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The association between polymorphism of TCF7L2 gene rs12255372 G/T and type 2 diabetes mellitus in Iraqi women suffering from menopause

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Abstract---Type 2 diabetes mellitus (T2DM) became the most prevalent health problem. Almost half of the world's people are ignorant that have diabetes. Menopause occurs as an important alteration in women through which take place the change in sex hormones, distribution in fat's body, and metabolism, altogether which participate in the metabolism disease such as type 2 diabetes mellitus. Several studies have appeared the association between the TCF7L2 gene and different diseases like type 2 diabetes mellitus (T2DM). This study aimed to detect the relation of the genetic variation polymorphism for the TCF7L2 gene (rs12255372 G/T) in Iraqi women menopausal with T2DM. The outcomes indicated the increased levels of biochemical characteristics including HbA1C, Cholesterol, Triglyceride, Prolactin, Progesterone, and Estrogen and the decreased level of HDL with significant differences ($P<0.05$). While there was no association between SNP for TCF7L2 gene (rs12255372 G/T) in patients with T2DM when compared with control ($P>0.05$). Although that there was a significant association between the biochemical characteristics and genotypes for this SNP. In conclusion that SNP (rs12255372G/T) for the TCF7L2 gene is not represented as a risk factor in Iraqi women of menopausal with type 2 diabetes mellitus.

Keyword---type 2 diabetes mellitus, transcription factor 7-like 2 (TCF7L2) gene, rs12255372 G/T, menopausal women.

Introduction

In the 21st century, Type 2 diabetes mellitus became the most prevalent health problem, it was recorded globally that up to 371 million individuals which have diabetes (IDF,2012). This number of diabetes cases is more than predictable and maybe more estimated in the 2030 year (Wild et al.,2004). Besides, 50% of the world's people are ignorant that have diabetes. Where diabetes was caused death for 4.8 million people in 2012 were the most of them below 60 years (IDF,2012). Menopause occurs as an important alteration in women through which take place the change in sex hormones, distribution in fat's body, and metabolism, altogether which participate in the metabolism disease such as type 2 diabetes mellitus, dyslipidemia, and cardiovascular disease (Jiang et al.,2019). The age timing of menopause plays an important role in the occurrence of these diseases, their morbidity, and mortality (Gold ,2011).

Also, the sex hormones in females are contributed regulating the lipid metabolism (Nikolic et al., 2016) and the homeostasis of glucose (Hao et al.,2015). The epidemiological studies demonstrated the status of menopause was influenced by the levels of estrogen (Pan et al., 2003) and is related to osteoporosis, urogenital atrophy, sexual dysfunction, psychiatric disorders, metabolic disorders, cardiovascular disease ,and obesity (Lobo et al., 2014; Utian and Wood ,2013; Archer et al.,2015). T2DM consider a multigenic metabolic disturbance that happens in the various age categories as a result of environmental and genetic factors (Malecki and Klupa,2005). There are various genes that were studied which anticipated to be as a risk factor in T2DM for the population. Opposite to predictions, there are many genes that have little effects concerning the disease risk and most with discordant results (Florez et al., 2003; Hattersly and McCarthy,2005).

The TCF7L2 gene (transcription factor 7-like 2) existed in10q25.3 at the human chromosome with long 215.9 kb (Zhou et al.,2019). Several studies have appeared the association between the TCF7L2 gene and different diseases like type 2 diabetes mellitus (T2DM), diabetes nephropathy, and nonalcoholic fatty liver (Morgan et al., 2020; Bhatt et al., 2020). Moreover, it was studied the association between single nucleotides polymorphisms for the TCF7L2 gene and the risk of breast cancer in the various populations (Goode et al.,2009; Conor et al., 2012; Naidu et al., 2012). Some one of studies illustrated on postmenopausal women with type 2 diabetes which have osteoporosis, the variation gene polymorphism of TCF7L2 gene can affect in the decline of level gene expression, and the inhibition of proliferation osteoblasts, consequently lead to the osteoporosis (Liu et al.,2021). The current study aimed to detect the relation of the genetic variation polymorphism for TCF7L2 gene in women menopausal with T2DM and also the association between the menopausal status and other clinical characteristics.

Methods

Samples and study design

All 100 blood samples collected from Iraqi women (November 2019 to March 2020) from Specialist Center for Deaf glands and diabetes diseases/ AL-Kindi Hospital /Baghdad/ Iraq. The study groups represented 75 patients (menopausal women with T2DM) and 25 healthy women as controls. Their ages ranged between 60-61 years.

Ethical considerations

The consent was taken from the participants and the collection of blood samples was approved by the ethics committee of the Iraqi Ministry of Health.

Biochemical characteristics detection

HbA1C detected by AFIAS/Avant medical. Netherlands. Further, Cholesterol, Triglyceride, HDL, Prolactin, Progesterone, and Estrogen were detected by using AccuBind ELISA kits from Monobind Inc. USA.

Genetic assay

Frozen blood in the EDTA tube was used to extract DNA via the use Quick-gDNA MiniPrep Zymo kit. The single nucleotide polymorphisms (SNP)for the TCF7L2 gene (rs12255372 G/T) was applied by using the custom SNP genotyping (assay ID: C__291484_20, catalog No. 4351379, Thermo Fisher Scientific, USA), further, TaqMan master mix was utilized from Kapa Biosystems, USA (Catalog: KK4701). SNP detected by using real-time thermal cycler, Sacace Biotechnologies, Italy.The reaction mixture composed of 0.5 µL of working stock of custom SNP genotyping (20×) , 5 µL of TaqMan master mix(2x), DNA sample (2 µL), and the final volume to 20 µL was completed by free nuclease water.Moreover, the conditions of custom SNP genotyping assay have included enzyme activation for 10 min at 95°C , denaturation for 15 seconds at 95°C for 40 cycles, and annealing/extension for 1 min at 60 °C for 40 cycles. These were applied according to the manufacturer.

The analysis of statistical

The clinical and biochemical characteristics were estimated as mean ± SE (standard error) using the SPSS program version 19.0 (SPSS Inc., Chicago, USA). T-test was conducted comparison between the control and patient groups when the p-value is less than 0.05. This means there are significant differences. Hardy–Weinberg equilibrium for the genotypes and alleles in the studies groups was calculated by chi-square with Fischer's exact probability. The odds ratio was estimated by logistic regression analysis.

Results

The clinical and biochemical characteristics of study groups

This study showed significant differences between control and patients according to HbA1C, Cholesterol, Triglyceride, HDL Prolactin, Progesterone and Estrogen levels($P<0.05$) whereas there were no significant differences in age between studies groups ($P>0.05$) (Table 1).

Table 1
Comparison the characteristics of clinical and biochemical between control and patients (mean ± SE)

Characteristics	Groups		Probability
	Control	Patients	
Age (Years)	60.40 ± 1.60	61.17 ± 0.85	0.657
HbA1C level (%)	4.05 ± 0.20	6.68 ± 0.12	1.25×10^{-19}
Cholesterol(mg/dl)	150.96 ± 5.20	430.17 ± 10.37	1.08×10^{-27}
Triglyceride (mg/dl)	150.56 ± 5.31	418.43 ± 11.95	1.50×10^{-22}
HDL (mg/dl)	54.52 ± 1.32	29.95 ± 0.89	9.96×10^{-26}
Prolactin (Unit)	16.16 ± 1.06	41.43 ± 0.79	9.6×10^{-31}
Progesterone (Unit)	13.84 ± 1.21	40.37 ± 0.77	3.80×10^{-32}
Estrogen level (Unit)	198.40 ± 20.62	525.04 ± 6.74	8.59×10^{-36}

Regarding, the results of the comparison between patients and control according to smoking status and blood group distribution there were appeared no significant differences (Table 2 and 3).

Table 2
Comparison between control and patients according smoking status

Smoking status	Control N (%)	Patients (%)	Probability
Smoking	0 (0.0)	5 (6.7)	0.185 NS
Not smoking	25 (100.0)	70 (93.3)	
Total	25 (100.0)	75 (100.0)	

Table 3
Comparison between control and patients according blood groups distribution

Blood group	Control N (%)	Patients N (%)	Probability
A+	3 (12.0)	13 (17.3)	0.529 NS
A-	1 (4.0)	5 (6.7)	0.627 NS
B+	3 (12.0)	7 (9.3)	0.70 NS
B-	2 (8.0)	3 (4.0)	0.427 NS
AB+	6 (24.0)	13 (17.3)	0.462 NS
AB-	1 (4.0)	8 (10.7)	0.313 NS
O+	8 (32.0)	25 (33.3)	0.902 NS

O-	1 (4.0)	1 (1.3)	0.409 NS
Total	25 (100.0)	75 (100.0)	

Association of the studied groups with other diseases

The results demonstrated significant differences with other diseases (P<0.05) as shown in table (4).

Table 4
Comparison between control and patients with other diseases

Genotypes and Alleles	Control No. (%)	Patients No. (%)	OR(CI:95%)	Chi-Square (x^2)	P-value
TT	15 (60.0)	33 (44.0)	0.52 (0.21 – 1.3)	1.923	0.166 NS
TG	3 (12.0)	18 (24.0)	2.3(0.64- 8.44)	1.627	0.202 NS
GG	7 (28.0)	24 (32.0)	1.2(0.41- 3.90)	0.140	0.708 NS
T	33 (66.0)	84 (56.0)	0.66(0.34- 1.27)	1.545	0.214 NS
G	17 (34.0)	66 (44.0)			
NS (P > 0.05).					

Analysis of genetic polymorphism

The results of genotypes and alleles for TCF7L2 gene (rs12255372 G/T) illustrated no significant differences between patients and control (Table 5).

Table 5
Comparison of genotype and allele frequency for TCF7L2 gene (rs12255372 G/T) between studies groups

Other diseases status	Control group	Patients group	Probability
Non	25 (100.0)	0 (0.0)	1.5×10^{-23} **
Blood pressure	0 (0.0)	34 (45.3)	4.3×10^{-5} **
Arthritis	0 (0.0)	29 (38.7)	2.2×10^{-4} **
Heart diseases	0 (0.0)	12 (16.0)	0.033 *
Total	25 (100.0)	75 (100.0)	
** (P < 0.001), * (P < 0.05)			

The relationship between some characteristics studied and SNP for TCF7L2 gene (rs12255372 G/T)

The outcomes in the table (6) demonstrated the distributions of HbA1C level by SNP (rs12255372 G/T) genotypes for TCF7L2 gene there were significant differences between control and patient groups in the TT, TG and GG genotypes(P < 0.05), while the outcomes indicated no significant differences between these genotypes in each group(P > 0.05).

Table 6
Distributions of HbA1C level in the studied groups by SNP (rs12255372 G/T) genotypes

Cholesterol level mean ± SE (mg/dl)			Probability
Genotypes	Control group	Patients group	
TT	155.0 ± 7.22 ^{B}	399.97 ± 15.64 ^{A}	6.60×10^{-13} **
TG	158.33 ± 18.68 ^{B}	439.61 ± 20.42 ^{A}	8.86×10^{-7} **
GG	139.14 ± 6.47 ^{A}	464.63 ± 16.53 ^{A}	6.64×10^{-13} **
** (P < 0.001), * (P < 0.05) The similar letters in column referred to non-significant differences (P > 0.05)			

In the table (7) demonstrates the results distributions of Cholesterol level by SNP (rs12255372 G/T) genotypes for TCF7L2 gene there were significant differences between control and patient groups in the TT, TG and GG genotypes. Also, the outcomes pointed significant differences in the control group for GG genotype compared to TT and TG (P < 0.05), whilst in patient groups appeared no significant differences between these genotypes (P >0.05).

Table 7
Distributions of Cholesterol level in the studied groups by SNP (rs12255372 G/T) genotypes

HbA1C level mean ± SE (%)			Probability
Genotypes	Control group	Patients group	
TT	4.10 ± 0.30 ^{A}	6.45 ± 0.19 ^{A}	3.54×10^{-10} **
TG	4.55 ± 0.50 ^{A}	6.94 ± 0.16 ^{A}	0.003 *
GG	3.74 ± 0.22 ^{A}	6.68 ± 0.22 ^{A}	2.53×10^{-9} **
** (P < 0.001), * (P < 0.05) The similar letters in column referred to non-significant differences (P > 0.05)			

Further, the tables (8 and 9) illustrated the results distributions of Triglyceride an HDL levels by SNP (rs12255372 G/T) genotypes for TCF7L2 gene there were significant differences between control and patient groups in the TT, TG and GG genotypes (P<0.05). Whilst, the findings suggested no significant differences between these genotypes in each group (P>0.05).

Table 8
Distributions of Triglyceride level in the studied groups by SNP (rs12255372 G/T) genotypes

Triglyceride level mean ± SE (mg/dl)			Probability
Genotypes	Control group	Patients group	
TT	152.20 ± 7.19 ^{A}	429.33 ± 18.62 ^{A}	6.76×10^{-13} **
TG	148.67 ± 14.45 ^{A}	410.06 ±25.58 ^{A}	0.0002 **
GG	147.86 ± 10.60 ^{A}	409.71 ± 19.94 ^{A}	3.66×10^{-8} **
** (P < 0.001), * (P < 0.05) The similar letters in column referred to non-significant differences (P > 0.05)			

Table 9
Distributions of HDL level in the studied groups by SNP (rs12255372 G/T) genotypes

Progesterone level mean ± SE (Unit)			Probability
Genotypes	Control group	Patients group	
TT	15.33 ± 1.46 A	39.70 ± 1.17 A	6.60×10^{-13} **
TG	11.0 ± 3.22 A	41.50 ± 1.54 A	6.42×10^{-10} **
GG	11.86 ± 2.61 A	40.46 ± 1.40 A	6.61×10^{-13} **
** (P < 0.001), * (P < 0.05) The similar letters referred to in column non-significant differences (P > 0.05)			

Table 10
Distributions of Prolactin level in the studied groups by SNP (rs12255372 G/T) genotypes

HDL level mean ± SE (mg/dl)			Probability
Genotypes	Control group	Patients group	
TT	54.40 ± 1.61 A	30.51 ± 1.14 A	6.61×10^{-13} **
TG	52.67 ± 5.78 A	28.56 ± 2.19 A	0.00002 **
GG	55.57 ± 2.60 A	30.21 ± 1.64 A	1.14×10^{-10} **
** (P < 0.001), * (P < 0.05) The similar letters in column referred to non-significant differences (P > 0.05)			

Besides, the outcomes in tables (10,11,and12) indicated to significant differences between control and patient groups in the TT, TG and GG genotypes in the distributions of Prolactin, Progesterone and Estrogen levels by SNP (rs12255372 G/T) for TCF7L2 gene(P < 0.05), whereas the outcomes pointed no significant differences between these genotypes in each group(P >0.05).

Table 11
Distributions of Progesterone level in the studied groups by SNP (rs12255372 G/T) genotypes

Prolactin level mean ± SE (Unit)			Probability
Genotypes	Control group	Patients group	
TT	16.0 ± 1.13 A	41.67 ± 1.17 A	6.60×10^{-13} **
TG	20.67 ± 2.40 A	40.11 ± 1.56 A	0.00009 **
GG	14.57 ± 2.60 A	42.08 ± 1.49 A	6.66×10^{-13} **
** (P < 0.001), * (P < 0.05) The similar letters referred to non-significant differences (P > 0.05)			

Table 12
Distributions of Estrogen level in the studied groups by SNP (rs12255372 G/T) genotypes

Estrogen level mean ± SE (Unit)			Probability
Genotypes	Control group	Patients group	
TT	180.53 ± 25.02 A	526.42 ± 10.82 A	6.60×10^{-13} **
TG	161.67 ± 51.60 A	517.06 ± 13.51 A	4.66×10^{-11} **

GG	252.43 ± 43.18 A	529.13 ± 11.33 A	9.43×10^{-13} **
** (P < 0.001), * (P < 0.05) The similar letters referred to in column non-significant differences (P > 0.05)			

Discussion

Type 2 DM spreads at high rates in women, and the status of postmenopausal is considered a risk factor for Type 2 DM; therefore, it should not be neglected postmenopausal women where Type 2 DM prevalence risk increased by 13.22% in women postmenopausal (Ren et al.,2019). Also, one of the studies illustrated the level of glucose increased following menopause, and the metabolic disorder, including dyslipidemia (cholesterol, lipids, hypertension) and obesity these represent the risk factors for type 2 DM (Zhou et al.,2010). This agreed with the current study, it was observed significant differences of the levels of the characteristics biochemicals in menopausal women with type 2 DM comparing to control group. TCF7L2 gene is considered one of the candidate genes in the synthesis and processing of insulin. Also, it is belonged to the transcription factors class.

TCF7L2 gene is known to display genes transcription including intestinal proglucan in β- pancreatic cells, adipocytes, and intestinal cells via the β-catenin heterodimerizing.TCF7L2 has strong association with type2DM in populations from the West African, Iceland, Italian, Polish, Danish, and U.S. Also, TCF7L2 gene has association with type2 DM on the Indian populations (Yadav et al.,2016). In the current study there were no association for TCF7L2 gene (rs12255372) with type 2DM in menopausal women. Also, Shi et al. (2014) they illustrated that SNP(rs12255372) no significant differences between gestational diabetes mellitus patient and control group. Previously, there were not found relation between the varients of TCF7L2 gene and type2 DM in independent researches in population Chinese, Pima Indians, and Emirati Arabs (Chang et al., 2007; Guo et al., 2007; Saadi et al.,2008). Further, a study showed that was performed in Arab population a fail or no relation of type2DM with SNPs for TCF7L2 (rs12255372 and rs7903146) (Alsmadi et al., 2008). While, several clinical research was performed in U.S.A at 27 centers, it was found that SNPs for TCF7L2 (rs122 55372 and rs7903146) were related with a rise risk of diabetes in individuals with decreased glucose tolerance (Florez et al.,2006).

Besides, in other studies on SNP (rs12255372) this SNP associated significantly with type2DM where the T allele for SNP rs7903146 and rs12255372 consider a risk factor for Turkish populations (Erkoc Kaya et al.,2017). Concerning the association of the genotypes for SNP (rs12255372) with the clinical and biochemical characteristics. It was found there are the little studies that studied the relationship between genotypes and biochemical and clinical characteristics, and these studies contrasted with the current study. Where Shokouhi et al. (2014) and Alami et al. (2012) showed that the GG and GT + TT genotypes for SNP (rs12255372) in control and type 2 diabetic groups had no differences in the clinical and biochemical characteristics when compared with these genotypes. Whereas in the current study there were significant differences. Thus, through a literature review about the relationship between women menopausal with type

2DM and genetic polymorphism for TCF7L2 (rs12255372), there was little published research for this relationship.

Conclusion

The results point that SNP (rs12255372G/T) for the TCF7L2 gene is not represented as a risk factor in Iraqi women of menopausal with type 2 diabetes mellitus. Although there are significant differences among the genotypes, the clinical and biochemical characteristics.

Declaration of conflicts

The authors declare no competing conflicts of interest

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