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Evaluation of E-selectin rs 5367 C/T Polymorphism in Iraqi Diabetic Foot patients

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Abstract .Diabetic foot is inflammation disease, including inflammation factors; such as E-Selectin. The study aimed to evaluate E-selectin gene SNP rs 5367 C/T polymorphisms with other factors in the Iraqi diabetic foot patients. The study was conducted on 100 Iraqi individual, 50 patients with diabetic foot and 50 as control group. 10ml of blood samples were taken to measured biochemical and genetic variations. The results showed significantly increasing in all biochemical parameter in patient compare to control group except HDL. As results shown no significant differences in the genotype distribution (TT, TC and CC) and allele frequency between the Diabetic foot patients and control groups. Additionally, the genotype TT recorded the highly ratio for patients and healthy individual (60-72%) which may consider the common genotype at Iraqi studied population. In conclusion, both homozygous genotype showed preventive fraction according odds ratio (0.58, 0.93), while CT genotype according odds ratio (1.39) consider as etiological fraction, but not related significantly with disease because all data still in agreement with Hardy-Weinberg so that research wok need more studied to improve a relationship between present locus with disease. Even that according to allele analysis T allele may preventive, while C allele could be etiological for disease.

Key word: E-selectin, rs 5367 C/T, Polymorphism, Diabetic foot.

1. Introduction

The diabetes is inflammation disease, because it is closely associated with other diseases such as kidney diseases; blindness, and heart attacks etc [1]. As well as, incidence indicated that foot infections in persons with diabetes ranges from a lifetime risk of 4% in all diabetic persons to 7% yearly [2]. People with a family history of the T2DM are at a higher risk of developing the disease since they share genetic background in addition to likely similar environments [3]. Diabetes mellitus is affected by Cellular adhesion Molecules of cells that increase the rate of lymphocyte binding with the lining of the blood vessels [4]. One of these Molecules (E-selectin). E-Selectin protein is encoded by SELE gene is composed of 14 exons located at position 1q24.2 of Chromosome 1, reverse strand [5].

E-selectin (CD62E) is surface glycoprotein molecule involved in adhesion of circulating leukocyte in activated endothelium and plays an important role in inflammation process [6], through regulates binding and extravasation of leucocytes from the bloodstream to sites of inflammation [7]. This inflammatory



cascade induces endothelial vascular dysfunction. Elevated levels of E-selectin, in particular, have recently been identified as a risk factor for type 2 diabetes [8]. As in Iraqi Diabetic patients [4, 9]

In addition, clinical studies in diabetic foot patients have reported associations between markers of endothelial activation and hyperglycemia, as well as other factors such as age, smoking, obesity, blood pressure, and blood lipids. Likewise, several experimental studies have found that hyperglycemia, glycated proteins, and cytokines can induce endothelial cell activation in vitro. However, the causative factors behind the development of endothelial dysfunction, and in particular altered adhesion molecule expression in diabetic foot, are not yet known [10]. In order to understand this relation present study has been design, to aimed investigate the association between the rs 5367 C/T polymorphisms in E-selectin genes and diabetes foot of Iraqi population.

2. Materials and methods

This control study conducted on 100 Iraqi subjects, age range (43- 94) for 50 patients (30 males and 20 female) that were diagnosed with T2DM having foot ulcers, who were periodic patients at the hospital follows Baghdad and Baqubah province , from January to June, 2018. The patients had been instructed about the purpose of the study and interested volunteers have been enrolled along with 50 healthy subjects (27 males and 23 female) that were taken as a control group. Diabetic patients were selected according to the World Health Organization 2016 guideline:

Fasting Blood Sugar (FBS) ≥ 126 mg/dL (7.0 mmol/L), or Glycohemoglobin HbA1c $\geq 6.5\%$ (48 mmol/mol) [11]. Diabetic patients younger than 18 years old, those with less than 6 months of follow-up or pregnant women, were excluded. Also subjects with the history other diseases while control group consisted of non-diabetic healthy individuals according to the laboratory finding of FBS (value < 90 mg/dL).

Venous blood samples (10 ml) were divided into two aliquots; one for biochemical tests and the other for DNA genotyping. Fasting Blood Sugar (FBS), Glycohemoglobin (HbA1c) and Lipid profile [Total cholesterol, Triglyceride (TG), high density lipoprotein (HDL-C), low density lipoprotein (LDL-C), very low density lipoprotein (VLDL-C)] were measured by enzymatic colorimetric methods with commercially available kits (Human, Germany and SPINREACT Company, USA). Body mass index weight/height (kg/m^2) and abdominal Circumference (cm) were calculated for both groups.

The genotypes were characterized using sequence specific primer (SSP) technique. Genomic DNA was extracted using gSYNCTM DNA Extraction Kit (Geneaid), the amplification of rs5367 C/T selectin SNP was done by designing sequence specific primers using PRIMER3PLUS software, the forward primer with and revers primer was used to obtain a PCR product size of 490 bp.

Table 1: shows the sequence of primers

Primers	Sequence	Tm	Size	References
F	5'-AGCGCTACTTAGTTTTTCAGCA-3'	57.96	490	Novel design
R	5'-CTTGGGAAACGTATTGCTGGA -3'	58.57		

Lyophilized primers were supplied by Alpha DNA (Canada), and were suspended according to the manufacturer's instructions. PCR reaction was performed using Go Taq® blue master mix (2X) of bioneer (korea) instructions for a final volume of 25 μL , as shown table (2).

Table 2: Materials used in polymerase chain reaction

Composition	Size
Master Mix (Maxime PCR PreMix)	5 l
Template DNA	4 l
Primers	2F,2R
Deionized Water	12 l
Total	25 l

Mix the above materials and transferred to the polymerase chain reaction device. Reaction was carried out with PCR thermocycler Black A. The PCR product (490bp fragment) was visualized by 1% agarose gel electrophoresis (stained by 5- μ L ethidium bromide).

Table 3: PCR Program for E-Selectin rs5367 C/T Genotyping:

Steps	Temperature (C ^o)	Time	Cycle number
Initial denaturation	95	5 min.	1
Denaturation	94	30 sec.	
Annealing	58	30 sec	30
Extension	72	45 sec	
Final extension	72	10 min.	1

3. Statistical analysis

Statistical analysis was performed using the SPSS software version 22 (SPSS Inc., Chicago, IL, USA). Each result was calculated as the mean \pm SE. The Hardy-Weinberg balance was used to check the sample with group representation. WINPEPI computer programs (version 11.63) was used to calculate differences in genotypic and allelic frequency between studied groups were evaluated using the Fisher's exact test or the χ^2 test as appropriate. The odds ratio (OR) was calculated together with its 95% confidence interval (CI). P<0.05 was considered to indicate statistically significant differences. [12].

4. Results and Discussion:

Diabetes mellitus (DM) is becoming a serious international public health problem. As

Table 4: Anthrobiological and Biochemical Parameters for study groups.

Parameters	Patients (Mean \pm SE)	Control (Mean \pm SE)
Age (year)	61.860 \pm 1.625	44.100 \pm 1.511
Gender (male/ female)		
Disease duration (year)		
BMI (kg/m ²)	26.458 \pm 0.638	23.244 \pm 0.418 *
Abdominal Circumference (cm)	89.060 \pm 0.869	76.540 \pm 0.799 *
FBS (mg/dl)	212.300 \pm 4.863	101.740 \pm 1.586 *
HbA1c (%)	9.660 \pm 0.18	4.966 \pm 0.70 *
Cholesterol(mg/dl)	202.980 \pm 3.395	187.340 \pm 3.083 *
TG(mg/dl)	214.500 \pm 3.250	157.040 \pm 2.579 *
HDL(mg/dl)	43.640 \pm 1.054	65.140 \pm 1.479 *
LDL(mg/dl)	116.440 \pm 3.660	90.792 \pm 3.549 *
VLDL(mg/dl)	42.900 \pm 0.650	31.408 \pm 0.516 *

* p < 0.001, BMI: body mass index, HbA1c: glycated hemoglobin, TC: Total Cholesterol, TG: Triglyceride,

LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol, VLDC:

Very low density lipoprotein

Reported by the International Diabetes Federation (2013), the world-wide diabetic population is now 382 million and will reach 592 million by 2035[13]. Present study showed significant increasing (p < 0.001) in, BMI, Abdominal Circumference in diabetic compared to controls (Table 4). These factors depending on the type diabetes and severity of diabetes, glycaemic control, nutritional status, age and other factors [14], Furthermore, abdominal Circumference provides a good indicator for obesity and T2DM [15]. All chemical parameters increase (p < 0.001) significantly in Iraqi patients that agree with previous locally studies about Iraqi patients [16, 17]. This elevation return to other causes such as Lack of insulin secretion

or Defects in insulin resistance or disorder of pancreas and metabolism especially the saccharides in the liver [18].

In the current study, it was observed that HbA1C levels of patients were significantly higher ($p = 0.001$) than controls. Previous studies consider that HbA1C level as a good indication for diagnosis Diabetic in patients sera [19]. In present study, that serum total cholesterol is increased ($p = 0.001$) in group of diabetes compared with the control, one of the possible reason that glycated LDL may contribute to the increase in serum cholesterol level in diabetics [20, 21], and this results were in agreement with results [22].

At the same time, that LDL-C were increased ($p = 0.001$) in diabetes as compared with the control group and this result were similar with results [23]. Refer to same of the possible reason such as reduced of lipoprotein lipase activity which lead to reduce of TG catabolism and most converted VLDL-c into LDL-c which a play role large important during from reduction of the convert LDL-c in the live tissues [24,25]. On the other hand, may be increased LDL-c result from defect linkage LDL-c with the receptors that stimulated with present insulin [26].

It is a well-known fact that low HDL-C is common in diabetes patients and may be a strong factor for coronary heart disease (CHD) [27]. Which is consistent with the present study results that low HDL-C is evident compared with the control group (Table 4). Serum triglycerides (TG) and VLDL-C are increased ($p = 0.001$) in diabetic patients and this is in agreement with result [28]. that The most common lipid abnormality in DM is the which is known to be an independent risk factor of coronary heart disease (CHD) [29]. It is due to increase in VLDL-C synthesis and an impaired VLDL-C catabolism [30]. Which result from reduced of lipoprotein lipase activity which lead to increased TG and VLDL in the same time [23,31] due to insulin deficiency, Since the enzyme activity requires insulin for activation [31].

5. DNA Genotyping results:

E-selectin polymorphism analysis showed non-significant frequency higher of all genotypes in patients group than control group according to Fisher's exact test. The genotype TT recorded highly ratio in both groups patients and control (60-72%) which made it the common genotype for this locus in Iraqi population. both homozygous genotype showed preventive fraction according odds ratio (0.58, 0.93), while CT genotype according odds ratio consider as etiological fraction. The analysis of the allele impact, including both genotypes that containing C allele showed no significant association in patient group comparing with control ($p = 0.449$). According OR, allele C tend to be risky allele with environmental fraction (7.00%), while allele T tend to be a preventive allele (24.20%) (Table 5). Frequency of genotype TT, TC and CC significantly increase in the groups of patients compared with control, which is a reflection of the results achieved in other study conducted on Iraqi patients [9]. While, Heterozygous TC genotype showed higher frequency in diabetic patients compared to control, that was consistent with in another studies [32,33,34].

Table 5: Genotypes and alleles frequency of the rs 5367 C/T E-selectin polymorphism
P values calculated with Fisher's exact test

Group Genotypes	Study groups		Odds Ratio	CI 95%	P value Fisher -test	preventive or etiological fraction
	Patient	Control				
T/T	24(60.0)	18(72)	0.58	0.19 to 1.73	0.36	30%
C/T	13(32.5)	5(20)	1.93	0.59 to 6.83	0.33	15.6%
C/C	3(7.5)	2(8)	0.93	0.13 to 8.35	0.821	0.5%
Alleles						
T n (%)	61(76.25)	41(82)	0.7	0.28 to 1.70	0.449	24.2%
C n (%)	19(23.75)	9(18)	1.42	0.59 to 3.58	0.449	7%

p 0.05 is not significant

According to Hardy –Weinberg equation for the expected frequencies of genotypes, no significant differences ($p < 0.05$) between observed and expected frequencies for both patients and control group. (Table 6), The analysis of rs5367 SNP of splice region Exon 9 showed that genotype frequencies were in agreement with the Hardy-Weinberg equation. Homozygous TT genotype was detected in 60.0% of T2DM patients, heterozygous CT genotype was detected in 32.5 % and homozygous CC genotype was detected in 7.5 %. The frequency of T allele was 76.25 % and the frequency of C allele was 23.75 %. In the control group, the TT genotype was detected in 72 % of subjects, CT in 20 % and CC in 8%.The frequency of T allele was 82 % and the frequency of C allele was 18 %. The present results were consistent with previous study [35], which showed that the common allele T is the most protective allele compared to the rare allele C that is considered as a dangerous agent in at least some cases. That may be related with sample size, the cultural of Iraqi population people who tend to inner consanguineous marriage.

Table 6: Expected Frequencies Genotypes of E-Selectin rs5367C/T Using Hardy-Weinberg equation

Groups			TT	CT	CC	T	C	X^2
Patients Genotypes	Observed	No.	24	13	3	61	19	0.42
		%	60.0	32.5	7.5	76.25	23.75	
	Expected	No.	23.26	14.49	2.26	Not detected		
		%	58.15	36.2	5.65	Not detected		
Control Genotypes	Observed	No.	18	5	2	41	9	2.6
		%	72	20	8	82	18	
	Expected	No.	16.81	7.38	0.81	Not detected		
		%	67.24	29.52	3.24	Not detected		

The expected frequencies of genotypes showed no significant differences ($X^2 < 3.84$) between observed and expected frequencies for both T2DM patients and control group.

The natural wild genotype of the most common allele of TT predominant genotype showed a non-significant difference in control compared with patients using a Fisher test and showed a protective genotype. Also heterozygous TC genotype showed non-significant difference compared to control with patients and showed a genetic pattern associated with the risk to get disease, as well as, shows the genotype CC are a protective genotype. Current study - agreed with a local studies on patients diabetes [4, 9], various genetic factors, including single nucleotide polymorphisms, may play a role in the development of diseases. One nucleotide replaces another nucleotide of the gene, which occurs in the gene in a region near the gene, which plays a direct role in the disease. As occurring in region Intron Variant of rs 5367 C/T [36].

In this study, results concluded have been significantly increasing in all biochemical parameter in patient compare with healthy individual except HDL. Also, increasing risk of diabetic foot diseases could be associated with C allele and the CT genotype, while the T allele and TT and CC genotypes were present more in-patient of diabetic foot that are thought to have been a protective effect (preventive fraction). These alleles dominating in population of this study compared to others at genetic levels, in addition, can be used these alleles as an indicator of genetic biomarkers that effect on either pathogenesis or protection from the diabetic foot diseases.

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