Student's unpaired t-test was used, except in gender comparison. T-test was applied on, (BMI = Body Mass Index; FBS = Fasting Blood Sugar; HDL = High-Density Lipoprotein; LDL = Low-Density Lipoprotein; highly sensitive C-reactive protein). * = Stands for significant differences between the groups (P < 0.05)

Table 2: Comparison of biochemical parameters in present genotype and null genotype of glutathione transferase enzyme (GSTT1) in Sudanese with type2 diabetes mellitus

GSTT1	Present (n = 59)	Null (n = 55)	P value
Glucose (mg/dl)	158.56±67.630	200.05±82.723	0.004**
HbA1c (%)	8.141±2.0587	8.838±2.0418	0.070
hs CRP (mg/l)	5.670±13.3667	29.830±72.3284	0.018*
Total serum cholesterol (mg/dl)	196.20±45.425	221.76±68.868	0.022*
Serum triglycerides (mg/dl)	135.59±62.175	148.00±58.045	0.274
HDL (mg/dl)	45.99±7.436	42.06±10.351	0.021*
LDL (mg/dl)	98.80±32.747	120.47±40.317	0.002**
BMI (kg/m2)	28.133±3.963	27.377±3.679	0.292

Comparisons were performed by the Student T-test. Data were written as mean ± SD. * = Stands for significant differences between the groups (P < 0.05)

Table 3: Comparison of biochemical parameters in present genotype and null genotype of glutathione S-transferase enzyme (GSTM1) in Sudanese with type 2 diabetes mellitus

GSTMI	Present (n = 71)	Null (n = 43)	P value
Glucose	183.66±78.375	170.19±76.994	0.372
HbA1c (%)	8.437±2.1852	8.544±1.8912	0.789
hs CRP (mg/l)	16.530±57.5736	18.642±42.907	0.836
Total serum cholesterol (mg/dl)	210.62±53.014	205.09±68.458	0.630
Serum triglycerides (mg/dl)	145.52±53.611	135.07±70.104	0.372
LDL (mg/dl)	116.44±41.555	97.40±27.928	0.004**
HDL (mg/dl)	43.83±8.916	44.53±9.581	0.691
BMI (kg/m^2)	28.026±3.301	27.328±4.591	0.386

Comparisons were performed by the Student T-test. Data were written as means ± SD.* = Stands for significant differences between the groups (P < 0.05)

Table 4: Distribution frequencies of genotypes of GSTT1 in Sudanese with T2DM and their control groups and the risk analysis of T2 DM

Genotype	T2DM	Control			
GSTT1	n (%)	n (%)	x2	OR (95%CI)	P value
Present (+)	59(51.3%)	47(71.2%)	-	1 Reference	
Null (–)	56(48.7%)	19(28.8%)	6.849	2.348(1.230-4.481)	0.012*
Total	115(100.0)	66(100.0)			

The comparisons were performed by the chi-square test. *= indicates significant differences between groups (P < 0.05).

Table 5: Distribution frequencies of genotypes of GSTM1 in T2DM and their control groups besides the risk analysis of T2DM

Genotype	T2DM	Control			
GSTM1	n (%)	n (%)	x2	OR (95%CI)	P value
Present (+)	68 (59.1 %)	42(63.6%)	-	1 Reference	-
Null (–)	47(40.9%)	24(36.4%)	0.357	1.210(0.210- 2.258)	0.636
Total	115(100.0)	66(100.0)			

Comparisons were performed by the chi-square test. * = Stands for significant differences between the groups (P < 0.05)

Table 6: Distribution frequencies of genotypes of GSTM1 and risk analysis of T2 DM complications

Genotypes	Present of T2DM complications	Absent of T2DM complications			
GSTM1	n (%)	n (%)	(x 2)	OR (95%CI)	P. value
Present (+)	56(63.6%)	12(44.4%)		1 Reference	
Null (–)	32(36.4%)	15(55.6%)	3.149	0.457 (0.191-1.096)	0.116
Total	88(100.0)	27(100.0)			

Comparisons were performed by the chi-square test. * = Stands for significant differences between the groups (P < 0.05).

The GSTT1-null genotype was associated with a 2.348-folds increased risk relative to the present genotypes with (P-value 0.012) and the incidence of GSTM1 null was associated with a 1.21 folds increase risk of having T2DM but insignificant (P value 0.636), while T2DM patients showed a lower frequency of GSTM1 present genotype compared to their controls (59.1%% versus 63.6%) as shown in (Table 5). The distribution frequencies of genotypes of GSTM1 and risk analysis of Type 2 DM complications were shown in (Table 6).

Discussion

Genetic factors play an important role in the pathogenesis of Type-2 Diabetes Mellitus (T2DM) and related complications and that genetically susceptible individuals are likely to develop the disease, when exposed to endogenous and environmental risk factors. Overproduction of Reactive Oxygen Species (ROS) and/or deficiency of antioxidant mechanisms depend on the balance between the generation of ROS and enzymatic or non-enzymatic systems of antioxidative protection, cause an increase of Oxidative Stress (OS). Several factors, such as hypercholesterolemia, glycation of protein, hypertension, diabetes, obesity and aging are risk factors for complications of the diseases, where OS is increased and antioxidant defenses are compromised. Antioxidant enzymes (GSTT1 and GSTM1) represent the second line of defense, which neutralizes lipid peroxidation products as reported by (Sharma *et al*., 2006). GSTM1 and GSTT1genotypes determine the enzymatic inactivity of the proteins encoded by these genes. The present study suggests that the null genotypes of GSTT1 and GSTM1 are risk factors of developing diabetes mellitus which are consistent with a study done by (Doney *et al*., 2005); which showed that GSTM1 and GSTT1 null genotypes seem to be genetic risk factors for diseases such as T2DM and its cardiovascular

complications. The conclusion of this research is also in agreement with a Brazilian study done by (Pinheiro *et al*., (2013). Many case control studies on GSTM1 and GSTT1 null genotypes had reported a risk associations between diabetes in general and GSTM1 and GSTT1 gene polymorphisms as stated earlier by (Yuille *et al*., 2002; Chen *et al*., 2005); while a study done in Romanian population showed that GSTM1 and GSTT1 gene polymorphisms had no associated with the risk of developing T2DM as mentioned by (Stoian *et al*., 2015).

The frequency of the GSTM1 null genotype in this study is 36.4%; which is lower than that reported in other populations like Egypt 58.62% by (Nowier *et al*., 2009), the United Arab Emrates 57.5% by (Bid *et al*., 2010) and Northern India 38.61 and 54.00% by (Raza *et al*., 2014a). Moreover; (Raza *et al*., 2014a); suggested that GSTM1 genes deletion can be a predictive marker for early identification of Indians at risk of T2DM. The frequency of the GSTTI null genotype of this study is (48.7% in T2DM patients) which is lower than what reported in Dubai (60.0%) by (Hossaini *et al*., 2008). On the contrary, the null genotype of GSTTI of Sudanese is higher than Egyptian (35.0%) (Amer *et al*., 2011). The genotype frequency of the positive GSTT1 and GSTM1 genes in this study are 71.2 and 63.6% respectively, which are significantly higher when compared with the Dubai study; where the positive GSTT1 and GSTM1 genes was 40.0 and 42.5%, respectively, as reported by (Hossaini *et al*., 2008).

Conclusion

In Sudanese patients with Type-2 Diabetes Mellitus GSTTI null and GSTM1 null genetic polymorphisms are risk factors for the development of diabetes mellitus and GSTTI null is a high risk factor for the development of T2DM complications. These results suggest that GSTT1 and GSTM1 present genotypes cooperatively play a protective role against the development of type 2 diabetes mellitus.

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Ethical Approval

Institutional ethical approval has been obtained to conduct this research study vides project reference number (medical-lab/2015/no.1) dated first September 2015.

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Declaration of Conflict of Interests

The authors declared that no potential conflicts of interest with respect to this research, authorship and/or the publication of this article.

Authors Contributions

Gaafar Mahmoud: Prepared the project proposal, collected the samples, did the lab work, did data analysis and wrote the paper.

Hisham Mohamed Abdelrahim: Prepared the project proposal, sample collection, data analysis and wrote the paper.

Abed Al Salam Aljahmany: Shared data analysis, wrote the manuscript and shared the consumables of the research.

Elteyeb Mohammed Ahmed Tayrab: Prepared the research project, shared sample collection, did the data analysis, collected the literature data, wrote the paper, the main supervisor of the research.

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