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STUDIES IN TYPE 2 DIABETIC PATIENTS ON CD36GENE AND THE LEVELS OF LIPPROTEIN IN IRAQ

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ABSTRACT

The aim of study to findig the relationship between the polymorphism of cd36 gene and the risk of developing diabetes type II (T2DM) in Iraqi patients. The single nucleotide polymorphism (SNP) in a gene cd36, and their receptors in patients with diabetes and hardening of the arteries, heart disease and blood vessels is a considerable importance as it is the gene responsible for the absorption of free fatty acids and antioxidant specificity a low-density, (LDLox). The study included eighty patients with T2DM patients with diabetes Type II and reviewers to diabetes of the center and /school of Medicin research at Mustansiriya University between April 2015 and April 2016, forty-sample of healthy ostensibly individuals 20 males and 20 females ranged in age study samples between (35-73 years). Information of Samples was collected in the form of a questionnaire for patients. DNA extract from blood samples collected from patients T2DM and healthy individuals using BIONEER kits, then the molecular diagnosis of exons using four special primers (exon 3 a molecular volume is 265 bp, Exon 4 a molecular volume is 358 bp and Exon 14 with amolecular volume is 313 bp and Exon 15 with a molecular volume is 250 bp) in cd36 gene using polymerase chain reaction program (PCR) and electrophoreses through a garose gel. The statistically analyzed of clinical results for systolic blood pressure (SBP), blood fasting sugar (FBS), sugar hemoglobin (% HbA1c), triglycerides (TG), low-density lipoprotein (LDL) and lipoprotein very low density (VLDL) in T2DM cases compared with healthy controls, as found significant differences on the level ($p < 0.05$). Sequence of gene

analysis in CD36 gene in Iraqis patient and healthy people have shown they have mutations deleted in exons 3 and 4 of Frameshift type and these mutations are responsible for the disease, type 2 diabetes, and to delete the 16bp area in exon 14 and the presence of a mutation in exon 15 is G> C all of these mutations caused T2DM. The genetic study of diversity in a gene associated with diabetes, and so is this gene is important in tracking the candidate T2DM.

KEYWORDS: Type 2 diabetes mellitus (T2DM), polymerase chain reaction (PCR), cluster of differentiation (CD36), Exons.

INTRODUCTION

Diabetes mellitus is a disease of carbohydrate metabolism, which presents as hyperglycemia in affected patients. A categorical classification scheme of the disease includes the following forms: type 1 and 2 diabetes mellitus, gestational diabetes mellitus, and monogenic diabetes (Fajans et al., 2011). The gene encoding *CD36* is located at chromosome 7, at locus q11.2. It is36 Kb long and is comprised of 15 alternatively spliced exons that are differentially regulated by several upstream promoters (Armesilla and Vega, 1994; Rac *et al*., 2007; Gautamand Banerjee, 2011). It was reported that hepatic insulin resistance with high plasma FFA and triglycerides occurred due to a homozygous disruption on the*CD36* locus (Ma *et al*., 2004; Nagarajan *et al*., 2012). In the present work an attempt was made to study the association of three single nucleotide polymorphisms (SNPs) in *CD36* gene with T2DM in a North Indian population. Since *CD36* is a fatty acid transporter in heart muscles and adipocytes, the discrepancy of*CD36* being protective depends on whether or not a pro-inflammatory environment generates pathologic *CD36* ligands. Under abnormal conditions such as obesity and hyperlipidaemia, ligands affect inflammatory and insulin signaling pathways *via CD36* (Nicholls *et al*., 2011). CD36 found on the surface of many cells in vertebrates and has the ability to endocytose oxidized LDL (OxLDL). (Noushmehr H et al., 2005 and Goldberg IJ et al., 2009). Several studies suggested the role of CD36 as an important regulator of the metabolic pathways involved in insulin resistance (Miyaoka K, et al., 2001 and Handberg A, et al., 2006). The pathophysiology of human CD36 deficiency in metabolic syndrome and atherogenesis has been explained (Yamashita S, et al., 2007).

CD36, an 88-kDa transmembrane glycoprotein receptor, is expressed on various cell types, including monocytes and macrophages; platelets; microvascular endothelial cells; adipocytes; epithelial cells in the kidney and cardiac myocytes (Febbraio M, Hajjar DP, and Silverstein

RLet al., 2002). CD36 belongs to the class B scavenger receptor family, which also includes scavenger receptor B1 and lysosomal integral membrane protein (Calvo D, et al., 1995). This function of CD36 provides an energy source for beta-oxidation to myocytes and lipid storage to adipocytes (Abumrad NA, et al., 1993).

METHODS

- **Subject**

The study included eighty T2DM outpatients from Diabetes Center for treatment and Research/Medical College at AL-Mustansiryah University, between April 2015 and April 2016, and forty apparently healthy individuals health 20 male and 20 female, age of subjects was between (35-70) years. Subjects information were collected in specific questioner forma.

- **Sample**

Collection 5 ml blood samples were equally distributed in two vials, 3 ml in M EDTA and the other in a plain vial for DNA extraction and biochemical estimations respectively. Serum was collected from the blood in plain vials after centrifugation for 10 min at 3000 g at 4°C. Estimations of plasma glucose (mg/dl), serum insulin (mg/dl) were done using commercially available Biolabo kits (France) and lipid profile (total serum cholesterol, TC; High density lipoprotein-cholesterol, HDL-C and serum triglycerides, TG) were done using commercially availableBiolabo kits (France) by double beam Kenzo 240TX Biolabo diagnostics and Determination of Glycosalated Hemoglobin (HbA1c) kit stanbio Glycohemoglobin.

- **DNA isolation**

DNA was extracted by using DNA extraction kit (BIONEER kit, Korea,) according to manufacturer's instruction

- **Detection of CD36 genes in type 2 diabetes mellitus (T2DM)**

The reaction of the PCR were used for detection of four exons on CD36 from patients with type2 diabetes mellitus in Iraq. Table 1 showed the primers used for detection of CD36genes and the PCR program was comprised of the following three steps: example exon3 initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 94°C for 1 min. annealing at 58°C for 70s, and extension at 72°C for 70s and the final extension for 6 min at 72°C. In PCR reaction, for cycling, a DNA thermo-cycler (Eppendorf Master cycler, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) was used. PCR was performed in total volume of 20µl and components are shown in Table 2. The amplified products were

visualized by ethidium bromide staining after gel electrophoresis of 10µL of the final reaction mixture in 2% agarose (see Figuer 1).

Table: 1. Primers used for detection of CD36 genes intype 2 diabetes mellitus (T2DM).

Exon	Primer sequence	Annealing temperature (°C)	Size of product (bp)	References
E3	□F 5□- GTGCTTAACACTAATTCACC-3□□□□□	56	*265*	
	R 5□- GATACAAAATTAGCAGTTACCATG-3			
E4	F:5□- GGTCCTTTTATCTGGTGACTCAAGGCTGC-3□□□□□□□□□□□	59	*358*	
	5□- TAAGTACATTTCAATACAATACAATGAC-3□			
E14	5□□□□F: - CATGTCTAGCCACTGATCATTTTT-3	56	*313*	
	5□□R:- TCAGGACTTTTCTGGATTTGG-3			
E15	F: 5□- CTGTCATAATCGCCTCATAAAGAC3□□□□□□□□□□□□□□□□□□□□	59	*250*	
	5□□R:-CAAATGTCTTTTTGTTCTTCTTCATCC-3			

Table: 2 PCR reaction components.

Components	Volume (µl)
Forward primer of four genes (IDT, USA)	2 (one of each gene, con. 10 pmol/ml)
Reverse primer of four genes (IDT, USA)	2 (one of each gene, con. 10 pmol/ml)
DNA template	2
Deionized Distilled water (Bioneer, Korea)	4
INTRON2X PCR Master Mix solution (i-MAXII) (Germany)	10
volume	**20**

RESULTS

Clinical analysis: The average age of the patients was 55.44±9.96 yr and their fasting and post-prandial glucose levels were 185.47 ± 72.45 and 275.39 ± 89.11 mg/dl, respectively. Total cholesterol (243.88 ± 24.18 mm/dl) and LDL-C (174.61 ± 20.10 mmHg) levels were slightly raised and HDL-C was low (45.30 ± 4.19 mmHg). However, no significant difference was observed in BMI, triglycerides and serum creatinine levels between the T2DM and control groups (Table 3).

Table: 3

Clinical parameters	Controls (n=40)	Patients (n=80)
Age (yr)	48.72 ± 9.75	55.44 ± 9.96
BMI (kg/m2)	21.66 ± 2.03	21.88 ± 3.27
Fasting plasma glucose mg/dI	89.83 ± 11.58	185.47 ± 72.45
Post-prandial plasma glucose (mg/dl)	172.30 ± 30.36	275.39 ± 89.11
Total cholesterol (mg/dl)	149.69 ± 39.51	243.88 ± 24.18
Triglyceride (mg/dl)	129.41 ± 59.78	118.65 ± 13.31

HDL-cholesterol (mg/dl)	59.98 ± 16.20	45.30 ± 4.19
VLDL-cholesterol (mg/dl)	29.19 ± 11.67	20.45 ± 4.29
LDL-cholesterol (mg/dl)	65.72 ± 46.31	174.61 ± 20.10

Values are expressed as mean ± SD

Results indicated in Figure (1) showed a sharp DNA bands were obtained after exraction and electrophoresis of genomic DNA from healthy controls and pateints with T2DM on agarose gel (0.7%). The concentration of DNA extracted from all samples was ranged between 37-125 ng/µl, while the purity was ranged between 1.8-2.41. This purity and concentration of DNA solutions were suitable and recommended for further genetic analysis by using PCR technique (Boesenberg-Smith*et al.*, 2012).

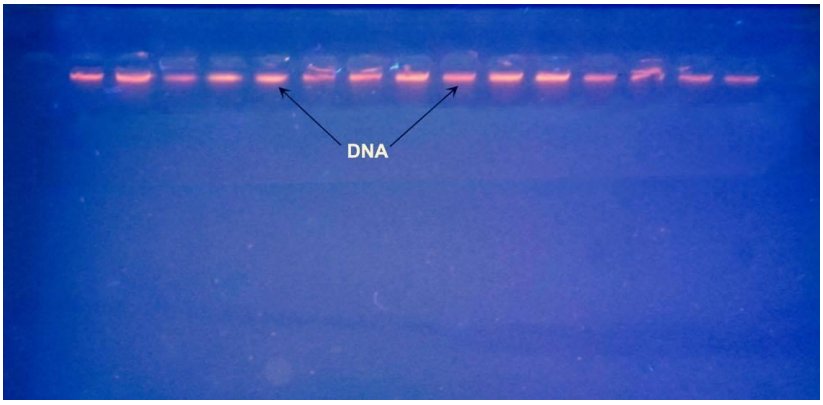
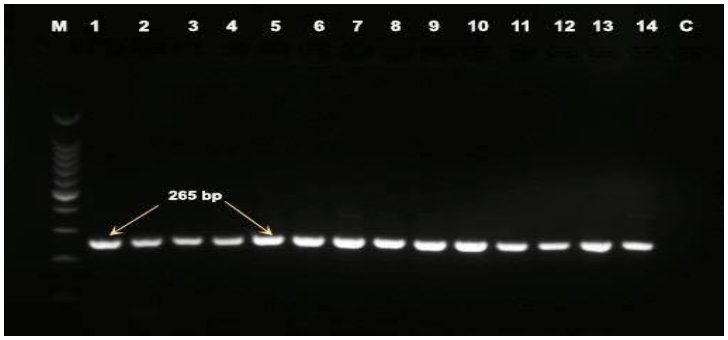
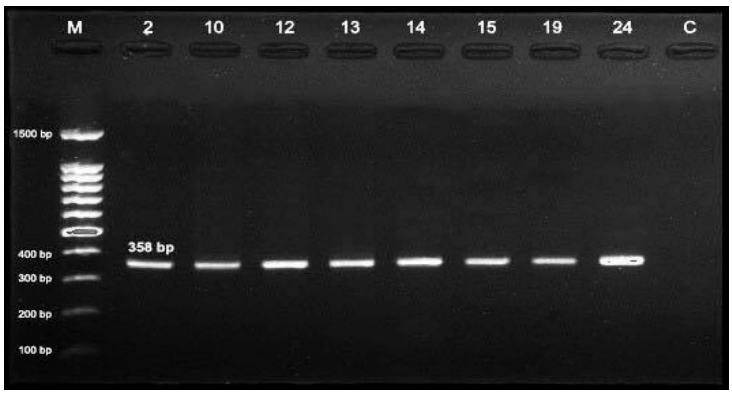


Figure: 1 Gel electrophoresis of DNA on agarose gel (0.7%) for 1 hour at 5v/cm^2. DNA extracted from blood sample of patients with T2DM. and healthy controls.

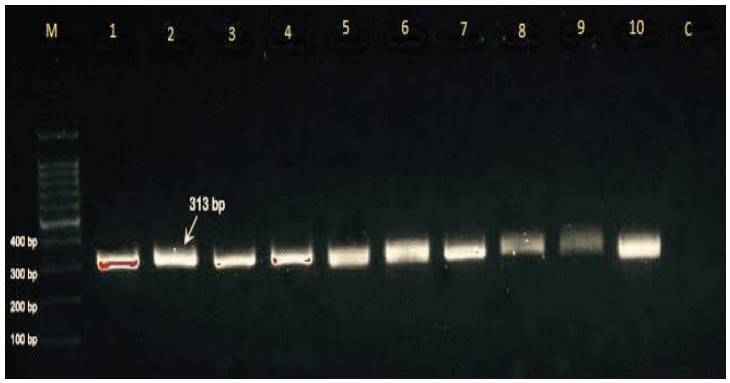
The Pcr products for Exons 3 (265 bp), 4 (358 bp) and 5 (199 bp) of *CD36* gene were amplified by polymerase chain reaction (PCR) using respective primers using Master Cycler ep Gradient (Eppendorf, USA). The primer sequences and PCR conditions are shown in (Table I). PCR was performed for 30 cycles using 0.5U Taq polymerase, 10 pmol/µl of each primer, and 200µM dNTP in 25µl reaction volume. The PCR products were checked on 1.5 per cent agarose gel along with 50 and 100 bp markers. The gels were documented and analyzed.



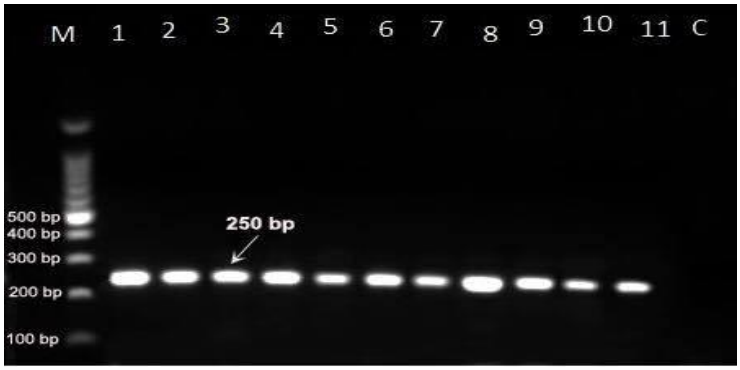
(A)



(B)



(C)



(D)

Figure (2) Detection of *CD36* gene by agarose gels showing PCR products of (A) Exon 3, (B) Exon 4,(C) Exon14, (D) Exon 15, Lane M: DNA ladder (100 bp);Samples of type 2 diabetes mellitus.

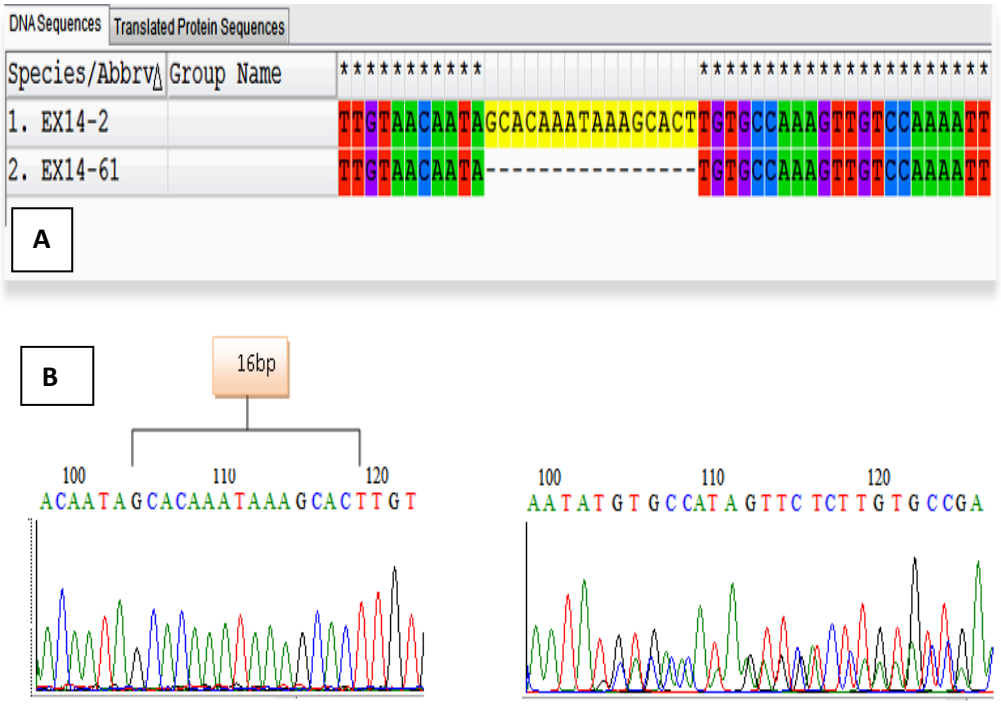


Fig.2. Multiple alignment using CLUSTAL-W and sequening showing Exon14 (16 bp del) in samples number (2,61).

DISCUSSION

The association between *CD36* over-expression and presence of atherosclerotic risk factors, particularly diabetes, shown in this study is in agreement with a common etiology of the disease (Saxena *et al*., 2012).Although SNPs Exon 14 (16 bp del) and Exon15 (G>C) lie in the 3'UTR region which attribute their role in post translation modifications, association of these polymorphisms in a Iraqis population.*cd36* being an important receptor molecule for modified lipoproteins plays an important role in the regulation of lipid metabolism. Studies have shown its involvement in diverse disorders such as insulin resistance, dyslipidaemia, hyperlipidaemia, atherosclerosis (Febbraio *et al*., 2001 and Furuhashi *et al*., 2003) and T2DM (Lepretre *et al*., 2004a; Lepretre *et al*., 2004b) Lipid abnormalities in CD36 deficiency might depend on the presence of diabetes since the total cholesterol and triglyceride levels in diabetic CD36 deficient patients were higher than in control subjects and non-diabetic CD36-deficient patients (Furuhashi *et al*., 2003). Several kinds of *CD36* gene mutations have been reported in CD36-deficient patients (Tanaka et al., 2001) one SNP rs1761667 (G>A) showed a highly significant association with T2DM (Love-Gregory *et al*., 2008; Banerjee *et al*., 2010).

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