Association of 11beta-hydroxysteroid dehydrogenase type 1 (HSD11B1) gene polymorphism with Type 2 Diabetes Mellitus in a sample Egyptian Population

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ABSTRACT
Background: Recently, numerous studies have associated polymorphisms in 11beta-hydroxysteroid dehydrogenase type 1 (HSD11β1) gene and T2DM risk. However, there is still a lack in the published literature evaluating the association between HSD11β1gene polymorphism and the risk of T2DM.
Objective: The Aim of the study was to assess the association of HSD11β1gene polymorphism (rs846910) with Type 2 diabetes mellitus (T2DM) in a sample of the Egyptian population.
Patients and methods: Our study was conducted on 100 subjects; their mean age was 50.26 ± 9.1 years old. They were selected from Diabetes Outpatient Clinics of our institution for 6 months in the period from June 2019 to December 2019. They were divided into two groups: 60 type 2 diabetic obese patients with BMI > 25 kg/m^2 (Group I) and 40 age- and sex-matched controls (Group II).
Results: There was no statistically significant difference between cases and controls in terms of HSD11β1 polymorphism (rs846910). The regression analysis showed that the HSD11β1 polymorphism did not significantly increase the risk of diabetes mellitus (DM). Detection of HSD11β1 gene polymorphism in the study showed that homozygous GG genotype was more prevalent than GA genotype. However, there was no significant difference between GG and GA in terms of fasting and postprandial sugar and in terms of lipid profile.
Conclusion: The present study showed no significant association between HSD11β1 polymorphism (rs846910) and T2DM. Moreover, the HSD11β1polymorphism (rs846910) is not correlated with glycemic status or lipid profile.
Keywords: HSD11B1 gene polymorphism, Single nucleotide polymorphism, T2DM, Obese.

INTRODUCTION

Diabetes mellitus is one of the fastest rising global health crises of the 21st century. In 2019, it is estimated that 463 million people have diabetes and this number is expected to reach 578 million by 2030, and 700 million by 2045. Egypt has been among the top ten countries for the number of people with DM with 8.9 million expected to project to 11.9 million in 2030. This carries a huge economic and social burden [1].

T2DM accounts for 90–95% of all diabetic population. The increased incidence of T2DM increases with age, obesity, and lack of physical activity. Obesity is the leading cause of insulin resistance, which is the fundamental risk factor for T2DM development [2]. While environmental factors could trigger the pathogenesis of T2DM, the development of T2DM is presumed to be largely genetically determined. The higher concordance rate for T2DM development in monozygotic twin pairs than dizygotic twins advocates the significant contribution of genetic factors [3]. To date, more than 50 genes have been studied for their T2DM susceptibility in various populations; a shred of rising evidence has linked polymorphisms in HSD11β1 gene and T2DM risk [4].

Recently, evidence has linked polymorphisms in HSD11β1 gene and T2DM risk. HSD11β1 encodes 292 amino acid proteins. HSD11β1 enzyme produces active cortisol from inert cortisone, thus plays a prominent role in glucocorticoid action [5]. Among many, Single Nucleotide Polymorphisms (SNPs) of the HSD11β1 gene, rs846910, in the 5' promoter region of the HSD11β1 gene, has been studied and the results showed a conflicting association with T2DM and metabolic syndrome in different populations [6]. However, there is still paucity in the published data that evaluates the association between HSD11β1gene polymorphism and the risk of T2DM. Therefore, this study aimed to evaluate the association of HSD11β1gene polymorphism (rs846910) with T2DM in a sample of Egyptian population.

PATIENTS AND METHODS

Our study was conducted on 100 subjects; their mean age was 50.26 ± 9.1 years old. They were selected from Diabetes Outpatient Clinics of our institution for 6 months in the period from June 2019 to December 2019. They were divided into two groups: 60 type 2 diabetic obese patients with BMI >

25 kg/m^2 (Group I) and 40 age- and sex-matched controls (Group II).

Ethical approval
An approval of the study was obtained from Ain Shams University Academic and Ethical Committee. Informed consents were obtained from all participants included in our study.

All patients recruited in this study were subjected to full medical history, family history of DM, medication list (metformin, oral hypoglycemic drugs, insulin, and statins). All subjects were physically examined (Body Mass Index (BMI), waist circumference (WC), systolic, and diastolic blood pressure. Laboratory investigations included FBS, 2 hours post prandial blood glucose, HbA1c, lipid profile, Fasting s. insulin, HOMA-IR, and PCR for analysis of HSD11β1 gene (rs846910).

Exclusion criteria: Patients with chronic liver disease, acute or chronic kidney disease, type 1 DM, known cases of connective tissue disorder, malignancy and infections were excluded from our study.

Patient's demographics and medical history were obtained from the patient's file or during the patients visit. BMI was calculated using the standard formula [weight (kg)/height (m^2)].

Whole blood samples were collected after an overnight fasting from all 100 subjects. Blood collected was split into three portions; the first portion was used to measure fasting blood glucose (FBG) and collected in Na fluoride-containing vacutainer tubes. The second portion was collected on EDTA containing vacutainer tubes to measure glycated hemoglobin (HbA1c %). The third portion of blood was centrifuged to separate serum for the measurement of insulin and lipid profile (triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C)) and isolation of DNA and analysis for HSD11β1 (rs846910) gene polymorphism by real-time PCR technique. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following equation: [fasting blood glucose (mg/dL) × fasting blood insulin (μU/mL)/405]. All routine work analyses were measured on the same day of the blood collection, the remaining samples were stored at -80°C till the time of assay for insulin and HSD11β1(rs846910) gene polymorphism.

Isolation of DNA and analysis for HSD11β1(rs846910) gene polymorphism was done by real-time PCR technique, which included:1) DNA Extraction by salting out method (DNA Quick reagents, Bio-serve.); 2) Amplification and allelic discrimination using TaqMan universal master mix II and ready-made TaqMan SNP genotyping assay for rs846910. TaqMan SNP genotyping assay for rs846910 contains sequence-specific forward and reverse primers to amplify the polymorphic sequence of interest.

DNA fragments containing the analyzed polymorphisms for rs846910 were amplified using the following primers and probes: F: 5'-CTC TGT TGC TTG TGC TTG ATT C-3', R: 5'-AGA GCA GGC TTT CAG CAG ATA C-3' and unlabeled probe: 5'-CTGGTGGGAATGATCAAATCAGAGAGA-3'.

The PCR was initiated with a 10-min denaturation at 950 ºC. Thermal cycling (60 cycles) consisted of denaturation at 950 ºC for 10 s, then 10 s annealing with a touchdown protocol covering a range of annealing temperatures from 680 ºC to 580 ºC (decreasing 180 ºC per cycle), and elongation at 720 ºC for 10 s. HRM acquisition and analysis After amplification, PCR products were denatured at 950 ºC for 1 min and cooled (2.50 ºC/s to 400 ºC) to form heteroduplexes. High-Resolution Melting Analysis was performed by heating from 558 ºC to 900 ºC, with 25 acquisitions per 10 ºC [7].

Statistical analysis
The collected data were revised, coded, tabulated, and introduced to a PC using Statistical Package for Social Science (SPSS 20). Data were presented and a suitable analysis was done according to the type of data obtained for each parameter. **Descriptive statistics:** Mean ± Standard deviation (SD) for numerical data and Frequency and percentage of non-numerical data. Mann Whitney test for abnormally quantitative variables, to compare between two studied groups. Chi-square test for categorical variables, to compare between different groups. Correlation analysis (using Spearman's method) was used to assess the strength of association between two quantitative variables. The correlation coefficient (denoted symbolically "rs" for Spearman's correlation) defines the strength and direction of the linear relationship between two variables. Probability (p) ≤ 0.05 was considered significant and < 0.01 highly significant. Significant relations were graphically represented by pie and scatter graphs.

RESULTS
The current study showed significant differences between cases and controls as regards SPB, DBP, WC and BMI. Patients had significantly higher SBP, DBP, WC, and BMI than the control group. Similarly, there were statistically significant differences between cases and controls regarding FBG, 2hPP and HbA1c (p < 0.01). Patients had significantly higher FBG, 2hPP, and HbA1c than the control group (Table 1). In addition, there were statistically significant differences between cases and controls in terms of total cholesterol, triglycerides (TG), LDL-C, and HDL-C. Patients had significantly higher total cholesterol level,

TG, and LDL-C than control group, while HDL-C was higher in control group (p <0.01) as shown in table (1).

Regarding our primary outcome, there was no statistically significant difference between cases and controls in terms of HSD11β1 (rs846910) gene polymorphism (p =0.447) (Table 2).

Also, the regression analysis showed that the HSD11β1 (rs846910) polymorphism did not significantly increase the risk of diabetes mellitus (OR 1.724, 95% CI (0.601 – 4.94)) as shown in table (3).

Moreover, there were no statistically significant differences between individuals with GG and individuals with GA in terms of SPB, DBP, WC and BMI [(p > 0.05) "Table 4"]. Similarly, there were no statistically significant differences between individuals with GG and individuals with GA regarding glycemic profile (FBG, 2 hours post prandial blood glucose and HbA1c), HOMA-IR and lipid profile (total cholesterol, triglycerides, LDL-C, and HDL-C) (p >0.05) as shown in table (4).

Table (1): Comparison between groups regarding demographic, Clinical and laboratory characteristics

Variables	Patient n= 60		Control n=40		P-value
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	
Age in years	50.26 ± 9.1	49.5 (42.5-55)	49.5 ± 5.99	48 (46.25-52)	0.719
SBP (mmHg)	130.6 ± 6.4	130 (130 – 132.5)	118.25 ± 7.48	120 (110 – 120)	<0.001
DBP (mmHg)	82 ± 6.7	80 (80 - 90)	76.5 ± 5.87	80 (70 – 80)	0.004
WC (cm)	120.57 ± 8.49	118 (116 - 125)	103.7 ± 6.4	105 (102 –176.25)	<0.001
BMI (Kg/cm2)	38.86 ± 7.2	37.87	27.63 ± 1.3	25.83	<0.001
FBG (mg/dl)	171.17 ± 7.6	145.5	81.15 ± 4.58	82	< 0.001
2hPP (mg/dl)	235.13 ± 2.4	234	95.7 ± 4.49	95.5	< 0.001
HbA1c %	8.87 ± 1.25	8.05	5.705 ± 0.24	5.8	< 0.001
Total Cholesterol (mg/dl)	172.43 ± 8.42	171	129.1 ± 8.35	131.5	< 0.001
Triglycerides (mg/dl)	154.23 ± 4.22	157.5	115.1 ± 8.7	112.5	< 0.001
LDL-C	71.7 ± 3.8	65	51.3 ± 8.24	50	< 0.001
HDL-C	45.2 ±2.7	45	53.7 ± 4.7	55	0.003

*Data are presented as mean ± SD, median (IQR), or number.

Table (2): Comparison between studied groups regarding Genotype data

Variables		Patient n= 60	Control n=40	P-value
HSD11β1	**GG**	46 (76.7%)	34 (85%)	**0.447**
	GA	14 (23.3%)	6 (15%)	

*Data are presented as number (%). HSD11β1: Hydroxysteroid 11-Beta Dehydrogenase 1

Table (3): Association analysis of all patients included in the study (n=60) and control (n=40) regarding Genotype data

Variables	Odds Ratio	95% CI	P-value
HSD11β1 polymorphism	1.7246	0.601 – 4.94	**0.447**

*Data are presented as mean ± SD, median (IQR), or number (%). HSD11β1: Hydroxysteroid 11-Beta Dehydrogenase 1; OR: Odds ratio

Table (4): Comparison between GG (n=80) and GA (n=20) patients regarding demographic, clinical and laboratory data

Variables	GG n= 80		GA n=20		P-value
	Mean ± SD	**Median (IQR)**	**Mean ± SD**	**Median (IQR)**	
Age in years	49.18 ± 7.1	48	52.42±10.4	50	0.257
Sex **Male**	38 (47.5%)		8 (40%)		0.252
Sex **Female**	42 (52.5%)		12 (60%)		
SBP (mmHg)	125.06 ± 6.4	130	127.14 ± 10.6	130	0.259
DBP (mmHg)	79.87 ± 6.6	80	79.52 ± 7.4	80	0.892
BMI (Kg/cm^2)	34.35 ± 1.1	33.29	34.1 ± 1.3	35.15	0.93
WC (cm)	114.35 ± 11.3	115	111.62 ± 12.7	117	0.532
FBG (mg/dl)	129.9 ± 9.5	103	150.48 ± 8.6	120	0.461
2hPP (mg/dl)	173.84 ± 9.6	125	196.24 ± 14.5	155	0.386
HbA1c %	7.45 ± 1.14	6	7.94 ± 1.7	7.5	0.459
HOMA-IR	3.4 ± 0.1	3.2	3.3 ± 0.4	3.1	0.571
Cholesterol (mg/dl)	155.66 ± 8.9	140	129.1 ± 8.35	131.5	0.642
TG (mg/dl)	138.26 ± 33.5	128	115.1 ± 8.7	112.5	0.808
LDL	65.47 ± 9.2	52	51.3 ± 8.24	50	0.238
HDL	48.64 ± 5.4	47	53.7 ± 4.7	55	0.945

*Data are presented as mean ±SD, median (IQR), or number (%).

DISCUSSION

The prevalence of DM has been growing all over the world throughout recent decades and the trend is set to progress as a consequence of population aging, urbanization, and associated lifestyle changes. In 2019, the number of deaths caused by diabetes and its complications is estimated to be 4.2 million and the annual global health expenditure on diabetes is estimated to be USD 760 billion and expected to reach USD 825 billion by 2030 worldwide [1]. T2DM, which accounts for the vast majority of diabetes cases, is a multifactorial disease with a strong genetic basis, with heritability estimates ranging from 30 to 70% [8, 9]. Thousands of germline genetic loci estimated to contribute to disease, including more than 200 that have been identified to date [10].

Overexpression of HSD11β1 involved in the interconversion of inert cortisone and active cortisol in metabolically active tissues, and is associated with insulin resistance, metabolic syndrome, T2DM and hypertension. The role of single-nucleotide polymorphisms (SNPs) of the HSD11B1 gene in the susceptibility to metabolic syndrome and T2DM has been reported [11]. However, the established association between HSD11β1gene (rs846910) polymorphism and the risk of T2DM is still unclear.

This study showed that there was no statistically significant difference between cases and controls in terms of HSD11β1 polymorphism (rs846910). The regression analysis showed that the HSD11β1 polymorphism did not significantly increase the risk of DM (OR 1.724, 95% CI (0.601 – 4.94)). Moreover, there were no statistically significant differences between individuals with GG and individuals with GA in terms of SPB, DBP, WC, BMI, glycemic profile (FBG, 2Hpp and HbA1c), HOMA-IR and lipid profile (total cholesterol, triglycerides, LDL-C, and HDL-C) (p > 0.05). In concordance with our findings, a systematic review was conducted to evaluate the association of HSD11β1 gene expression in abdominal adipose tissue and HSD11B1 polymorphisms with T2DM. The authors reported that most of the included studies showed no significant associations between HSD11B1 polymorphisms and T2DM [12]. The association between HSD11β1 polymorphisms and T2DM and metabolic phenotypes was studied in Koreans. The results showed no association between common polymorphisms of HSD11B1 and T2DM [13]. Correspondingly, the correlation of common SNPs in HSD11β1 (rs12086634), (rs1000283) and H6PD (rs6688832), (rs17368528) with T2DM and metabolic syndrome were studied in Korean research and no substantial link of these SNPs with T2DM and metabolic syndrome was found [14]. Moreover, the association of HSD11β1gene variants with diabetes, hypertension, and obesity was examined in a longitudinal population study of American Indians (N=918). The results showed that the associations between HSD11β1 gene variants with diabetes were not statistically significant [15].

On the contrary, the association of HSD11β1 (rs12086634 and rs846910) gene polymorphisms were assessed with T2DM. A total of 616 subjects were enrolled. The association analysis indicated that HSD11β1 rs12086634 TG and HSD11β 1 rs846910 AG contributed to an increased risk of T2DM [16]. Similarly, genetic association studies in Pima Indians were done to study the association between HSD11β1 gene polymorphisms with T2DM. The results showed that the genetic variations in the HSD11B1 gene were associated with T2DM, plasma insulin concentrations, and insulin action [6].

The exact causes of such heterogeneity in the published literature, regarding the association between HSD11β1 gene polymorphisms with T2DM, are unclear. However, this difference can be attributed to many methodological differences. Different populations, whether healthy subjects or T2DM patients, were included in the above-mentioned studies. Moreover, the type of polymorphism was substantially different among the published studies. Another explanation is the differences in sample size.

The limitations of our study were the small sample size, which may be not sufficient to detect association between the SNP (rs846910) polymorphism and type 2 diabetes. Second, rs846910 polymorphism was the only screened single-nucleotide polymorphism (SNP) for evaluation of the relationship between HSD11β1gene polymorphism and T2DM while other SNPs may have strong associations with diabetes or metabolic syndrome in Egyptian population. Therefore, the probability of a positive association between HSD11β1gene polymorphism and diabetes remains.

CONCLUSION

The present study showed no significant association between HSD11β1 polymorphism (rs846910) and T2DM. The HSD11β1 polymorphism did not significantly increase the risk of diabetes mellitus in obese individuals.

List of Abbreviations:

HSD11β1: 11beta-hydroxysteroid dehydrogenase Type 1
SNP: single-nucleotide polymorphism
DBP: Systolic blood pressure
DBP: Diastolic blood pressure
WC: Waist circumference
BMI: Body mass index
FBG: Fasting blood glucose
HbA1c: Glycated hemoglobin

HOMA-IR: Homeostatic model assessment-insulin resistance
TG: Triglyceride; LDL: Low-density lipoprotein;
HDL: High-density lipoprotein
T2DM: type 2 diabetes mellitus
DM: Diabetes Mellitus.

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