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A STUDY OF THE RELATIONSHIP BETWEEN PC-1 GENE POLYMORPHISM AND INSULIN RESISTANCE IN PATIENTS WITH DIABETES TYPE 2 IN THI-QAR GOVERNORATE

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Annotation: The research study was carried out between December and May 2023 at the Biotechnology Research Unit of Thi-Qar at Mazaya University College. was the current study aimed to identify the genetic polymorphisms of 120 samples to investigate the possible association of PC-1 gene polymorphism with the diabetes mellitus. The observed PCR amplicons were subjected to the DNA sequencing procedure. The discovered variants were then localized based on where they were located within the reference genomic DNA sequences. The recent findings showed that the samples under investigation had genetic variations that were unevenly distributed. Besides, the rs1044498, A248C,also the high frequency variant is usually detected in three polymorphic modes, AA, AC, CC. Homozygous AA pattern discovered in the majority of patients (62.5%) and normal specimens (60%). However, each observed SNP was taken a particular pattern of variation differ from its mate in terms of the investigated samples. Ultimately, a statistical analysis is required to evaluate the possible association of the SNPs is tested samples, with the diabetes mellitus. As a result, the PC1 polymorphism was found to be associated with type 2 diabetes mellitus and insulin resistance in the current investigation.. Mutations in the PC-1 gene were recorded in the bank gene(NCBi) with accession numbers. LC768831, LC768832, LC768833, LC768834, LC768835. LC768836, LC768837 LC768838, LC768839, LC768840, LC768841, LC768842, LC768843.

INTRODUCTION

The chronic illnesses and health difficulties brought on by diabetes mellitus (DM) are a concern for people all over the world. A rise in hyperglycemia, often known as high blood sugar, is the metabolic condition known as diabetes mellitus (DM). By 2030, more than 280 million adults will have diabetes worldwide, and by 2050, that number will rise to more than 400 million (Shaw *et al*., 2010). Diabetes is commonly divided into categories (Thomas and Philipson ., 2015). These include autoimmunemediated type 1 diabetes causes insulin deficiency; mellitus, which develops as a result of pancreatic injury; diabetes linked to particular genetic disorders; and a general category known as type 2 diabetes, in which insulin production is impaired and resistance to insulin actions is typically present but not always (American, 2020). Type 1 diabetes results in insulin deficiency. Growing understanding of significant variances in the physiological mechanisms underpinning these different categories has the potential to improve outcomes by making some therapeutic modalities more accessible (American, 2021). Body's incapacity to create or react to insulin is what distinguishes the metabolic syndrome, which includes type 2 diabetes (T2DM). In addition to hypertension, dyslipidemia (low lipid levels), and insulin resistance, which is directly related to the development of T2DM, a major risk factor for metabolic syndrome (Zafa *et al*., 2019). Attacks on the brain and other heart and circulatory (CVD) illnesses (Martins et al. 2019). Estimates of the high prevalence of a

metabolic disorder and insulin resistance range from 10% to 40% in younger individuals and developing nations (Ansarimoghaddam *et al*., 2018) . In comparison to other T2DM diabetes has the highest prevalence and accounts to over 90% of the worldwide diabetes prevalence (Santoro *et al*., 2021). In the event that insulin production levels fall for whatever cause or cells develop resistance to insulin, T2D disease will result (Chaudhury *et al*.,2017). But because they contribute to insulin resistance and type 2 diabetes (T2D), genetic and environmental variables that control how cells respond to insulin are also crucial to be studied (de Veciana *et al*.,2017). According to the International Diabetes Federation (IDF), diabetes is an endocrine system illness that will affect more than 693 million persons worldwide by the year 2045, up from 50% in 2017 (Cho *et al*., 2018). Diabetes of type 2 is a clinical syndrome that causes high blood sugar levels, which is due to genetic and environmental risk factors. Diabetes is one of the biggest challenges facing scientific health (Ogurtsova *et al*.,2017). Unhealthy changes in biological clock and Additionally contributing to metabolic syndrome and insulin resistance is lifestyle. Genetic variables also influence a person's likelihood of developing insulin resistance and the metabolic syndrome (Brown and Walker, 2016). Ectonucleotide Pyrophosphatase-Phosphodiesterase 1 (ENPP1) is another name for the ***PC1*** gene. It is found on the long arm of chromosome 6 (6q23.2) which has 25 exons and 24 introns, and it encodes for a protein that influences the sensitivity of the body to insulin. It is an endogenous transmembrane glycoprotein that can be found in the endoplasmic reticulum (ER) as well as on the plasma membrane. It is possible for the (***PC1***) to suppress the autophosphorylation of the insulin receptor (IR with direct engaging with the insulin receptor subunit, hence preventing further downstream signaling, despite the fact that its function is not fully understood. (Vickers,. 2017; Pappalardo *et al*., 2017).

MATERIAL AND METHODS

Ethical statement

Before the start of the study, institutional approval was obtained, Nasiriyah Teaching Hospital, Thi- Qar, Iraq.

DNA extraction

Genomic DNA was isolated from whole blood using a Geneaid Blood DNA extraction Kit (of Korean origin) after blood was collected in EDTA tubes. in accordance with industrial requirements. Primer-based Polymerase Chain Reaction (PCR) was used.

(F \ 5-TCAGAGTGGCCATGGTAGTG-3; R \ 5-TGTAAAGCCCCGCTAAGACG-3) rs1044498, a polymorphism in PC1. Digested products and marker DNA (350 bp ladder) were resolved on a 2% agarose gel electrophoresis for scale interpretation.

RESULTS

A total of 80 cases of T2DM and 40 healthy controls comprised the population analyzed in our study. The demographics and clinical parameters of all participants are shown in Tables 2. The results showed that there was a significant difference in patients with T2D, smokers and non-smokers (OR=0.013), and the duration of infection (OR=0.000), while there was no significant difference in gender, age, and blood pressure as in a Table 1.

The AA frequency appeared without a significant difference, amounting to OR = 1.1, and the frequency CC appeared with a significant difference, amounting to(OR = 0.8) as in a Table 2.

Characteristics	Case NO:80	Control:40	p Value
Age	56.95±16.13	41.97±11.33	0.387
Sex (%)			
Woman Man	46.25 53.75	37.5 62.5	0.362
Smoking (%)			
Smoker Ex-smoker	48.75 51.25	25 75	0.013*
blood pressure (mmHg) Non-blood pressure (mmHg)	41.25 58.75		0.118
Family history(%)			
exist does not exist	56.25 43.75		0.264
The duration of the injury(%)			
less than a year 10 - 1 20 - 11	7.5 57.5 35		0.000*

Table 1. **Description of Anthropometric, clinical, for all volunteers**

PC-1,248 A>C	Case=80	Control=40	OR	P.V
AA	50(62.5%)	24(60%)	1.1	0.8
AC	2(2.5%)	0(0%)	2.5	0.5
CC	28(35%)	16(40%)	0.8	0.5
AG	0	0	1	1
GG	0	0	1	1

Table 2: Genotype frequency among diabetes mellitusand control

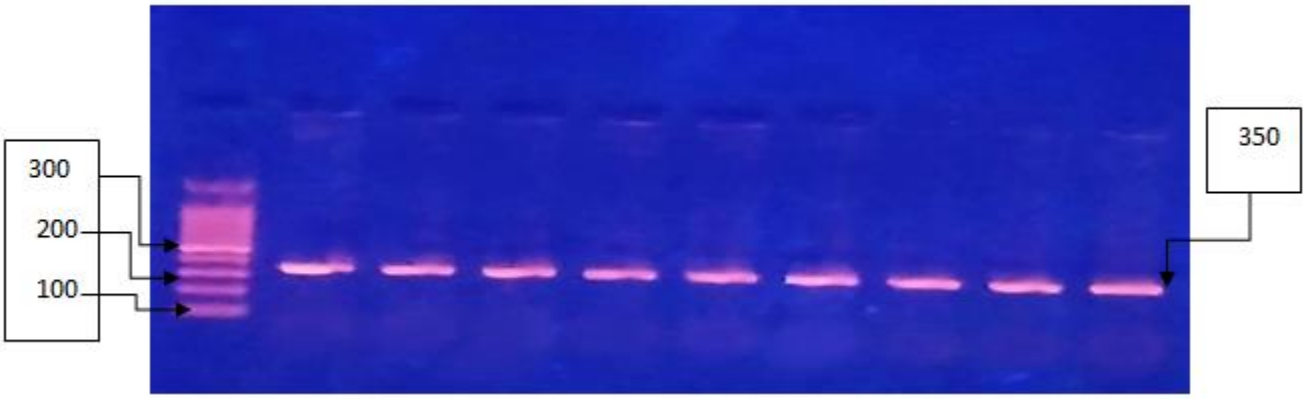

Figure 1. Show Electrophoresis of PCR products on 2% agarose gel. ***PC-1*** gene and bundle appearance at the base pair 350bp

Discussion

The results of the current study based on the sequencing analysis of the (***PC-1***)To elucidate the positions of the targeted SNPs with regard to their deposited SNP database of the sequenced 350bp fragment, the corresponding positions of the PC-1 gene was retrieved from the dbSNP server. To find out the nature of these SNPs, a graphical representation was performed concerning the PC-1dbSNP database within chromosome no. 6 (Gen Bank Acc. NC_000006.12).).By reviewing all the detected fourteen SNPs in the dbSNP engine.

More than 180 million people worldwide suffer from diabetes mellitus, which reaches pandemic proportions. Global projections for 2010 call for an additional growth of about 50%, with the largest increases expected in the developing nations of Africa, Asia, and South America (Zimmet *et al*., 2011).

It was also known as Ectonucleotide Pyrophosphatase/Phosphodiesterase 1 (ENPP1) and Plasma Membrane Glycoprotein 1 (***PC-1***). It was situated on the long arm of chromosome 6 (6q23.2) and encoded for the protein that affected insulin sensitivity, among other things. Both the endoplasmic reticulum (ER) and the plasma membrane had a grade II transmembrane glycoprotein.

The molecular mechanism underlying the phenomenon was initially linked to a single synonymous SNP, K121Q17, which allowed the Q allele to have a "gain-of-function" effect that was several times more potent than the K allele in reducing insulin stimulation of IR autophosphorylation, IR substrate-1 phosphorylation, phosphatidylinositol 3-kinase activity, glycogen synthesis, and cell proliferation (Costanzo *et al*,.2001).

Though, the role of ***PC-1*** was not fully understood , it was able to inhibit the autophosphorylation of insulin receptors by interacting directly with α-subunit insulin receptors , thereby blocking subsequent downstream signals (Johnson , 2006; Pappalardo *et al*., 2017).

The results of current study recorded that anassociation between ***PC-1*** 248 A>C SNP polymorphism with diabetes mellitus patients (P. value= 0.0001).

But it has been shown that excessive ***PC-1*** expression inhibits the action of insulin receptors in tyrosine kinase and, as a result, cellular communication in a number of cells. There is still some confusion regarding the ***PC-1*** protein's functions (Maddux and Goldfine, 2000).

The results of current study were C>T (rs1044498) polymorphism a significantly associated with diabetes mellitus; The present results incorporated with different studies documented the polymorphism is linked to metabolic syndrome, T2DM, and insulin resistance. (Bhatti *et al*., 2010; Daoming, Marchenko *et al*., 2018). However, other research has not confirmed a genetic link between the ***PC-1*** K121Q polymorphism and insulin resistance (Rasmussen *et al*., 2000). According to Sortica *et al*., (2015), the ***PC1*** K121Q polymorphism was a misleading functional alteration since the 121Q variant more strongly binds insulin receptors than the 121K variant does (Costanzo *et al*., 2001). Additionally, genetic association studies have confirmed and shown that ENPP1 polymorphisms are linked to a risk for T2D, obesity, and a number of metabolic disorders. (Zhao *et al*., 2011).

Failed to confirm a link between rs1044498 and type 2 diabetes in the Chinese Han population (Zhao *et al*., 2011). The positive associations were often reported from White (Abate *et al*., 2005; Moore *et al*., 2009) and African (Chandalia *et al*., 2007) populations; while the negative results were found in Moroccan (El Achhab *et al*., 2009) Chinese, (Chen *et al*., 2006) Japanese (Keshavarz *et al*., 2006) and Korean (Seo *et al*., 2008) populations. Indeed, demographic variation must be considered, particularly in the case of a complex metabolic condition. Furthermore, T2D and obesity should be attributed to multifactorial etiology, rather than a single gene disease, as a result of changes in several gene products.

In present results of genotype distribution of ***PC-1*** gene documented that the AA genotype was identified in the majority of case samples (62.5%), and controls (60%), while AC pattern was detected only in case samples (2.5%). The present results were incorporated with results of . Research done in China also revealed that T2DM or any of the characteristics of the metabolic syndrome have no correlation with ***PC-1*** K121Q polymorphotype distribution (Daoming *et al*., 2006). The present findings differed from those of Abdo Albegali *et al*. (2019), who found a correlation between ***PC-1*** and the incidence of diabetes type 2 in the Punjabi population of Pakistan. While many studies have showed no link by this allele and illness risk (Rasmussen *et al*., 2000), prior research has demonstrated a correlation between the Q allele of ***PC1***

(rs1044498) and insulin resistance(IR) (Gu *et al*., 2000). Very few reports about the role of ***PC1*** risk alleles in disease have come from the South Asian continent (Prakash *et al*., 2013).

Positive findings and proven genetic connections were found in a meta-analysis of the ***PC1*** K121Q polymorphism and T2DM risk (Abate *et al*., 2005; Hamaguchi *et al*., 2004) , specific molecular mechanism of this gene's risk factor is still unknown (Abdo Albegali *et al*., 2019).

Many diseases, including different genetic polymorphisms and risk alleles in this gene have been linked to coronary heart disease, polycystic ovarian disease, obesity, risk of diabetic kidney disease, coronary artery calcification, ischemia of the heart, and T2DM. (Di *et al*., 2018; Li *et al*., 2016; Pappalardo *et al*., 2017).

Some studies recorded relation between the same rs1044498 and other disease that detectesuch as: (Zhang *et al*., 2021) Hemodialysis patients in the participants recruited from one hospital in China showed the variations of ENPP1 (rs1044498). According to a review, T2DM patients in Asia were the basis for the rs1044498 variation.

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