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Potential Role of *Peroxisome Proliferator-activated Receptor Gamma Coactivator-1 Alpha (PGC-1α)* Gene Polymorphism in Patients with Type 2 Diabetes Mellitus

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ABSTRACT

Background: *Peroxisome Proliferator-activated Receptor Gamma Coactivator-1 Alpha* (*PGC-1α*) is a cellular modulator of oxidative and lipid metabolism. It has a vital role in the regulation of mitochondrial activity. Patients with type 2 diabetes mellitus (T2DM) have mitochondrial abnormality and reduced mitochondrial numbers within the cells of skeletal muscles.

Objective: To evaluate the correlation between *PGC-1α* gene polymorphism and T2DM in Egyptian patients attending Zagazig University Hospitals.

Patients and Methods: This was a case-control study including 136 participants of both sexes recruited from the Zagazig University Hospitals. The participants were divided into two equal groups: control group with normal individuals and T2DM group with 68 participants in each group.

Results: The incidence of *A* allele was significantly higher in T2DM group. Also, there were statistically significant elevations in waist hip ratio (WHR) and fasting blood sugar (FBS) among carriers of *GA* and *AA* genotypes. However, there was no significant correlation between gene polymorphism and lipid profile in T2DM group. After applying multivariate analysis, *A* allele carriers and increased values of WHR, FBS, HOMA-IR and low-density lipoprotein-cholesterol (LDL-c) were detected in T2DM.

Conclusion: We concluded that *A* allele of *PGC-1α* polymorphism was a possible predictor of T2DM occurrence in Egyptian patients. Additionally, WHR and FBS were significantly higher among carriers of *GA* and *AA* genotypes

Keywords: *PGC-1α*, polymorphism, Type 2 diabetes mellitus.

INTRODUCTION

Diabetes mellitus is defined by the World Health Organization as a metabolic abnormality accompanied with alteration of glucose, protein, and lipid metabolism accompanied by increased glucose level, and insulin resistance. The most prominent type is type 2 diabetes mellitus (T2DM) representing nearly 90% of all cases with diabetes mellitus [(1)].

T2DM affects nearly 300 million people worldwide nowadays, and more than 590 million individuals are expected to develop T2DM by 2035 [(2)]. Egypt comes 10th in the countries with high diabetes mellitus incidences with 7.5 million diabetic cases estimated by the International Diabetes Federation with expected elevation by 2035 up to 13.1 million cases [(3)].

Peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1α) is a potential coactivator of multiple transcriptional factors that has a variety of biological activity in various tissues. It has an oxidative metabolism regulator activity especially in reactive oxygen species production. Any alteration in *PGC-1α* expression may alter the metabolic processes, which influence thermogenesis, adipogenesis, and gluconeogenesis which could result in insulin resistance[(4)].

PGC-1α is a key regulator of metabolic adaptations as it regulates the expression of key enzymes involved in β-oxidation, cellular energy metabolism regulation, and various aspects of glucose metabolism, including glucose production and utilization, hepatic gluconeogenesis, and glucose uptake in skeletal muscles [(5)].

AIM OF THE STUDY

The present study aims to evaluate the correlation between *PGC-1α* gene polymorphism and T2DM in Egyptian patients attending Zagazig University Hospitals**.**

PATIENTS AND METHODS

This case-control study included 136 participants of both sexes (68 participants in each group) recruited from the Internal Medicine Department, Zagazig University Hospitals.

Ethical consent:

The study design was approved by the Institute Review Board of the Ethical Committee of Faculty of Medicine, Zagazig University (ZU-IRB #6705/27-2-2021).

Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

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Group I (control group): This group included 68 normal apparently healthy volunteers not suffering from any diseases that might interfere with the present study.

Group II (diabetic group): This group included 68 patients with T2DM. The ages of cases were between 35-65 years old.

The exclusion criteria included liver, renal, or heart failure. Patients with malignancy and cases with Type 1 DM were also excluded from the study.

All cases were subjected to complete history taking, complete clinical and physical examination, and the estimation of fasting blood glucose (FBS), HOMA-IR, glycated hemoglobin (HbA1c), lipid profile and serum creatinine. Also, all participants were subjected to the detection of Gly482Ser polymorphism in *PGC-1α* gene by polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP).

Detection of *Gly482Ser* polymorphism in *PGC-1α* gene:

G-spin ™ Total DNA Extraction Kit (iNtron bio-tehnology, Seongnam-Si, Gyeonggi-do, Korea) was used for total DNA extraction from blood, as described by the manufacture. The extracted DNA absorbances at 260 and 280 nm wavelengths were determined using Milton Roy Spectronic 3000 Array to determine DNA concentration and purity.

The amplification was performed using the thermal cycler PTC-100 device (AG Eppendorf. Inc. Mastercycler, Hamburg. Germany) according to the following protocol: initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 57°C for 30 seconds, extension at 72°C for 30 seconds, and final extension at 72°C for 10 minutes. The used primers were: Forward 5' GGGCAGATTTGTTCTTCCA 3' and Reverse 5' GTCATCAAACAG GCCATCC 3' PCR products were digested using the restriction enzyme Hpall. The *PGC-1a* gene polymorphism showed two possible alleles, allele G and A with three available genotypes: G/G, G/A, A/A.

The digests were subjected to electrophoresis on a 2.5% agarose gel then stained with ethidium bromide and visualized with UV transilluminator. DNA yields allele (G) was cleaved by Hpall and gave 182-bp and 97-bp bands, and allele (A) wasn't cleaved by this enzyme [(6)].

Statistical analysis

Statistical Package for the Social Sciences (SPSS version 20.0) software was used for data analysis and collection. Quantitative data were represented by mean ± standard deviation (SD). Difference and association of qualitative variable were performed by Chi square test (X^2). Differences between quantitative independent groups was performed by t test. P value was set at <0.05 for significant results and <0.001 for highly significant results.

RESULTS

Regarding the basic characteristics of the studied groups, there was no significant variations in age or sex (P=0.829 and 0.501, respectively), while blood pressures (both systolic and diastolic) were remarkably higher in T2DM groups as compared with controls (P<0.001 for each) (Table **1**).

Table (1): Basic characteristics of the studied groups

Variables	Studied groups		t test/ X^2#	P
	Controls N =68	Type 2 DM N =68		
Age Mean ± SD	60.2 ± 6.8	60.7 ± 5.2	0.122	0.829
Sex				
Male N (%)	18 (58.1)	14 (45.2)	1.38#	0.501
Female N (%)	13 (41.9)	17 (54.8)		
Systolic blood pressure (mm\Hg) Mean ± SD	118.6 ± 8.96	155.2 ± 11.6	20.9	<0.001**
Diastolic blood pressure (mm\Hg) Mean ± SD	79.5 ± 8.6	99.6 ± 8.7	13.5	<0.001**

****:** highly significant difference.

There were highly statistically elevations of body mass index (BMI), waist hip ratio (WHR), fasting blood sugar (FBS) and glycated hemoglobin (HbA1C) levels in T2DM group as compared to the control group (P<0.001 for each). Remarkable higher levels of total cholesterol, triglycerides (TG), and low-density lipoprotein- cholesterol (LDL-c) were observed in T2DM group than control group (P<0.001 for each). However, regarding high-density lipoprotein-cholesterol (HDL-c) levels, the relation between the groups was not remarkably different (P=0.05) (Table **2**).

Table (2): Biochemical and blood lipid data of the studied groups

Variables	Studied groups: Controls N =68	Type2 DM N =68	t test	P
	Mean ± SD			
BMI (Kg\m^2)	24.9 ± 4.23	29.3 ± 5.22	5.41	<0.001**
WHR	0.93 ± 0.12	1.66 ± 0.22	24.2	<0.001**
Waist circumference (cm)	85.5 ± 8.91	113.7 ± 11.4	16.1	<0.001**
FBS (mg\dL)	88.3 ± 2.65	145.2 ± 6.34	68.3	<0.001**
HbA1C (%)	5.33 ± 0.95	9.12 ± 1.34	19.1	<0.001**
HOMA IR	1.52 ± 0.31	4.26 ± 1.01	19.7	<0.001**
Serum creatinine (mg\dL)	0.74 ± 0.12	1.1 ± 0.13	16.8	<0.001**
TG (mg\dL)	132.5 ± 14.4	211.6 ± 10.6	36.5	<0.001**
HDL-c (mg\dL)	45.7 ± 6.1	43.6 ± 6.3	1.91	0.05
LDL-c (mg\dL)	122.8 ± 21.5	138.1 ± 15.1	4.83	<0.001**
Cholesterol (mg\dL)	195.2 ± 25.1	224.4 ± 31.7	5.98	<0.001**

BMI: body mass index**; WHR:** waist hip ratio**; FBS:** fasting blood sugar; **HbA1C:** glycated hemoglobin; **TG:** triglycerides; **HDL-c:** high density lipoprotein- cholesterol; **LDL-c:** low density lipoprotein- cholesterol**;** **: highly significant difference

Regarding the distribution of *Gly482ser gene* polymorphism among the studied groups, it was shown that the incidence of *A* allele was significantly higher in T2DM group (19.6%) compared to the control group (7.4%) (OR=5.5, 95% CI=1.04–10.8 and P=0.007) as shown in (Table **3**).

Table (3): Different genotypes and allele distribution of Gly482ser gene polymorphism among the studied groups

Genotype	Controls (n=68) N	%	Type 2 DM (n=68) N	%	OR (95% CI)	P
GG	60	88.2	47	69.1	1	Ref.
GA	6	8.8	17	25	2.2 (0.7-6.7)	0.01*
AA	2	2.9	4	5.9	7.9 (0.59-57.8)	0.12
A allele	10	7.4	25	19.6	5.5 (1.04-10.8)	0.007*
G allele	126	92.6	111	80.4		

*: significant difference between the groups

The current study revealed that BMI, WHR, FBS, HbA1C and HOMA-IR were higher among carriers of *GA* and *AA* genotypes, but there was a significant difference only regarding WHR and FBS (P=0.008 and 0.02, respectively) (Table 4).

Table (4): The relation between gene polymorphism and the biochemical data among type 2 diabetic patients

Variables	Type 2 diabetic patients (n=68): GG N =47	GA N =17	AA N=4	F test*	P-value
	Mean ± SD				
BMI (Kg\m^2)	28.3 ± 1.19	28.9 ± 4.2	26.8 ± 2.32	1.33	0.27
WHR	0.97 ± 0.17	1.12 ± 0.16	1.02 ± 0.09	5.12	0.008*
Waist circumference (cm)	109.2 ± 9.91	110.5 ± 6.65	111.6 ± 10.1	0.22	0.81
FBS (mg\dL)	137.5± 10.6	149.5 ± 23.3[a]	146.3 ± 15.8[a]	4.24	0.02*
HbA1C (%)	9.13 ± 0.62	9.14 ± 0.16	9.11 ± 1.22	0.007	0.99
HOMA-IR	3.95± 0.28	4.15 ± 0.61	4.11 ± 1.21	1.26	0.29
Serum creatinine (mg\dL)	0.97 ± 0.02	0.99 ± 0.11	0.99 ± 0.12	0.74	0.84
TG (mg\dL)	197.5 ± 20.6	201.5 ± 23.3	200.3 ± 25.8	0.22	0.81
HDL-c (mg\dL)	42.4 ± 5.62	41.3 ± 2.16	41.1 ± 4.22	0.37	0.68
LDL-c (mg\dL)	138.5 ± 32.8	137.5 ± 25.1[a]	137.1 ± 31.1	0.009	0.99
Cholesterol (mg\dL)	223.9 ± 24.9	224.5 ± 27.1	223.5 ± 30.5	0.004	0.997

BMI: body mass index**; WHR:** waist hip ratio**; FBS:** fasting blood sugar; **HbA1C:** glycated hemoglobin; **TG:** triglycerides; **HDL-c:** high density lipoprotein- cholesterol; **LDL-c:** low density lipoprotein- cholesterol; *: significant difference. **a:** Significant difference compared to G/G genotype.

After applying multivariate analysis, *A allele* carriers, increased WHR, FBS, HOMA-IR and LDL-c were possible predictors in T2DM as shown in (Table **5**).

Table (5): Logistic multivariate regression analysis of significant predictors for occurrence of type 2 DM

Variables	Unstandardized coefficients		Standardized coefficients	t-test	Sig.
	B	Std. Error	Beta		
***GA* genotype**	0.121	0.218	0.042	1.12	0.124*
***A* allele**	-0.009	0.212	-0.008	-4.38	0.01**
BMI (Kg\m^2)	-0.005	0.122	0.121	0.512	0.231*
WHR	0.502	0.432	-0.003	-5.22	0.02**
FBS (mg\dL)	0.0018	0.234	0.984	4.34	0.004**
HbA1C (%)	-0.002	0.445	0.231	1.98	0.13*
HOMA-IR	0.012	0.116	0.453	4.87	0.01**
TG (mg\dL)	0.011	0.118	0.342	1.306	0.914*
LDL-c (mg\dL)	0.098	0.069	0.597	2.415	0.03**
Cholesterol (mg\dL)	-0.029	0.028	-0.088	-0.38	0.118*

BMI: body mass index**; WHR:** waist hip ratio**; FBS:** fasting blood sugar; **HbA1C:** glycated hemoglobin; **TG:** triglycerides; **LDL-c:** low density lipoprotein- cholesterol; ***:** insignificant difference; ****:** significant difference.

DISCUSSION

Diabetes Mellitus is a global public health problem with a steadily increasing prevalence over the last few decades, making it one of the leading causes of morbidity and mortality in adults. Approximately half of all diabetics in the world are undiagnosed [(7)]. T2DM is a set of chronic disorders characterized by a dysregulated glucose metabolism caused by β-cell dysfunction in combination with systemic insulin resistance [(8)].

PGC-1α belongs to a superfamily of transcriptional coregulators associated with transcription factors, most of which are nuclear receptors that do not directly bind to DNA. It plays an important role in the regulation of energy and glucose metabolism by strongly modulating mitochondrial biogenesis and activity [(9)].

In our study, blood pressures (both systolic and diastolic) were remarkably higher in T2DM group as compared with controls, while other parameters did not show any remarkable variations. So, our results support the earlier findings of **Zhang *et al.* [(10)]**, who revealed that cases with T2DM had increased blood pressure than the control group.

The current study found that there were highly statistically significant elevations in BMI, WHR, FBS and HbA1C levels in T2DM group as compared to the control group. Our results were in line with that of Sun *et al.*, who concluded that diabetic cases had statistically significantly higher WHR, and BMI than normal cases [(11)].

Remarkable higher total cholesterol, TG, and LDL-c levels were observed in our cases than controls (P<0.001), but regarding HDL-c levels, the relation between the groups was not remarkably different. Similar to our findings, **Shokouhi *et al.* [(12)]**, reported that T2DM cases had lower levels of HDL-C and higher levels for BMI, cholesterol, glucose, TG, HbA1C, WHR, LDL-C, HOMA-IR and insulin than healthy controls.

In this study, the incidence of *A allele* was higher in T2DM group (19.6%) than the control group. Similarly, **Shokouhi *et al.* [(12)]**, demonstrated that the frequencies of *A* allele of *Gly482Ser* polymorphism were remarkably different between cases (14%) and control (4%) groups. In the same line, a Chinese study performed by **Zhang *et al.* [(10)]**, showed that the *A* allele was correlated with T2DM pathogenesis. There was a higher *A* frequency in T2DM cases than in controls (40.1% vs 29.3%, P=0.0002). Whereas the *G* allele was negatively correlated with diabetes mellitus susceptibility.

Regarding HOMA IR, **Fanelli *et al.* [(13)]** demonstrated that a remarkable variation between insulin resistance and *PGC-1α Gly482Ser* variants (P< 0.007) was detected. The *A* allele was a remarkable marker of reduced insulin sensitivity (P < 0.02) which makes it a possible determinant of insulin sensitivity reduction.

Also, the A allele carriers have decreased clearance of non-esterified fatty acids (NEFA) as a consequence of lipid oxidation abnormality. Elevated NEFA decreases glucose disposal and insulin signaling which lead to T2DM development [(14,15)].

In contrary, an Asian Indian study conducted by **Vimaleswaran *et al.* [(16)]**, showed no remarkable variations in the genotype or allelic distribution between T2DM and normal cases regarding *Gly482Ser* polymorphism. Also, **Muller *et al.* [(17)]**, did not revealed any significant correlation between *Gly482Ser* variants with either T2DM or obesity. Discrepancies between our results and those of others may be attributable to the differences in ethnicity, study design and population stratification.

The current study revealed that BMI, WHR, FBS, HbA1C and HOMA-IR were higher among carriers of *GA* and *AA* genotypes, but there was a significant difference only regarding WHR and FBS. **Fanelli *et al.* [(13)]**, demonstrated that heterozygous and homozygous carriers of the *Gly482Ser* SNP had significantly higher HOMA IR, and significantly higher fasting plasma insulin. There was no difference between homozygous Ser/Ser genotypes and heterozygous Gly/Ser compared to metabolic and clinical parameters. On the other hand, **Sun *et al.* [(11)]**, denied the correlation between the *Gly482Ser* polymorphism and parameters of insulin resistance.

In the present study, no statistically significant correlations were detected between *PGC-1α Gly482Ser* polymorphism and lipid profile among the studied T2DM cases. **Sun *et al.* [(11)]** found that TG levels were the most elevated test in T2DM *Ser/Ser genotype c*arriers compared with the others. They concluded that total cholesterol, HDL-c and LDL-c were not different in different genotypes, and no remarkable correlations were observed.

In our study, after applying multivariate analysis, *A* allele carriers, elevated WHR, FBS, HOMA-IR and LDL-c levels were considered potential predictors of T2DM.

CONCLUSION AND RECOMMENDATIONS

We concluded *A* allele of *PGC-1α* polymorphism is a possible predictor of T2DM occurrence in Egyptian patients. Additionally, WHR and FBS were significantly higher among carriers of *GA* and *AA* genotypes. We recommended further studies are needed to fully clarify the role of *PGC-1α* gene polymorphism and its plasma levels in the development of T2DM by investigation of other populations including larger sample sizes, multiple ethnic groups and other *PGC-1α* polymorphisms.

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