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THE INSULIN RESISTANCE AND ITS RELATION TO PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-GAMMA (PPAR) POLYMORPHISM IN DIABETES MELLITUS TYPE II IN EGYPT

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Abstract

Pathogenesis of type 2 diabetes mellitus (T2DM) and development of insulin resistance are described by multi-stimuli factors. Peroxisome proliferator-activated receptor-γ2(PPAR-gamma2) polymorphism might play a vital role in type 2 diabetes mellitus and insulin resistance. The adipose tissue-released cytokines as interleukin-1β (IL-1β) and Tumor Necrosis Factor-Alpha (TNF-α) may be contributory factors. Homeostatic model assessment (HOMA IR) is an approximating equation for insulin resistance from fasting glucose and insulin concentrations divided by a constant. HOMA-IR has been observed to have a linear link with the glucose clamp and minimum model estimations of insulin sensitivity/resistance in several studies of distinct people. The goal of the study is to determine the relation between PPAR-γ2, TNF-α, IL-1β and HOMA-IR with T2DM in Egypt.

Key Words: PPAR-γ, TNF-α, T2DM, IL-1β, HOMA-IR **,** Polymorphism

Introduction

Type 2 diabetes mellitus (T2DM) is a series of metabolic disorders elucidated by high blood glucose levels, which results from deficiency in insulin excretion or action or both leading to complications (Saxena M, *et al.,* 2009).Diabetes mellitus is predicted to reach nearly 5% of the world's population (about 366 million) in 2030 in the proportion of people >65 years of age (Wild, S., *et al.,* 2004).

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that are portion of the super family contains receptors for steroid hormones, thyroid hormones, retinoic acid and fat-soluble vitamin A and D. The main role of PPARs is to adjust glucose, energy balance, fatty acid and lipoprotein metabolism, cell proliferation and differentiation, inflammation and atherosclerosis (Grygiel-Górniak B, 2014). PPAR γ was the first gene reproducibly related to T2DM. The relation between the substitution of alanine by proline at codon 12 of PPARγ2 (Ala12 allele) and the risk for T2DM has been vastly studied since (Yen CJ, *et al.,* 1997), first notified this polymorphism.

A large and different family of small, low molecular weight cell signaling proteins acting as intermediate complex interaction is called "cytokines", which involve interleukins and interferons (Banerjee M., Saxena M., 2012). The adipose tissue-released cytokines as interleukin-1 (IL-1) and Tumor Necrosis Factor-Alpha (TNF alpha) may be contributory factors (Wieser V, *et al.,* 2013). The pro-inflammatory IL-1 family members; IL-1α and IL1β were among the first identified cytokines (Dinarello CA , 2011). IL-1alpha looks to play a more important role than IL-1beta in the development of atherosclerosis in mice (Freigang, S., *et al.,* 2013). However, the role of IL-1alpha in patients with type 2 remains to be investigated. IL-1β, a key inflammatory mediator during T2D, stimulates insulin resistance, impairs β-cells function, and causes apoptosis (Karstoft K, Pedersen BK, 2016).

Through all pro-inflammatory biomarkers, TNF- α first identified to be included in the pathogenesis of insulin resistance, and glucose related abnormalities that linked to T2DM (Imai Y, *et al.,* 2013). TNF-α has a pivotal role in the development of insulin resistance as it decreases the expression of glucose transporter type 4 (GLUT4) which is an insulin-regulated glucose transporter and located mainly in adipocytes, skeletal and cardiac muscles (Olson, A.L., 2012) .High level of TNF-á in circulation is related to the development of insulin resistance and diabetes (Swaroop J.J., *et al.,* 2012).

HOMA IR was first reported in 1985 by (Matthews, *et al.,* 1985). It is a method used to quantify insulin resistance and beta cell function from fasting glucose and insulin (or C-peptide) concentrations. It was calculated by the form: serum insulin (μIU/ml) × FPG (mg/dl)/405 (Bergman, *et al.,* 1985).

In this study, we are going to assess the correlation between PPARγ2 (Ala12 allele), TNF-α, IL-1β and HOMA-IR with T2DM.

Materials and Methods

Study population

This study was done on 110 patients with type 2 diabetes mellitus aged 28-87 years compared with 30 healthy controls aged 20-55 years.

Biochemical analysis

Blood samples was collected from the patients and healthy control after a 10-h fasting on sodium fluoride tubes, and plasma was separated by centrifugation at room temperature for fasting blood glucose and fasting insulin. After 2-h of meal, peripheral blood was collected from the patients and healthy control for post-prandial glucose test. Fasting blood glucose and post-prandial blood glucose were examined enzymatically using kit provided by (Spinreact, Spain). Fasting insulin was measured by commercially kit (Chemux Bioscience, Inc). After centrifugation, whole blood was stored at -20 for DNA extraction.

Genomic DNA extraction and genotyping

DNA was extracted from whole blood samples according to the method of (Medrano, *et al.,* 1990). The final preparation of genomic DNA used for PCR technique was free of RNA and protein and had an $A_{260} \backslash A_{280}$ ratio more than 1.7 in all samples.

A single PCR product was produced using a commercial Kit (MyTaq™ Red Mix) and visualized on the agarose gel after exposure to UV light. PCR was performed through 35 cycles by the following steps: denaturation at 94°C for 30 sec; annealing at 60°C for 30 sec; and extension at 72°C for 30 sec. A single product of 270 bp was produced as shown in Fig. 1. The PCR product was digested with restriction enzyme BstU-I at 37æ%c for 15 minutes, then applied to a 2.5% agarose gel and stained with ethidium bromide. Two different patterns were observed, a non-cut (non-polymorphic) for wild (Pro/Pro) variant, cutted (polymorphic) for heterogeneous type (Pro/Ala) as shown in Fig. 2.

Determination of TNF-α and IL-1β level by using Western blotting method (Harlow, E.D. and Lane, D, 1988) .

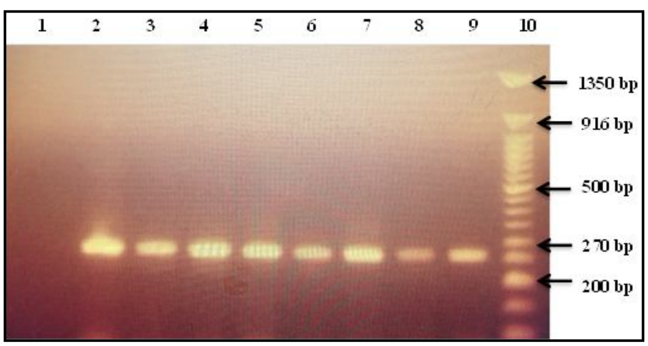

Fig. 1: Electrophoretic pattern of PCR products. Lane (1- 9) the PCR products at 270 bp, lane (10) 50 bp ladder.

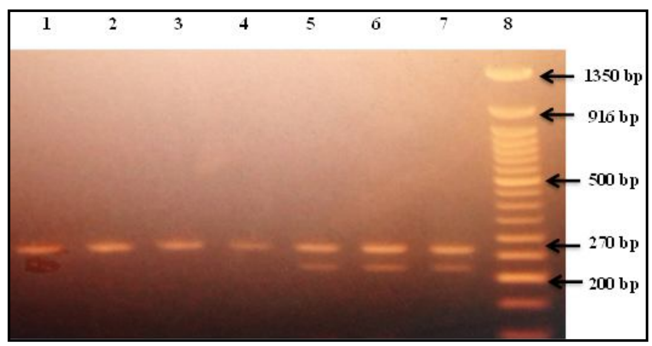

Fig. 2: Electrophoretic pattern of PCR product digest. Lanes (1- 4) wild pro/pro shows one band at 270 bp, lanes (5-7) shows heterozygous pattern with 2 bands at 270, 227 bp, lane (8) 50 bp ladder.

Table 1: Biochemical characteristics of study subjects.

Control	Diabetic female post- menopause	Diabetic female pre- menopause	Diabetic male	Characteristic
30	47	30	33	n
91.83 ± 2.47	148.9±9.098	168.2±10.16	158 ± 9.59	Fasting blood sugar(mg/dl)
119.9 ±3.951	264.4±13.73	259.2±14.24	260.9±17.01	Post prandial blood sugar(mg/dl)
5.193±0.1979	14.81 ±1.341	15.06 ± 1.549	11.5 ± 1.39	Fasting insulin (μIU/ml)
29.65 ±1.022	31.94±0.736	31.92±0.894	30.18±1.016	BMI
1.163±0.0422	5.447±0.638	6.547±0.902	4.509±0.596	HOMA-IR

Statistical analysis

Data analysis was all done using software GraphPad Prism7. Quantitative inputs were given by mean and standard deviation, while qualitative data were given by frequency distribution. Chi Square used to examine the significant difference for proportion and calculation of Odds ratio, and one way ANOVA test for multiple comparison. The probability of < 0.05 was used as a cut off point for all significant tests.

Results

Biochemical variables

The biochemical parameters of the T2DM patients (male, female pre-menopause, female post-menopause) and controls are summarized in table 1.

Genotypes and allele frequencies

The genotype distribution and the allele frequencies between the control and T2DM patients are shown in table 2. The frequencies of GG, GC, and CC genotypes in control group were 80%, 16.67%, 3.33% while in T2DM patients males were 84.85%, 12.12%, 3.03% and in T2DM patients female pre-menopause were 96.67%, 3.33%, 0% where in T2DM patients female post-menopause were 89.36%, 10.64%, 0%.

Determination of TNF-α and IL-1β using Western Blotting shows significant between T2DM and healthy control (P<0.0001) as shown in table 3.

Discussion

Type 2 diabetes mellitus (T2DM) is a general health problem in the world with a high diffusion which is the most noticeable disease in developing countries (Morita, *et al.,* 2005). Gene association studies have identified several common variants implicated in T2DM. One of it is Peroxisome Proliferator Activated Receptor γ2 (PPARγ2). A Pro12Ala polymorphism at extreme amino terminus of PPAR-γ2 gene has been studied but its effect on obesity and insulin sensitivity is unclear (Allan F. Moore, Jose C. Florez, 2008). In several additional studies, the Ala12 allele was related to lower BMI, improved insulin sensitivity, and reduced risk of type 2 diabetes (Deeb, *et al.,* 1998). Although most studies have shown a statistically significant Type2 Diabetes reduction given by Ala variant (Zeggini, *et al.,* 2005; Ghoussaini, *et al.,* 2005), some others have not (Badii, *et al.,* 2008; Bouassida, *et al.,* 2005) suggesting variability in the contribution of this variant to the risk of T2DM.

Table 2: Genotypes distribution of PPARã2 (Ala12 allele) polymorphisms in healthy control and T2DM patients.

Total	Ala/Ala (CC)	Pro/Ala (GC)	Pro/Pro (GG)	Group
30	1 (3.33%)	5 (16.67%)	24 (80%)	Control
33	1 (3.03%)	4 (12.12%)	28 (84.85%)	Dia. male
30	- (0%)	1 (3.33%)	29 (96.67%)	Dia.F. pre
47	- (0%)	5 (10.64%)	42 (89.36%)	Dia.f. post
30 110	Control 1 Cases 1 (0.91%)	Control 5 Cases 10 (9.09%)	Control 24 Cases 99 (90%)	Total no.

Table 3: Correlation of TNF-alpha and IL-1β in patients with type2 diabetes mellitus and healthy volunteers.

P value	control	T2DM	characteristic
<0.0001*	1.014 ± 0.015	6.024 ± 0.086	TNF-alpha
<0.0001*	1.082 ± 0.072	6.562 ± 0.4506	IL-1β

* Statistically highly significant.

The present study aimed to study the Pro12Ala polymorphism in PPARγ2 gene in Egyptian population. Various biochemical parameters were analyzed in controls and Type 2 diabetics. The association of this polymorphism with T2DM was studied. The relationship of this polymorphism with insulin and other biochemical parameters were also studied.

Association of Pro12Ala and Type 2 Diabetes mellitus

On genotype analysis, Pro/Pro homozygotes were 24 (80%) in controls and 99 (90%) in cases that include males, females pre-menopause and females post-menopause. The number of Pro/Ala heterozygotes were 5 (16.67%) in controls and 10 (9.09%) in cases. The number of Ala/Ala homozygotes were 1 (3.33%) in controls and 1 (0.91%) in cases that was a male. These results did not detect any statistically significant between the Pro12Ala SNP and T2DM. This result corroborates the findings of study done on South Indian population from Chennai (Radha, *et al.,* 2006). Another study on South Indian population reported no association of Pro12Ala SNP with metabolic syndrome (Vimaleswaran, *et al.,* 2007). Also, a study was done on Palestinians was unable to explain a significant association of Pro12Ala variant and T2DM (Ereqat, *et al.,* 2009). The finding of the present study differs from a study done on Caucasians, where an association of this polymorphism was found with the insulin sensitivity (Altshuler, *et al.,* 2000). The difference in reports of various studies may suggest that the effect of genetic variation may be restricted to particular ethnic groups. This may also be due to the influence of other genetic variants in the candidate gene or the interaction of certain yet uncharacterized genetic factors with environmental factors.

Association of TNF-α and IL-1β with Type 2

Diabetes mellitus

In 1993, tumor necrosis factor (TNF) was known as a pro-inflammatory yield of adipose tissue that is produced from models of diabetes and obesity, providing proof for a functional relation between obesity and inflammation (Hotamisligil, *et al.,* 1993). TNF-α interferes with the signals of the activated insulin receptor, promoting insulin resistance (Ouchi, *et al.,* 2011) . TNF-α was related to homeostasis model assessment (HOMA-IR) in some studies (Abbatecola, *et al.,* 2004; Löfgren, *et al.,* 2000), but not in others (Koistinen, *et al.,* 2000; Bruun, *et al.,* 2003). Our study confirms the correlation between TNF-α and HOMA-IR as it shows high levels of TNF-α and HOMA-IR in T2DM compared with control. Our results are in agreement with some studies (Abbatecola, *et al.,* 2004; De Rekeneire, *et al.,* 2006) but not with others (Bruun, *et al.,* 2003; Bastard, *et al.,* 2002).

High plasma IL-1β levels were linked to hyperglycemia and insulin resistance in T2DM patients (Donath, M.Y., Shoelson, S.E., 2011). Hyperglycemia is recognized to stimulate the production and release of IL-1â in various cell types (Maedler, *et al.,* 2017) and IL-1β may result in islet β-cells dysfunction, reduce insulin secretion and thus raise the risk of diabetes (Maedler, *et al.,* 2017; Herder, *et al.,* 2015). Our results show high levels of IL-1β and HOMA-IR in T2DM compared with control.

In summary, our study shows non-significant association between the Pro12Ala SNP and T2DM, but TNF-α, IL-1β and HOMA-IR have a significant association with T2DM.

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