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Research Article

The Role of Soluble L-Selectin with Polymorphism in Iraqi Arabs Patients with Diabetes Mellitus Type 2

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ABSTRACT

Diabetes mellitus type 2 [DMT2] is a disturbance of metabolism and complex diseases influenced by environmental, genetic agents, and linked with inflammation, happens when the pancreas either does not use the insulin as it should or the body does not make enough insulin, lead to insulin resistance [IR] alongside with gradual loss of ß-cell secretory ability. The aim of this study was to investigate the role of soluble L-selectin (sL-selectin) in diabetes mellitus type 2 patients in Iraqi Arabs patient. Study includes seventy six Iraqi Arabs patients (male and female) having newly diagnosed type 2 diabetes mellitus (T2DM), with Fifty three Iraqi Arabs healthy subjects matched in age, sex and ethnic group. Patients and healthy subjects were genotyped, by PCR-RFLP analysis, and mesure serum level of L-selectin by enzyme-linked immunosorbent assay (sandwich ELISA) test include 65 patients and 23 controls. The statistical analysis of serum level of sL-selectin in study groups showed that the mean of sL-selectin level high significantly increased in patients group (10.708±1.1007) compared to control group (7.055±0.767) respectively. Thus, our results suggest soluble L-selectin play a role in the development of DMT2 in Iraqi Arabs patients. Present results showed that genotype PS associated with increase the susceptibility of DMT2.

Keywords: L-selectin (CD62, SELL), Soluble L-selectin, Diabetes mellitus type 2 (DMT2), Single Nucleotide polymorphism (SNP) P213S (rs2229569). Enzyme-linked immunosorbent assay (ELISA).

INTRODUCTION

CD62L L-selectin is cleaved enzymatically from the leucocyte superficial membrane after cell activation and released into the blood stream, sL-selectin is active functionally[1]. SL-selectin half-life is a long in animal models, suggesting sL-selectin levels might remain raised for many hours afterward shedding the protein[2]. The determination of the concentration of soluble L-selectin can be beneficial for the monitoring and diagnosis of certain diseases such as DMT2, atherosclerosis, and cardiovascular disease (CVD)[3].

SELL gene location on chromosome one (1q24.2), and the total length of the SELL gene 27kbp, which is containing of eight introns and nine exons[4]. CD62L, a leukocyte adhesion molecule, is a type one membrane protein constitutively expressed on leukocytes[5] inclusive eosinophils, lymphocytes (subsets of memory T cells & naive T cells), monocytes, neutrophils, immature thymocyte, and hematopoietic progenitor cells[6]. L-selectin roles mediate both lymphocyte homing to peripheral lymph nodes and first recruitment leukocyte accumulation at locations of inflammation[7]. SELL plays important roles in marked adhesion, rolling leucocytes followed by activation, and regulation of immune cell`s homing to the infected tissues[8]. SL-selecting gene polymorphisms Proline 213 Serine (P 213 S) contribute to susceptibility to DMT2[9]. Polymorphism P213S (a C/T transition in exon six) of the gene symbolizes a good nominee in order to assess this danger. This genetic various determine a P 213 S interchange in the protein domain one and so seem to impact the interaction among endothelium and leucocytes[10].

MATERIALS AND METHODS

Iraqi Arabs patients (76) male and female having newly diagnosed T2DM were examined by the AL- Yarmouk Teaching Hospital. We selected patients who suffer from DMT2 less than six years, no history of heart disease, hyperlipidemia, no thyroid dysfunction, hypertension, renal disease, and were free of acute sickness and infection at time of sampling, their age range was among (26-77) years, with (53) Iraqi Arabs healthy subjects matched in age range was among (24-71) years, ethnic group and sex. Laboratory test were include:

Anthropometric measurements included

Central Obesity (CO) (Abdominal circumference) Waist to hip bones ratio (WHR) was measured by tool and recorded in (cm), weight (kg), height (m), and body mass index (BMI) was calculated by dividing the body weight in Kg the square of the height in (cm) according to the following equation:- BMI = Weight (Kg) / [Height (cm)] 2

Chemical parameters include

Fasting blood glucose (FBG), Uric acid (UA), Lipid profile (Total Serum Cholesterol T-c, Serum Triglycerides TG-c, Very Low Density Lipoprotein-Cholesterol VLDL-c, and Serum High Density Lipoprotein Cholesterol LDL-c) were

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measured in serum by auto analyzer ARCHITECT c4000 Methods Potentiometric, Photometric, and Turbid metric, and Glycosylated hemoglobin (HbA1c), this test from blood samples that collected in EDTA tubes from patients and controls. HbA1c was measured by Genius PA54 Specific Protein Analyzer method Nephelometry. While S-VLDL, S-LDL, and Atherogenic index of plasma (AI) detected mathematically by using the formula

*S.VLDL-C= TG - TC/5 in mg/d

*S-LDL-cholesterol= Total cholesterol-HDL-cholesterol-Triglycerides

*Atherogenic Index= $\frac{\text{Serum – total cholesterol}}{\text{HDL-cholesterol}}$

c- Immunological test

5The quantity determinations of serum L-selectin levels were performed by enzyme-linked immunosorbent assay (sandwich ELISA) kit, following the manufactures protocol of Shanghai Yehua Biological Technology Company. Developed color reaction was measured as OD 450 units on an ELISA reader. The concentration of serum L-selectin was determined by using standard curve constructed with the kit's standards over the range of 0.2-60 ng/ml.

d- DNA genotyping

The genomic DNA was extracted from the peripheral venous blood of patients and control (2ml) in tube containing ethylene diaminetetraacetic acid (EDTA), using the protocol of Genomic gSYNC™ DNA purification Kit and then stored at (-20°C). The Pro213Ser (rs2229569) SELL gene polymorphism genotyping was detected by Polymerase chine reaction and Restriction fragment length polymorphism (PCR-RFLP) technique. 186 bp was amplified, using the following primers: F 5' - TGA TTC AGT GTG AGC CTT TG – 3' And R 5' - CTT GAC AGG TTG GTT CTG – 3'.

Table [1] shows the PCR mix working solution

The PCR program has an initial denaturation at 94 ^{0}C for 5 minutes 1 cycle, 35 cycles at (94^{0}C Denaturation for 30 second, 58^{0}C Annealing for 45second and 72^{0}C Elongation for 45 second),and a final step at 72^{0}C final Elongation for 5 minutes 1 cycle. The quality of amplicons has been checked by means of electrophoresis (agarose 2%, TBE 1x). The genotypes spread within the genotypes were determined by digestion of (20µL) PCR product with (0.5 µL) of *Hph1* enzyme (New England Bio labs, Inc. Beverly, USA) for a 3 hour at 37°C followed inactivation at 65 °C for 10 minutes. The restriction fragments have been viewed in UV light, after electrophoresis in 4% agarose gel stained with ethidium bromide and run at 70volt/cm one hour, genotype PP yielded one fragment 186bp, genotype SS yielded two fragment 141 and 45, genotype PS yielded three digestion fragments of 186,141, and 45.

Table 1: The mixture of working solution.

Working Solution	
Go Taq Green Master Mix	25 µl
Primer Reverse	1.5 µl of each primer
Primer Forward	1.5 µl of each primer
DNA template	5 µl
Free Nuclease water	17 µl
Final volume 50µl	

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS version 21 computer software (statistical package for social sciences) and Microsoft Office Excel (Microsoft Office Excel for windows; 2010). Data were analyzed by using t-test (independent t-samples t-test) used to value significant difference among means between two groups. ANOVA (DUNCAN, LSD) test to test the significance of difference in means between more than two groups .Proportions were compared by chi-square.P $\leq$ 0.05*, p $\leq$ 0.01** was considered statistically significant, while p > 0.05 was considered non-significant. The association among study parameters compared by correlation.

RESULTS

The statistical analysis of parameters in study groups is shown in table [2], central Obesity (CO) were significantly higher in patients group (43.8816 ± .65640) compared to control group (40.8302±.68295). The mean of BMI significantly below (P $\leq$ 0.05) in patients group (31.1495±.82175) compared to control group (28.3927±.74754) respectively. The mean of parameters (FBG, HbA1c) were significantly higher in patients group (204.5658 ±8.36986, 9.0026±.19779) compared to control group (90.2642±1.38155, 4.9557 ±.08304) respectively. While Atherogenic index (AI) was non - significantly higher in patients group (5.4550±0.23063) compared to control group (4.8079±0.24218).

The statistical analysis of serum level of sL-selectin in study group's table [3] showed that the mean of sL-selectin level high significantly increased in patients group (10.708±1.1007) compared to control group (7.055±0.767) respectively.

The amplified DNA products were digested with Hph1 enzyme. PCR-RFLP analysis has showed the presence of all three genotypes of P213S polymorphism in the L-selectin gene. The presence of a restriction site for Hph I enzyme (213ser allele) and the amplicon digestion generates two fragments of 141bp and 45 bp. When the restriction sites is not created (pro213 allele), the amplicon is not digested and it maintains its size of 186 bp Figure [1].

Distribution of Genotype polymorphism in table [4].The data show that the genotypes (PP, PS, SS) recorded different between patients and controls. The homozygous genotype PP in patients (14.5 %) and in controls (32.1 %), while the heterozygous genotype PS and homozygous SS genotype were in patients (54.0 %) (31.5 %) and in controls were (16.9 %) and (51.0 %). There were high significant differences according to Chi square test (p$\leq$ 0.05). The data showed that the total genotypes (PP, PS, SS) recorded different. The homozygous genotype PP recorded (21.7 %), while the heterozygous genotype PS and homozygous SS genotype recorded (38.8 %) (39.5 %). There were high significant differences according to Chi square test (p$\leq$ 0.05).Within group the data showed that the

Table 2: Statistical analysis of parameters in patients and control.

Parameters	Patients group Mean ± SE	Control group Mean ± SE	t- test	P –value
CO(cm)	43.8816 ± .65640	40.8302±.68295	3.141	0.002**
BMI (kg/m^2)	31.1495±.82175	28.3927±.74754	2.365	0.020*
FBG(mg/dl)	204.5658 ±8.36986	90.2642±1.38155	13.474	0.000**
HbAlc (%)	9.0026±.19779	4.9557 ±.08304	18.866	0.000**
AI (%)	5.4550±.23063	4.8079±.24218	1.890	0.061NS

Significant differences $p \leq 0.05$*, $p \leq 0.01$**, non_ significant $p > 0.05$.

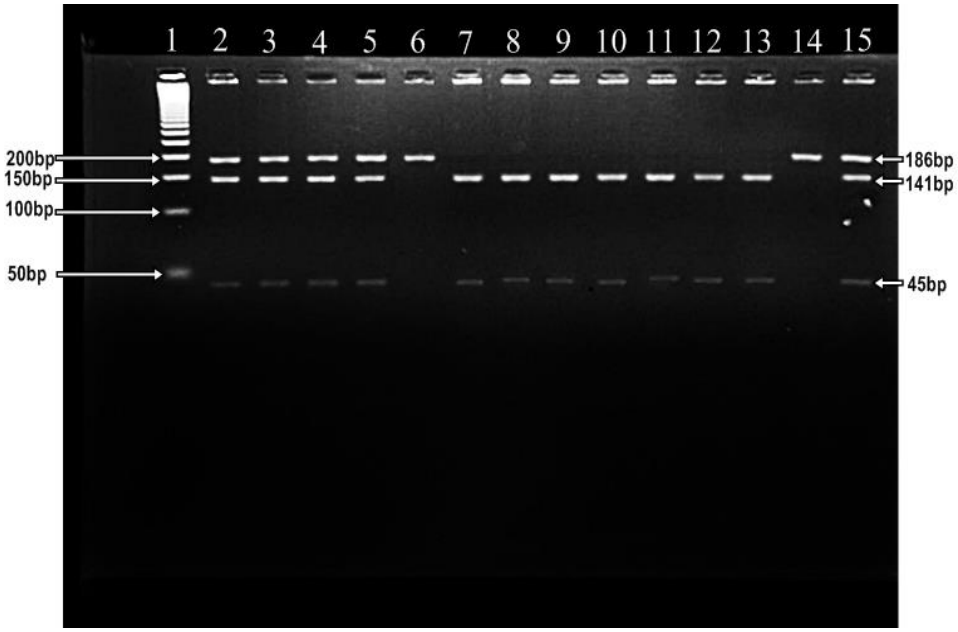

Figure 1: Electrophoresis in 4% agarose gel stained with ethidium bromide and run at 70volt/cm one hour, the electrophoresis results for Pro213Ser polymorphism (lines 1: DNA leader 50 bp; lanes 2,3,4,5,15 :ProSer genotype; lanes 6,14: ProPro genotype ;lanes 7,8,9,10,11,12,13: SerSer genotype).

Table 3: Statistical analysis of serum level of sL-selectin in patients and control.

Characteristics	Patients group Mean ± SE	Control group Mean ± SE	t- test	P –value
SL-selectin(ng/ml)	10.708±1.1007	7.055±0.767	2.723	.008**

Significant differences $p \leq 0.05$*, $p \leq 0.01$**, non_significant $p > 0.05$.

genotypes (PP, PS) and (SS, PS) high significant differences according to Chi square test ($p \leq 0.05$).
$X^2 = 18.427$** df = 2 $p \leq 0.05$ (Significant differences) for each genotype groups.
$X^2 = 14.736$** df = 1 $p \leq 0.05$ (Significant differences) for PP and PS genotype.
$X^2 = 13.438$** df = 1 $p \leq 0.05$ (Significant differences) for SS and PS genotype.
Anthropometric features in study groups according to genotypes show in table [5], Patients genotype group PP showed significant mean of BMI (28.92±1.80) compared to both other genotype groups, while the mean of BMI was non-significant between both other genotype groups (31.40±0.94, 31.73±1.89) respectively. Control groups for genotype showed non-significant mean of BMI among them. The comparison between patients and control for PP, PS, and SS genotype group showed non-significant mean

Table 4: Genotype distribution of P213S polymorphism in DMT2 patients and control.

Genotypes	Patients		Controls	
	NO.	%	NO.	%
PP	11	14.5	17	32.1
PS	41	54.0	9	16.9
SS	24	31.5	27	51.0

of BMI. Patients all genotype groups showed non-significant mean of CO (44.27±1.48, 44.00±0.93, 43.50±1.18) respectively. Control genotype group PS showed high significant mean of CO (42.66±1.81) compared to both other genotype groups (40.11±1.08, 40.66±0.99) respectively, while the mean of CO was non-significant between both other genotype groups (40.11±1.08, 40.66±0.99) respectively. The comparison between patients and control for genotype groups PS, SS

Table 5: Statistical analysis of Anthropometric characters according genotypes.

Characteristics		Genotypes		
		PP NO. 28 Mean ± SE	PS NO. 50 Mean ± SE	SS NO. 51 Mean ± SE
BMI (kg/m^2)	Patients	28.92±1.80 b	31.40±0.94 a	31.73±1.89 a
	Controls	27.72±1.26 a	28.36±1.27 a	28.82±1.17 a
P-value		.580 NS	.155 NS	.187 NS
CO(cm)	Patients	44.27±1.48 a	44.00±0.93 a	43.50±1.18 a
	Controls	40.11±1.08 bc	42.66±1.81 a	40.66±0.99 b
P-value		.029 *	.542 NS	.071 NS

Significant differences $p \leq 0.05$*, $p \leq 0.01$**, non_significant $p > 0.05$.
The different letters at the same row mean significant differences.

Table 6: Statistical analysis of some chemical parameters in patients and control according genotypes.

Chemical Parameters		Genotypes		
		PP NO. 28 Mean ± SE	PS NO. 50 Mean ± SE	SS NO. 51 Mean ± SE
FBG(mg/dl)	Patients	233.09±22.5 a	209.78±11.88 a	182.58±12.77 b
	Controls	186.94±9.01a	89.22±2.54 b	89.48±1.59 b
P-value		.039 *	.000 **	.000 **
HbAlc (%)	Patients	10.07±0.71a	8.66±0.22b	9.09±0.33b
	Controls	4.802±0.14 b	4.91±0.29 ab	5.06±0.09 a
P-value		.000 **	.000 **	.000 **
AI (%)	Patients	4.83±0.36 b	6.05±0.34 a	4.71±0.33 b
	Controls	4.28±0.27 b	5.15±0.57 a	5.02±0.39 ab
P-value		.234 NS	.259 NS	.557 NS

Significant differences $p \leq 0.05$*, $p \leq 0.01$**, non_significant $p > 0.05$.
The different letters at the same row mean significant differences.

Table 7: Distribution of sL-selectin level in patients according genotypes.

Chemical Parameters		Genotypes		
		PP NO. 17 Mean ± SE	PS NO. 36 Mean ± SE	SS NO. 31 Mean ± SE
sL-selectin (ng/ml)	Patients	12.21±3.77 a	9.29±1.51 a	12.13±1.61a
	Controls	6.33±1.08 a	7.72±2.60 a	7.39±1.20 a
P-value		.225 NS	.776 NS	.026 *

Significant differences $p \leq 0.05$*, $p \leq 0.01$**, non_significant $p > 0.05$.
The different letters at the same row mean significant differences.

showed non-significant mean of BMI, while showed significant mean of CO in genotype group PP. The statistical analysis of some chemical parameters study group's according genotypes is show in table [6], Patients each genotype groups showed significant mean of FBG. Control genotype group PP showed significant mean of FBG (186.94±9.01) compared to both other genotype groups (89.22±2.54, 89.48±1.59) respectively, while genotype group PS showed non- significant mean of FBG (89.22±2.54) compared to genotype group SS (89.48±1.59). The comparison between patients and control for each genotype groups showed significant mean of FBG. Patients PP genotype group showed high significant increased level of HbA1c (10.07±0.71) compared to PS and SS genotype groups (8.66±0.22, 9.09±0.33) respectively, while the mean of HbA1c was non-significant between both other genotype groups (8.66±0.22, 9.09±0.33) respectively. Control genotype group PS showed non-significant mean of HbA1c (4.91±0.29) compared to both other genotype groups (4.802±0.14, 5.06±0.09) respectively, while the mean of HbA1c was significant HbA1c between both other genotype groups (4.802±0.14, 5.06±0.09) respectively. The comparison between patients and control for each genotype groups showed high significant mean of HbA1c. Patients PS genotype group showed significant mean of AI (6.05±0.34) compared to both other genotype groups (4.83±0.36, 4.71±0.33) respectively, while the mean of AI was non- significant between both other genotype groups (4.83±0.36, 4.71±0.33) respectively. Control genotype

Table 8: The correlation between the studies parameters.

Parameters	SL-selectin		CO		BMI		AI	
	R	P-value	R	P-value	R	P-value	R	P-value
FBS	0.06	0.65	0.03	0.76	0.15	0.19	-0.07	0.51
HbA1c	0.03	0.80	0.11	0.34	0.24	0.06	0.05	0.62
AI	0.004	0.97	-0.05	0.67	0.08	0.48	------	------

Significant differences $p \leq 0.05$*, $p \leq 0.01$**, non_significant $p > 0.05$.

group SS showed non-significant mean of AI (5.02±0.39) compared to both other genotype groups (4.28±0.27, 5.15±0.57) respectively, while the mean of AI was significant between both other genotype groups (4.28±0.27, 5.15±0.57) respectively. The comparison between patients and control for each genotype groups showed high non-significant mean of AI.

The data showed in table [7] Patients of each genotype groups showed non-significant level of sL-selectin (12.21±3.77, 9.209±1.52, 12.13±1.61) respectively. Control each genotype groups showed non-significant level of sL-selectin (6.33±1.86, 7.72±2.60, 7.39±1.20) respectively. The comparison between patients and control for PPand PS genotype groups showed non-significant level of sL-selectin, while patients SS genotype group showed significant differences (12.13±1.61) compared to control genotype groups (7.39±1.20). The correlation between the studies parameters are shown in table [8]. The mean of sL-selectin negative and no significant correlation between the means of sL-selectin and all parameters. The mean of CO, BMI and AI showed negative and non- significant correlation with all parameters.

DISCUSSION

The comparison between study groups according to anthropometric parameters showed significant increased (CO) & (BMI) in patients compared to control. Adipose tissue releases a high amount of non_esterified fatty acids (NEFA), glycerol, pro inflammatory cytokines (such as IL-18 & IL-6), and hormones, in obese patients. These elements are involved, in the evolution of (IR) insulin resistance[11]. Thus increase (CO) and (BMI) linked with increased rate dangers of Diabetes mellitus type 2. While in previous report showed the same parameters decreased level in Iraqi Arab patient[12,13]. Present results are consistent with a local study showed significant body mass index in patients compared to control[14].

The mean of FBG and HbA1c in present data indicated that were high significant patient compared to control in diabetic, these result agree with previous study in Iraqi demonstrated that the combination of FBG and HbA1c identifies persons who are at danger of progression to T2DM[12,13]. Present results are agree with a local study showed significant FBG and HbA1c in patients compared to control[15]. Pancreatic β-cells encourage proliferation from short term exposure to growing glucose concentrations; after a prolonged exposure to increased glucose concentrations led to suppress the proliferative ability[16]. Rise the glycation of common proteins like hemoglobin, creating Hemoglobin A1c (HbA1c) when glucose concentrations increased. BbA1c is known to correlate with blood glucose levels over the lifetime of the RBC about 120 days[17].

The present data indicated that the mean of AI was non-significant in diabetic patient compared to control, these results agree with previous Iraqi study which showed AI non-significant in patients group compared to healthy control[13], while dis agree with another previous Iraqi study showed AI significantly higher in diabetic group compared to healthy control[12].

The present data show the mean of sL-selectin level high significantly increased in patients group with DMT2 compared to control group, this result disagree with a previous results showed the serum level of sL-selectin is decreased in patients with type 2 diabetes[18], Patients with type II diabetes had sL-selectin levels which did not differ from controls[19], While agree with previous results in ischemic stroke patients showed soluble L-selectin level were increased in patients group[20], and with previous results in benign thyroid diseases and papillary thyroid carcinoma (PTC) showed significantly higher in patients with both as compared to the healthy individuals[21].

Selectins play main roles in the first step in leukocyte recruitment and stealth, which is essential in the key process of atherosclerosis[22]. The high serum levels of L-selectin have been linked with different diseases in which the micro inflamation has an important part DMT1, atherosclerosis[19]. The increasing of serum levels of L-selectin happens as a consequence of its rapid cleavage on the leucocytes superficial in the neighboring zone of the trans membrane domain. So the association among leucocytes and endothelial cells can be rapidly damaged and they can roll at the endothelium level. When inflammation happens, the SELL expression decreases on the leucocytes superficial, entailing rise of the serum level of sL-selectin[23]. SL-selectin serum levels have been shown to rise in acute inflammatory conditions, while have low levels in chronic disease suggesting an important role for soluble Lselectin in alter chronic inflammatory diseases. Modulating soluble Lselectin so presents a potential therapeutic aim for addressing skin injury in patients with diffuse systemic sclerosis dSSc[1].

The P213S polymorphism may affect the levels of soluble L-selectin. The serum levels of L-selectin SS genotype carriers was significant than no carriers (PP and PS genotypes) in patients with DMT2 compered to control. Local study showed PS genotype possible risk factor in patients with DMT2[24].

All parameter of present study showed non-significant correlation with sL-selectine indented risk factors; however a functional relationship could be among them. That relationship may causes the progression of diabetes, farther studies showed is down.

In summary, we found that the soluble L-selectin level in serum was significantly associated with risk of DMT2 in Iraqi Arab patients. Statical analysis showed high mean of SL-selectin in SS genotype group that preface to infected disease.

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