Study the Exons 5 and 6 GSTP1 SNPs and Correlation with Physiological Growth Hormone and TAO-C Levels in Iraqi patients with T2DM

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Abstract

Background: Type 2 Diabetes Mellitus (T2DM) is a complicated, miscellaneous, not autoimmune, and multiple gene inheritances metabolic disease condition, in which the body is not able to produce sufficient insulin and is characterized by irregular glucose homeostasis. This study aimed to evaluate the effect of Exons 5 and 6 of Glutathione S-Transferase P1 GSTP1 Ile/Val 105 (I105V) and Ala/Val 114 (A114V) SNPs and Correlation with Physiological Growth Hormone levels in Iraqi Patients with T2DM. Methods: A total number of 60 patients with T2DM and 30 subjects as control were used. The ELISA and spectrophotometer assessed GH and TAO-C levels, respectively, while GSTP1 I105V and A114V SNPs was an estimate were estimated by PCR-ARMS and restriction fragments by Alw26I. Results: The results showing statistically significant differences (p-value<0.05) in TAO-C (U/l) between T2DM and control group while not showing significant differences in GH (pg/ml) (p-value>0.05) between both groups. The genetic analysis results suggested a significant difference (p-value<0.05) between T2DM and control groups in AA, AB, BD, CC, and CD genotypes probability distribution that represented GSTP1 SNP while not significant differences in AC, AD, BC, and DD genotypes (p-value>0.05) between both groups. Conclusion: GSTP1 I105V and A114V may

behave as risk factors to the incidence of T2DM by decreasing levels of TAO-C.

Keywords: T2DM, GSTP1, Growth hormone, A114V, I105V SNPs

Introduction

The prevalence of diabetes mellitus (DM) is gradually elevated in the world (19). As per a global report on diabetes released by WHO on 6th April 2016, the prevalence of DM in the adult population worldwide was 8.5% in 2014, which is nearly twofold that (4.7%) in 1980 (1). DM is a syndrome, not a single disease, it was the main cause of suffering and morbidity (2). DM remains one of the most public health challenges worldwide imposing emotional costs and significant financial on sufferers' families as well as the community (3). In general, about 450 million people worldwide (8.8% of adults aged 20–79 years) are expected to have DM(4,5). And there is a study confirming the widespread prevalence of type 2 diabetes among adolescents and children (6) . According to the World Health Organization (WHO), DM will be the 7th main cause of death in 2030 (7). The report of the International Diabetes Federation (IDF) in 2015 Diabetes Atlas showed about (415) million adults around the world suffer from DM and in 2040 predict this number to increase to 642 million, The same report also referred that about five million people died because of DM in 2015 and 5-20% of overall health expenditure on adults was spent on management of DM and its associated complications (8). Additionally, countries that have not been the generally associated high prevalence of DM have recorded significant increases in prevalence recently, one of this region in the world of a high prevalence of DM middle east about 10% of adults whiles about twenty million people in Africa have DM with predicting this number to double by 2035 (9). Most cases of DM involve several genes, each of them being a small supporter to increased likelihood to become type 2 diabetic (10), if one of the identical twins has diabetes, the other has a chance to

develop diabetes during his lifetime is more than 90%, however, and for a non-identical twin is 25–50%. As of 2011, more than 36 genes had been determined which cause the risk of T2DM. All of these genes together still only account for 10% of the total heritable ingredient of the disease (11). The GSTP1 gene, which is located on chromosome 11q13, consists of 7 exons and 6 introns. There are two polymorphisms in GSTP1 that have been exposed to a transposition for codon 105 (Ile/Val) and 114 (Ala/Val) in exons 5 and 6 (12) and most of the genes associated with DM are concerned with beta cell functions. The aim of this study is to evaluate the effect of exons 5 and 6 of Glutathione S-Transferase P1 GSTP1 Ile/Val 105 (I105V) and Ala/Val 114 (A114V) SNPs and Correlation with Physiological Growth Hormone levels in Iraqi Patients with T2DM.

Materials and methods

Study design:

This study included 60 patients with T2DM (40 male and 20 female) at range of age (30-60 Y) and 30 subjects as control group with matching in age and genders.

Determination of GH levels:

GH levels (pg/ml) were estimated by use of ELISA technique while TAOC (U/l) was performed by use spectrophotometric method. The protocols was performed depending on instruction of manufactures.

Genotyping analysis

Genomic DNA was extracted from peripheral whole blood of all subjects (T2DM and CONT) who participating in this study by using the genomic DNA mini kit (Invitrogen™ PureLink™ Genomic DNA Mini Kit) that providing an efficient method for purifying of total DNA from whole and frozen blood. PCR-ARMS was performed by used unique primers for GSTP1 Ile/Val 105 (I105V) and Ala/Val 114 (A114V) SNPs analysis of genotyping and *Alw26I* as restriction enzyme (RE), as shown in table 1.

Table-1: primers of ARMS-PCR method of GSTP1 Ile/Val 105 (I105V) and Ala/Val 114 (A114V) SNPs gene that used in genotyping analysis

Primer sequence (5'→3')		Amplicon length	RE bands *(Alw26I)*
GSTP1	F: ACC CCA GGG CTC TAT GGG AA	988 bp	343, 322, 260, 73 bp
GSTP Ala:	R: TCA CAT CAT CCT TGC CGG		
GSTP Val:	R: TCA CAT CAT CCT TGC CGA		

PCR was carried out in a total volume 25 µl of reaction mixture with Taqman polymerase and carried by the thermocycler (bio-rad) and subjected to denaturation at 95 Cº for 4 min, followed by 35 cycles of 95 Cº for 20 sec, annealing temperature of 59.9 Cº for I105V (58.6 Cº for A114V) for 30 second and the final extension phase at 72 Cº for 5 min. After PCR, the product was electrophoresed on 1% gel and the PCR products were observed with Ala114 and Val114 bands, which were kept inoculated by the RE *(Alw26I)* enzyme at 37°C for overnight and then electrophoresed on 1.5% gel (Figures 1 and 2) . The results of the initial PCR determined the site of SNP position 114 of two alleles Ala114 and Val114, and the end of the Alw26I enzyme after PCR product revealed SNP position 105 of two Val105 and Ile105 alleles . Results on the observed fracture PCR products electrophoresis with four bands at positions (73, 260, 322, and 343 bp), the single-nucleotide mutant defines the Ile105, the probability of each of the (GSTP1*A or GSTP1*D) alleles and in the presence of five bands at positions (73, 93, 250, 260, and 322 bp) represent the single-nucleotide mutation Val105, which was the probability of each of the (GSTP1*B or GSTP1*C) alleles. In the six bands observation at positions (73, 93, 250, 260, 322, and 343 bp), the pair showed the status of the heterozygote Ile105/Val105. The final PCR product was photo documentation on UV analyzer.

Statistical Analysis

The Hardy Weinberg equation was used to genotypes analysis and to determine the significant differences between the study groups as related with genotype and allele frequencies was used the chi-square test in both T2DM and control groups.

Results

Clinical characteristics of T2DM group is shown in table 2:

Table-2: Characteristics of T2DM group

Clinical variables	No = 60	Percentage (%)	p-value
Age			
30-44	34	57	0.009
45-59	26	43	
Gender			
M	39	65	0.003
F	21	35	
BMI			
≥30	32	53	0.065
<30	28	47	

The results suggesting highly significant differences in TAO-C levels between T2DM and control group (p-value< 0.05) while the results not showing significant differences in GH levels between both groups (p-value>0.05), as showing in table 3:

Table-3: TAO-C and GH levels in study group

Groups	GH (pg/ml) mean± SD	P-value	TAO-C(U/l) mean± SD	P-value
T2DM n=60	329±33	0.192	10.19±1.8	0.0000**
CONT n=30	319±28		19.6±1.2	

The results suggesting the highly negative correlation (r=-0.665) between ages of T2DM group with levels of TAO-C (U/l), as shows in figure1:

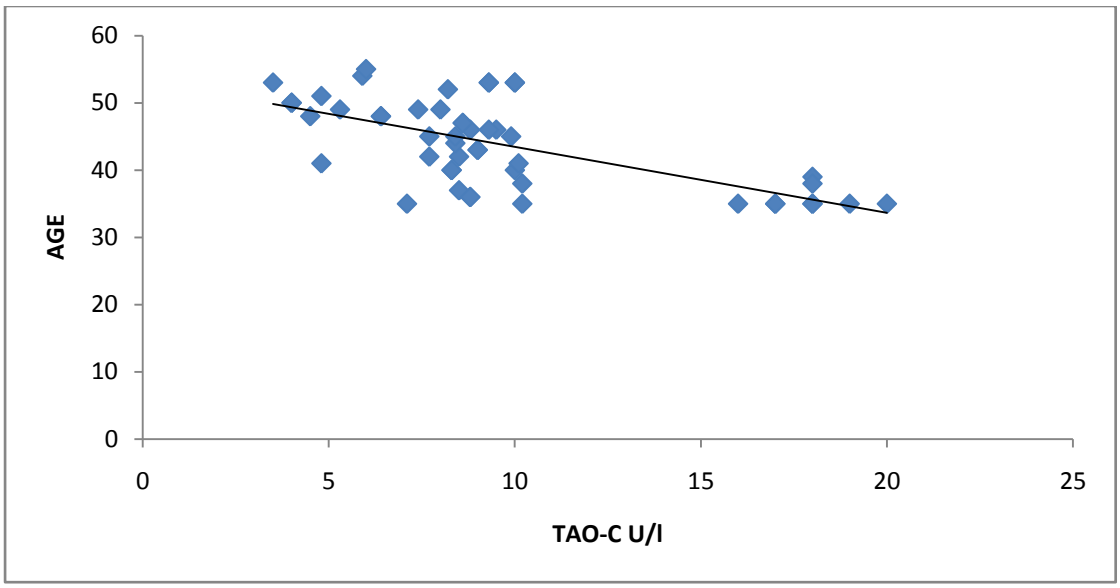

Figure-1: correlation between age with TAO-C in T2DM group

For genotyping analysis, PCR-ARMS used to amplification and restriction of target sequence of GTST1 gene, as showing in figures 2 and 3:

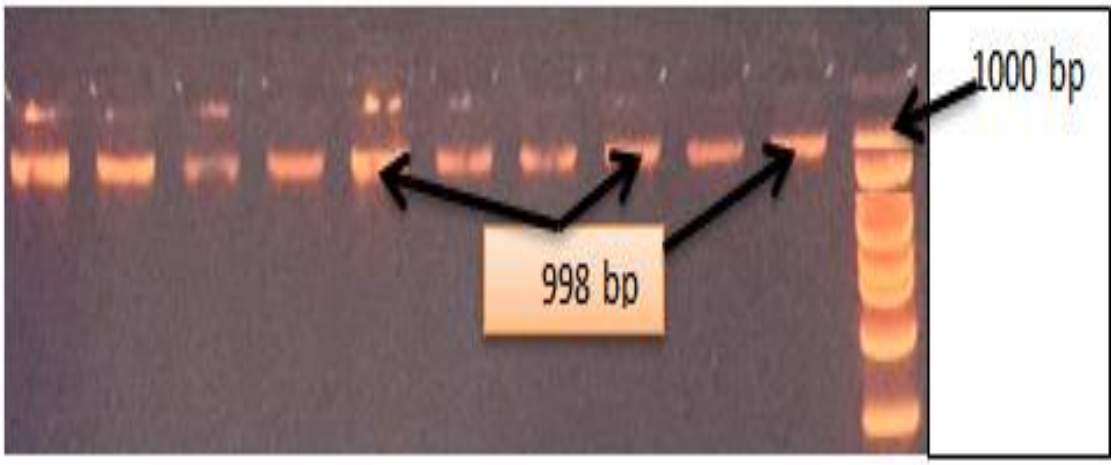

Figure-2: Electrophoresis of the GSTP1 Genotype Ala114 and Val114. Lines 1-5 are associated with genotype Ala114 and lines 6-10 correlated to genotype Val114 that are observed in all samples. Lane M is DNA marker

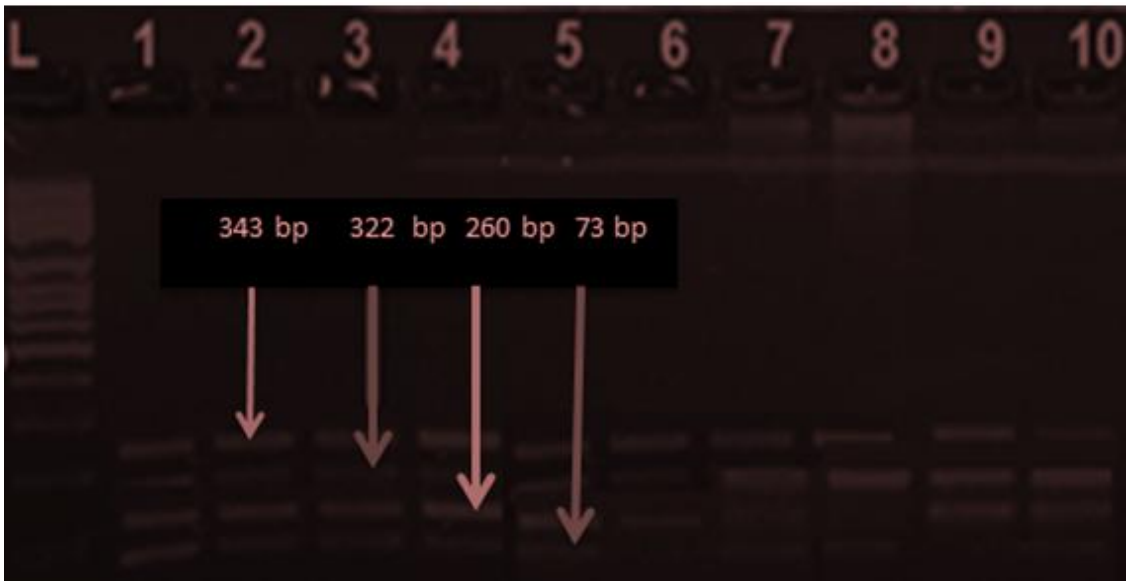

Figure-3. Electrophoresis Results after Incubation with the Alw26I Enzyme. In the presence of 4 bands at positions at lane (1-10): 343, 322, 260, and 73 bp the SNP defines the Ile105, the probability of each of the (GSTP1*A or GSTP1*D) alleles.

The results suggesting statistical differences (odd ratio) in A114A, V114V, and A114V between T2DM and control groups, as showing in table 4:

Table-4: Comparison of A114V genotypes incidence in T2DM and control groups

Genotypes	CONT n=30 (%)	T2DM n= 60(%)	Total	OR (CI 95%)	p-value
A114A	6 (20)	12 (20)	18	1.0 (reference)	0. 0000*
A114V	15 (50)	34 (57)	49	4.49(2.01-7.08)	
V114V	9 (30)	14 (23)	23	3.44 (1. 77-5.12)	0.0000*
TOTAL	30	60	90		
HWE(Alleles frequency) (p+q=1)					
A114	45%	47%		3.68 (1.32-5.76)	0. 0000*
V114	55%	53%			

In this study, the results indicated that the A114A homozygote genotype implied a statistically significant effect (P-value=0.0000) (OR=4.49(2.01-7.08)) for the risk of T2DM. In addition, we observed a significant relation between GSTP1 A114V genotype in exon 5 and the risk of T2DM. Moreover, those with the homozygous V105V genotype, the odds

ratio for T2DM was 3.44 times higher than that for the control group in this study.

The results suggesting statistical differences (OR) in I105I, I105V, and V105V between T2DM and control groups, as showing in table 5:

Table-5: Comparison of I105V genotypes incidence in T2DM and control groups

Genotypes	CONT n=30 (%)	T2DM n= 60(%)	Total	OR (CI 95%)	p-value
I105I	8 (28)	16 (42)	24	1.0 (Reference)	0. 0012*
I105V	18 (30)	23 (30)	41	4.46(1.06-7.07)	
V105V	4 (42)	21 (28)	25	2.67 (1.10-5.70)	0.0067*
TOTAL	30	60	90		
HWE(Alleles frequency) (p+q=1)					
I105	56%	46%	1.65 (0.94-3.39)		0. 019*
V105	43%	54%			

In this study, the results indicated that the I105I homozygote genotype implied a statistically significant effect (P-value=0.0012) (OR=4.46(1.06-7.07) for the risk of T2DM. In addition, we observed a significant relation between GSTP1 I105V genotype in exon 5 and the risk of T2DM. Moreover, those with the homozygous V105V genotype, the odds ratio for T2DM was 2.67 times higher than that for the control group in this study.

Table 6, showing the genotype probability distribution and risk T2DM that associated with GSTP1 genotype and allele frequency:

Table-6: The genotype distribution inT2DM and control groups

Genotypes Probability	T2DM N=60	CONT N=30	OR(95%CI)	p-value
AA	3	1	0.68(0.33-1.92)	0.021
AB	4	2	0.48(0.41-1.73)	0.043
AC	5	3	0.65(0.23-0.67)	0.078
AD	5	3	0.67(0.34-0.78)	0.066
BB	6	4	0.87(0.33-0.78)	0.078
BC	10	5	1.0 (Reference)	-
BD	8	3	1.56(0.77-2.79	0.01
CC	10	4	1.34(0.89-3.44)	0.012
CD	6	3	1.09(0.55-2.67)	0.022
DD	3	2	0.45(0.34-0.88)	0.076

The genetic analysis results suggested that significant difference (p-value<0.05) between T2DM and control groups in AA, AB, BD, CC, and CD genotypes probability distribution that represented GSTP1 SNP while not significant differences in AC, AD, BC, and DD genotypes (p-value>0.05) between both groups.

Discussion

Glutathione S-transferase (GST) belongs to a superfamily of phase II detoxification enzymes, which play an important role in protecting cells from damage caused by endogenous and exogenous compounds by conjugating reactive intermediates with glutathione to produce less reactive water-soluble compounds (12). In the present study, we determined the frequencies of two polymorphisms in exon 5 and exon 6 of the GSTP1 gene in 60 patients with T2DM and 30 normal individuals from Babylon province/ Iraq. The results of the present study indicated a significant difference in the frequency of GSTP1 Ile/Val genotypes in exon 5; whereas, there was also a significant difference in GSTP1 Ala/Val genotypes in exon 6 with the risk of T2DM. Moreover, we found that GSTP1 AA, AB, BD, CC, and CD increased in T2DM compared to the control group. Studying GSTP1 polymorphism also showed a

significant association between GSTP1 Ile/Val genotype and T2DM in exon 5; also significant relation was found between GSTP1 Ala/Val genotypes with exon 6 and T2DM. Considering the different types of alleles, this polymorphism in GSTP1 AA, AB, BD, CC, and CD showed a significant relation with T2DM. Recent studies have reported the relation between GSTP1 Val105 polymorphism and other types of diseases such as cancers (13-15). Garte et al., (2001) were reported that GSTP1 Val/Val genotype is uncommon and exists in 5% of Caucasians (16). Johansson et al., (1998) said that the form of the GSTP1 enzyme has been reported to be 2–3 times less stable than the Ile105 form (17). Cheng-Gang et al., (2012) were showing that individuals with GSTP1 Val/Val genotype had significantly better survival in hepatocellular carcinoma patients (18), while Ramachamdran et al., (2000) suggested that genotype is associated with worse outcomes in basal cell carcinoma (19). Lu et al., 2011 were reported in a meta-analysis study that individuals with GSTP1 Ile105/Val 105 genotype increase susceptibility to breast cancer in the Asian population (20), while, Zhang et al., (2011) and Hamzah et al.,(2020) and other studies were showing a good response and light toxicity were also observed in breast cancer patients carrying GSTP1 Ile105/Val 105 or Ile 105/Ile105 genotype (21-26).

Conclusion:

GSTP1 I105V and A114V may have risk factors to the incidence of T2DM by decreasing levels of TAO-C.

Conflict of interest

No potential conflict of interest relevant to this manuscript was reported.

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