Editorial Article

Skin AGEs and Complications of Diabetes Mellitus

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According to the International Diabetes Federation (IDF) statistics, it is estimated that every 7 seconds someone is dying due to diabetes mellitus (DM) or its complications.[1] Major complications of DM include cardiovascular disease, diabetic nephropathy, diabetic neuropathy, and retinopathy.[2] These complications are also linked with substantial morbidity and poor quality of life.[1,2] Therefore, timely diagnosis of DM and its complications is crucial. In this context, skin advanced glycation end products (skin AGEs) have received increased attention during the last years.[3]

During hyperglycaemic state, glucose forms a non-enzymatic process with plasma proteins, lipids, or nucleic acids, which is termed the "Maillard reaction".[4] This reaction results in the formation of endogenous AGEs. In addition to them, exogenous AGEs are of extreme importance. Indeed, dietary AGEs are by-products of heat-processed food consumption and are partially absorbed as such.[5] Smoking is also a major source of exogenous AGEs.[6] AGEs are metabolised via extracellular proteolysis as well as intracellular uptake and degradation in tissue macrophages, and Kupffer cells in the liver.[7] Once degraded intracellularly, AGEs are further metabolised to second generation AGEs and excreted by kidneys.[7]

AGEs accumulate with increasing age. Nevertheless, their accumulation is exacerbated by many pathologic conditions, such as DM, cardiovascular disease, Alzheimer's disease etc. In such conditions, AGEs are not merely a disease manifestation, but instead a factor contributing to the pathophysiology.[8]

Measurement of AGEs can be accomplished by several different methods. These include ELISA using monoclonal or polyclonal antibodies, high performance liquid chromatography (HPLC), and mass spectrography.[9] For tissue-bound AGEs, the gold standard remains as tissue biopsy, but it is invasive and very demanding.

In recent years, AGE-related autofluorescence has been used as a non-invasive option to measure AGEs. This method uses the autofluorescence, which is inherent in some major AGEs (e.g. pentosidine).[10] In order to achieve quick, inexpensive and non-invasive measurements, the AGE reader (Diagnoptics, Groningen, The Netherlands) was produced.[10,11] This is a portable device that illuminates a skin surface of approximately 4 cm^2 at the volar side of the dominant arm, guarding against surrounding light with an excitation light source. Emission and reflected extraction light are measured with a spectrometer using a glass fibre. [10,11]

Increased skin autofluorescence (SAF) has been linked with microvascular complications of DM such as nephropathy, neuropathy and to a lesser extent retinopathy.[10-12] In subjects with type 2 DM, this method was also able to predict likelihood of developing these microvascular complications except for retinopathy.[12]

SAF can contribute to the timely and accurate identification of the future risk for diabetic

peripheral neuropathy (DPN).[13] In high-risk subjects, close monitoring, multifactorial treatment and education should be intensively offered to avert further risk of complications, notably foot ulcerations.[13,14]

In conclusion, skin AGEs are increasingly being appreciated for the timely diagnosis of chronic DM complications. For this purpose, they merit further widespread utilisation, to improve patient outcomes.[13-15]

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Original Article

Relationship Between E – Selectin Gene Polymorphism and Carotid Artery Atherosclerosis in End Stage Renal Disease

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Abstract:

Background: Adhesion molecules like the members of selectin family participate in the interaction between leukocytes and the endothelium. They are also involved in the pathogenesis of atherosclerotic processes. E-selectin is a cell surface glycoprotein that mediates the adhesion of leucocytes to vessels endothelium, an important early step in the atherosclerotic process. End-stage renal disease (ESRD) is a highly atherogenic disease, but it is unknown whether genetic polymorphism(s) in the E-selectin gene plays a role in the severity of arterial damage in this condition. **Objective:** The aim of the study was to test the relationship between the Leu 554 Phe polymorphism of the E-selectin gene and the severity of carotid atherosclerosis in End stage renal disease patients. **Methods:** In this study, we tested whether the Leu554Phe variant in the E-selectin gene is linked to carotid atherosclerosis as estimated by intima-media thickness (IMT), cross-sectional area and carotid stenosis using carotid ultrasonography in 70 well-characterized ESRD patients. The frequency of this polymorphism was also measured in a population sample of 30 healthy controls of the same geographical area using genetic analysis of Leu554Phe polymorphism of E-selectin gene by restriction fragment length polymorphism **Results:** A total of 14.3% patients had the CC genotype, 78% had the CT genotype, 7.14% had the TT genotype and this distribution did not differ from that in the control population. Intima-media thickness (IMT) (P =0,926) and cross-sectional area (P = 0.567) were not significantly higher in patients with the T-allele than in those without this allele. Furthermore, the degree of carotid stenosis was not significantly higher (P = 0.593) in patients with T-allele than in CC patients. On multivariate analyses including the traditional and non-traditional risk factors, the Leu554Phe polymorphism was confirmed as not an independent correlate of IMT (P = 00926), cross-sectional area (P = 0.567) and carotid stenosis (P = 0.593). **Conclusion:** In ESRD, the Leu554Phe polymorphism of E-selectin gene is not associated with the severity of carotid atherosclerosis, suggesting that genetically-determined alterations in the E-selectin molecule doesn't render ESRD patients with this gene variant particularly susceptible to the detrimental effects of inflammation on the arterial wall.

Key Words: E- selectin, Leu554Phe variant, carotid stenosis, ESRD,

INTRODUCTION

Atherosclerosis is a multifactorial disease that involves the interaction of environmental and genetic factors; it is the single most common cause of death and disability worldwide [(1)]. Accelerated atherosclerosis has been noticed in patients with CKD. It was also noticed that atherosclerotic lesions developed in the early stages of CKD [(2).]

Cardiovascular diseases are 3.5–100 times higher in patients who are on maintenance dialysis than the general population [(3)]. End-stage renal disease patients have also much greater mortality from myocardial infarctions (MIs) [(4)]. The mortality of patients receiving dialysis reaches 60 % in the first year after a first myocardial infarction.

Numerous pathophysiologic observations led to the postulation of the response-to-injury hypothesis of atherosclerosis, which initially stated that endothelial desquamation was the key event in atherogenesis [(5)]. Now, it is considered that the pathophysiology of atherosclerosis is due to endothelial dysfunction rather than endothelial denudation. Whichever process is at work, each characteristic lesion of atherosclerosis represents a different stage in a chronic process of inflammation that takes place in the arterial wall [(6).]

The first morphologic phenomenon observed in plaque formation is the adherence of monocytes to the endothelial surface [(7)], monocytes adherence are triggered by a number of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), P-Selectin and E-Selectin. Moreover, there are T-lymphocytes that tend to accumulate in atherosclerotic lesions [(8).]

This adhesion is followed by monocytes migration into the intima, this migration is induced by the presence of certain mediators called chemo attractants in the intima as monocyte chemoattractant protein-1 (MCP-1) which is released from the endothelium [(9)].

In the intima, monocytes are activated, converted to macrophages and may divide. Macrophage cell division is triggered by macrophage colony stimulating factor (M-CSF), which can augment expression of macrophage scavenger receptors leading to uptake of modified lipoprotein particles and formation of lipid laden macrophages or foam cells [(10)].

The macrophage foam cells produce a range of inflammatory cytokines including tumor necrosis factor alpha, metalloproteinases and the pro coagulant tissue factor that potentially stimulates thrombus formation when in contact with blood [(11)].

Atherosclerosis in chronic kidney disease:

The increased risk of atherosclerosis in CKD patients is the result of interaction between traditional risk factors and specific uremic and dialysis related risk factors [(12)]. Moreover, the prevalence of the traditional risk factors is greater in dialysis dependent patients than in the general population [(13)].

Cardiovascular risk factors in chronic kidney disease:

Risk factors can be divided into the following:

I- Traditional risk factors [(14)] :

Traditional risk factors include the following: age , sex, family history , obesity, cigarette smoking, hypertension, diabetes mellitus dyslipidemia and sedentary life style

II- Non traditional risk factors [(15)]:

They include homocysteine ,lipoprotein (a), asymmetric dimethyl arginine (ADMA), advanced glycation end products (AGEs) ,oxidative stress , infections and inflammation.

III- Other potential risk factors specific to ESRD

Risk factors related to ESRD include disturbance of glucose metabolism, anaemia [(16)], calcium-phosphate disorders [(17)], coagulation equilibrium imbalance [(18)], vitamin E-deficiency [(19)], hypoalbuminaemia [(20)],haemodynamic overload (plasma volume expansion, arteriovenous fistula) [(21)].

IV- Risk factors related to dialysis [(21)]:

Endotoxin, bioincompatibility of membranes, fluctuation in blood pressure and fluctuation in serum electrolytes level.

Inflammation and atherosclerosis

Atherosclerosis is considered as a chronic inflammatory process as suggested by Ross. Inflammation mediates all stages of atherosclerosis from initiation through progression and thrombotic complications.

Inflammation determines the fragility of the fibrous cap of the plaque, and its thrombogenic potentiality. T cells stimulate matrix metalloproteinases production such as proteinase, collagenase and also the production of the pro-coagulant tissue factors .Macrophage-derived matrix metalloproteinase digests the extracellular matrix of the plaque resulting in fibrous cap instability and plaque rupture. This in turn leads to the exposure of the tissue factor-rich lipid core to the circulating blood components, and thrombosis occurs [(22)].

Adhesion Molecules:

The adherence of circulating leucocytes to endothelial cells and the subsequent trans-endothelial migration to the vascular intima is one of the key events in the initiation of atherosclerosis [(23)]. These processes are mediated chiefly by different cellular adhesion molecules (CAMs), which are released on the surface of vascular endothelial cells in response to pro-inflammatory cytokines, oxidants or oxidized LDL-cholesterol [(24)]. Increased expression of CAMs have been described on endothelial cells, vascular smooth muscle cells and macrophages in human atherosclerotic plaques. Soluble types of these adhesive molecules are thought to be shed from activated cells and can be measured in peripheral blood. Plasma concentrations of these soluble forms of CAMs are elevated in septicemia, autoimmune diseases and allograft rejection [(24)].

Cell adhesion molecules fall mainly under four broad families selectins, immunoglobulin superfamily, integrins and cadherins [(25)]. The members of immuoglobulin superfamily are related to immunoglobulins and are often expressed on cell surface. They are the biggest group of adhesion molecules including more than 70 members, e.g., intercellular adhesion molecules (ICAM), and vascular cell adhesion moleucle-1 (VCAM-1) [(25)].

Members of the selectin family play an important role in the adhesion of leukocytes to activated endothelium. This produces characteristic rolling motion of the leucocyte on the endothelial surface. The selectins can be classified according to their site of expression into: P-selectin (platelet), L-selectin (leucocyte) and E selectin (endothelium) [(26)].

E-selectin:

E-selectin was described as an antigen that was induced on cultured human umbilical vein endothelium after stimulation by interleukin 1 (IL-1) and that was contributed to the adhesion of neutrophils, previously described as endothelial leucocyte adhesion molecule-1 [(27)].

E-selectin has a molecular weight of approximately 94.000 dalton. It is composed of an array of discrete protein domains; an amino terminal, C-type lectin domain, a single epidermal growth factor (EGF) like domain and from 4-9 short consensus repeats (SCR), a single membrane spinning region and a cytoplasmic tail with a 589 amino acids as shown in Figure (1) The terminal lectin domain and an epitope within the EGF like region are important for ligand binding [(28)]. The human E-selectin gene *(SELE)* is located on chromosome 1q. It consists of 14 exons and 13 introns, spanning approximately 13kb [(29)] .

E-selectin is expressed only by cytokines activated endothelial cells, cytokines such as interluekin-1 and tumor necrosis factor alpha induce ICAM, VCAM, and E-selectin. Interleukin-1 mRNA and tumor necrosis factor protein have been demonstrated in smooth muscle cells and macrophages of atherosclerotic plaques. At the endothelial cells, E-selectin as other selectins mediate leukocyte rolling which is the initial step in leukocytes adhesion to the endothelial cells [(30)].

E-selectin is maximally released 2-4 hours after cell has been activated. Within the next 24-48 hours, E-selectin is removed from the cytoplasmic membrane by shedding into the circulation [(31)] Plasma E-selectin levels were increased in patients with hypertension, hyperchlosterolemia and diabetes mellitus, all the three disorders are important risk factors for atherosclerosis, it was expressed by endothelial cells overlying atherosclerotic plaques as well as by adventitial vessels [(32)].

Several lines of evidence indicate that besides environmental factors, the genetic background has an important influence on inflammation-induced arterial damage. The E

selectin genomic location was previously known to be linked to blood pressure. Moreover the polymorphism in the E-selectin gene (Leu554Phe) hinders the E selectin shedding from the endothelium was believed to be associated with coronary atherosclerosis [33].

In patients with ESRD, it is still unknown whether the genetic background plays a role in atherosclerosis and inflammation. It is still a relevant question because in uremia, the factors predisposed to atherosclerosis do not correspond to those in the general population. Adhesion molecules have a crucial rule in the development of atherosclerosis, Therefore attention was given on Leu554Phe polymorphism as it enhances the anchoring of E-selectin to the endothelium [34]. This alteration in amino acids sequence in E-selectin gene that might amplify the inflammation process on the vessel wall.

AIM OF THE STUDY

The aim of the study was to test the relationship between the Leu554Phe polymorphism of the E-Selectin gene and the severity of carotid atherosclerosis as estimated by intima- media thickness (IMT), cross-sectional area and carotid stenosis using carotid ultrasonography in End stage renal disease (ESRD) patients.

SUBJECTS AND METHODS

The present study was conducted on 100 subjects selected from the kidney dialysis unit, El-Mowasah hospital, Alexandria Main University Hospital.

The design of the study consist of two groups:

Group (I): included 70 dialysis patients of age ranged between 20 to 70 years old ,who have been on regular dialysis treatment (RDT) for at least 6 months and who were free of overt infections (fever, infected vascular access , peritonitis or exit-site infection) but suffered from other chronic disease as hypertension , diabetes and cardiac diseases.

Group (II): included 30 healthy age and sex matched as patients who were considered as control group for genetic data analysis, and who were free from acute or serious chronic diseases such as cancer, hypertension, diabetes and Coronary heart disease.

The following was done to all the enrolled subjects:

I- Thorough history taking and Complete clinical examination including:

1. Body mass index.

The patients were grouped according to their body mass index (BMI) into underweight (<18.5 kg/m2), average weight (18.5–24.9 kg/m2) and overweight (>24.9 kg/m2).

2. Vital signs.

- **Heart rate**

It is measured by palpation of the radial artery pulsation, number of heart beats per minute.

- **Blood pressure**

In hemodialysis patients pre dialysis and post dialysis BPs were calculated as the average value of all recordings [12 measurements (i.e. 3 per week)] taken during the month preceding the study. The mean value of pre dialysis and post dialysis BPs was then obtained for each patient and it was considered for global statistical assessment.

II- Laboratory investigations including:

1- Fasting blood glucose is performed during the mid-week non-dialysis day [35].

2- Blood urea and serum creatinine.

3- Haemoglobin level [36].

4- Fasting Lipid profile (Total cholesterol, serum triglycerides, low density lipoproteins cholesterol and high density lipoproteins cholesterol) [37].

5- Serum calcium, phosphrous and PTH [38].

6- CRP, measured by nephelometry (high sensitivity)[39].

7- Genetic analysis of Leu55Phe polymorphism of E-selectin gene by restriction fragment lengh polymorphism [40].

I- Carotid artery ultrasonography for vascular assessment:

Doppler studies were performed in longitudinal and transverse planes using anterior, lateral and posterior approaches by both B-mode imaging and power Doppler imaging. Assessment of atherosclerosis is done by measurements of:

1- Carotid intima media thickness [41]:

Measurement of carotid IMT bilaterally was done by B-mode 7 MHZ probe [ultrasound examination was done using LOGIQ 7 PRO, GE (General Electric Medical System).

2- Degree of stenosis [42].

3- Cross section area, was calculated by the standard formula [43].

Statistical Analysis of the data [44]:

Data were statistically described in terms of mean ± SD, median (interquartile [IQR] range) or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student's t test for independent samples in comparing two groups when normally distributed and Mann-Whitney U test for independent samples when not normally distributed .

Comparison of numerical variables between more than two groups was done using the one-way analysis of variance (ANOVA) test with post hoc multiple two-group comparisons in normal data, and the Kruskal Wallis test with post hoc multiple two-group comparisons in non-normal data. Association between qualitative data was done using the Chi-square test .

Correlation between various variables was done using the Pearson moment correlation equation for linear relation in normally distributed variables and Spearman's rank correlation equation for non-normal variables. P-Values less than 0.05 were considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

RESULTS

I- Demographic data:

This study included 100 subjects, which were divided into two groups.

Group (I): included 70 dialysis patients, who have been on regular dialysis treatment (RDT) for at least 6 months and who were free of overt infections but suffer from other chronic disease as hypertension , diabetes and cardiac diseases, which were considered as risk factors of atherosclerosis. This group was then subdivided according to the genotype distribution of Leu554Phe E selectin gene polymorphism on basis of absence or presence of risk allele (T) into **(TT/CT)** versus **(CC).**

Group (II): included 30 healthy age and sex matched as patients who were considered as control group for genetic data analysis, and who were free from acute or serious chronic diseases **Table (1)**.

II- Genotype distribution:

The genotype distribution of the Leu554Phe polymorphism of the E-selectin gene did not deviate from Hardy–Weinberg equilibrium either in patients (CC genotype, 14.3%; CT genotype, 78.6%; TT genotype, 7.1%) or in control healthy subjects (CC genotype, 13.3%; CT genotype, 83.3%; TT genotype, 3.3%) **Tables (2)**.

IV- Carotid artery ultrasonography for measuring the indicators of atherosclerosis:

1- Carotid intima media thickness (IMT):

In Patients, IMT ranged 0.60 – 1.6 mm with a mean of 1.08 ± 0.67 mm while in control, it ranged between 0.61 – 0.89 mm with a mean of 0.76 ± 0.10mm, there were statistically significant difference between two groups as regard IMT, where P<0.001 (P significant as P<0.05).**Table (3)**

2- Cross section area:

In Patients, cross section area ranged 1.227-40.7 mm^2 with a mean of 21±19.7 mm^2 while in control, it ranged between 13.85-46.56 mm^2 with a mean of 30.205 ± 16.355 mm^2, There were statistically significant difference between two groups as regard cross section area, where P<0.001 (P significant as P<0.05). **Table (3)**

3- Degree of stenosis:

In Patients, degree of stenosis ranged 0.0 – 20.0%with a mean of 2.29 ± 4.87 %while in control ,it ranged 0.0 – 10.0% between with a mean of 0.50 ± 2.01% ,There were

statistically significant difference between two groups as regard degree of stenosis, where P=0.040 (P significant as P<0.05). **Table (3)**

V- E-selectin Leu554Phe polymorphism and carotid atherosclerosis

When patients were categorized on the basis of the presence/absence of the risk allele (i.e. TT and CT vs CC patients), no significant difference emerged between the two groups as for demographic and somatometric data or for clinical and biochemical parameters. **Table (4)**

Relation of presence of the risk allele (TT/CT) and indicators of carotid atherosclerosis:

1) Intima media thickness:

Patient's intima Thickness in the Patients with CT or TT genotypes ranged between 0.60 – 6.1 mm with a mean of 0.888 ± 0.712 mm while in Patients with CC genotypes it ranged between 0.60 – 1.8 mm with a mean of 0.850 ± 0.375 mm. There were no statistically significant difference between patients with presence of risk allele (TT/CT) and patients with the absence of risk allele (CC) as regard IMT where P=0.926 (P significant as P<0.05).**Table (5).**

2-Cross section area:

Patient's Cross section in the Patients with CT or TT genotypes ranged between 1.227-40.7 mm^2 with a mean of 21±19.7 mm^2 while in Patients with CC genotypes it ranged between 5.123-40.1 mm^2 with a mean of 20.996±19.836 mm^2. There were no statistically significant difference between patients with presence of risk allele (TT/CT) and patients with the absence of risk allele (CC) as regard cross section area, where P=0.567 (P significant as P<0.05).**(Table (5)**

3- Degree of stenosis:

Patient's degree of stenosis in the Patients with CT or TT genotypes ranged between 0 – 20 % with a mean of 2.25 ± 4.642 % while in Patients with CC genotypes it ranged between 0 – 20 % with a mean of 2.5 ± 6.346%. There were no statistically significant difference between patients with presence of risk allele (TT/CT) and patients with the absence of risk allele (CC) as regard degree of stenosis, where P=0.593 (P significant as P<0.05).**(Table (5)**

E- Selectin gene and carotid atherosclerosis multiple regression analyses:

Since the association between E-selectin genotypes and carotid atherosclerosis could be confounded by other risk factors, multiple regression analyses were performed adjusting for all variables which were associated with the three indicators of carotid atherosclerosis. In these analyses, mentioned risk factors (cofounders) were confirmed as a positive correlate of IMT, cross sectional area and the severity of carotid stenosis **(Table 6).**

Table (I): Comparison between the two studied groups according to demographic data

	Cases (n = 70)		Control (n = 30)		Test of Sig.	p
	No.	%	No.	%		
Sex						
Male	40	57.1	13	73.3	χ^2=	0.205
Female	30	42.9	17	56.7	1.608	
Age (years)						
Min. – Max.	22.0 – 74.0		27.0 – 55.0		t=	0.055
Mean ± SD.	51.06 ± 13.52		47.30 ± 5.84		1.941	
Median	52.50		48.50			

χ^2: **Chi square test** t: **Student t-test**

p: p value for comparing between the two groups

Table (II): Comparison between the two studied groups according to E-selectin gene polymorphism

	Cases (n = 70)		Control (n = 30)		χ^2	^{MC}p
	No.	%	No.	%		
E-selectin						
TT	5	7.1	1	3.3		
CC	10	14.3	4	13.3	0.437	0.918
CT	55	78.6	25	83.3		
HWE	0.053		0.139			

χ^2: **Chi square test** MC: Monte Carlo test

p: p value for comparing between the two groups

HWE: p value for comparing observed and expected data according to Hardy-Weinberg between the two groups

Table (III): Comparison between the two studied groups according to the indicators of atherosclerosis

	Cases (n = 70)	Control (n = 30)	U	p
INT thickness (mm)				
Min. – Max.	0.60 – 1.6	0.61 – 0.89		
Mean ± SD	1.08 ± 0.67	0.76 ±0.10	397.50*	<0.001*
Median	1.0	0.77		
Cross section (mm^2)				
Min.– Max.	1.227– 40.7	13.85– 46.56	3.871*	
Mean ± SD.	21 ± 19.7	30.205± 16.355		<0.001*
Median	16.20	17.0		
Degree of stenosis %.				
Min. – Max	0.0 – 20.0	0.0 – 10.0	863.0*	0.040*
Mean ± SD.	2.29 ± 4.87	0.50 ± 2.01		
Median	0.0	0.0		

U: **Mann Whitney test**t: **Student t-test**

p: p value for comparing between the two groups *: Statistically significant at p ≤ 0.05

Table (IV): Main demographic, somatometric, clinical and biochemical characteristics of the study population divided on the basis of E-selectin Leu554Phe polymorphism

	Patients with CT or TT genotypes (n=60)	Patients with CC genotypes (n=10)	P-Value
Age	51.38±13.581	49.10±13.658	0.817
Sex: *n (%)*			
Male	35(58.3%)	5(50%)	0.735
Female	25(41.7%)	5(50%)	
BMI	25.258±2.316	25.300±2.611	0.518
Smoker *n (%)*	10(16.7%)	4(40%)	0.104
DM *n (%)*	21(35%)	5(50%)	0.483
Systolic	128±18.208	116±21.705	0.988
Diastolic	81.33±9.994	72±12.293	0.878
Duration of RDT	6.277±4.032	6.200±3.190	0.654
HR	65.10±6.262	64.50±4.972	0.510
Hemoglobin	10.682±1.392	10.730±0.874	0.118
WBCs	6.543±3.286	6.350±3.177	0.785
Cholesterol	168.27±45.217	147.30±28.706	0.104
Triglyceride	177.40±109.46	140.00±50.323	0.465
LDL	106.27±24.378	112.20±17.492	0.276
HDL	60.42±9.230	66.30±4.191	0.095
Ca	8.658±0.983	9.190±0.690	0.436
PO_4	5.243±2.072	5.74±2.560	0.361
Ca X PO_4	45.660±19.731	52.625±23.405	0.322
PTH	553.692±405.294	685.840±316.997	0.228
Albumin	3.833±0.275	3.620±0.561	0.617
Creatinine	10.026±2.644	9.630±2.008	0.475
Urea	129.95±26.602	128.5±28.602	0.934
FBG	90.242±16.273	88.00±13.333	0.265
CRP	8.086±8.814	6.455±6.852	0.569
IMT	0.888±0.712	0.850±0.375	0.926
Cross-sectional area	21±19.7	20±19.836	0.567
Degree of carotid stenosis	2.25±4.642	2.50±6.346	0.593

Data are mean ±SD or percent frequency, as appropriate

Table (V): Distribution of studied sample according to different parameters

	Patients with CT or TT genotypes (n=60)	**Patients with CC genotypes (n=10)**
Int. Thickness(mm)		
Min.	0.60	0.60
Max.	6.1	1.8
Mean	0.888	0.850
S.D	0.712	0.375
P Value	**0.926**	
Cross section (mm^2)		
Min.	1.227	5.123
Max.	40.7	40.1
Mean	21	20.996
S.D	19.7	19.836
P Value	**0.567**	
Degree of stenosis%		
Min.	0	0
Max.	20	20
Mean	2.25	2.50
S.D	4.642	6.346
P Value	**0.593**	

Table (VI): Multiple regression analyses

	Beta regression coefficients	**Partial *R***	**P-Value**
IMT (Multiple R= 0.744, P <0.001*)			
BP	0.007	0.339	0.005*
$Ca \times PO_4$	0.015	0.672	<0.001*
Cross-sectional area (Multiple R= 0.934, P <0.001*)			
Age	0.010	0.545	<0.001*
BP	0.022	0.759	<0.001*
BMI	0.020	0.251	0.043*
FBS	0.254	0.454	<0.001*
Carotid stenosis (Multiple R= 0.909, P <0.001*)			
Smoking	0.633	0.259	0.033*
BP	0.029	0.906	<0.001
LDL	0.022	0.759	<0.001
Serum urea	0.20	0.251	0.043

DISCUSSION

There are 17 mutations that have been detected in adhesive molecules, five of which resulted in an amino acid substitution. In E-selectin, exchange at Ser ^{128} Arg in epidermal growth factor (EGF) domain and Leu ^{554} Phe in membrane domain, and a DNA mutation from guanine to thymine (position 98) are examples of mutations due to amino acids substitutions [45].

In this study, Leu^{554}Phe E-selectin polymorphism has been studied, in which the mutation in exon 10 leads to the substitution of leucine (Leu) by phenylalanine (Phe) at amino acid 554, (Leu = allele-C; Phe=allele-T). This mutation is believed to delay the shedding of E-selectin from vascular endothelium [45].

In the present study, the distribution of *SELE* rs5355 C and T genotypes did not show significant difference in both ESRD patients on dialysis and control groups. The genotype distribution of our studied Egyptian population group was identical to those reported by MS Issac et al. [46] however, they were different from those reported previously in Iranian [47] and Italian populations [48].

Three well established indicators of carotid atherosclerosis were studied , namely intima media thickness (IMT), cross-sectional area [49], and arterial stenosis [50], These indicators reflect different aspects of the atherosclerotic process. Our statistics showed lack of association between *SELE* rs5355 C>T genotypes and presence of carotid atherosclerosis in ESRD patients. This finding is in concordance with results reported previously by Isaac MS et al. [46] This result is contrary to results found by Testa et al, [47] who reported that IMT, CSA, and the degree of carotid stenosis were significantly higher in patients with CT and TT genotypes (i,e., in patients with T allele) when compared to CC patients.

Robert Bernat et al 2012, studied the relationship of Leu554Phe polymorphism for atherosclerosis and Long-Term Outcome after Percutaneous Coronary intervention (PCI) with Stenting, The study included 190 patients with standardized clinical follow-up over 5 years, which were initially treated with PCI, The long term clinical outcome was defined by the major adverse cardiac events MACE: death, target vessel revascularization (PCI or coronary bypass grafting, CABG) and myocardial infarction. T allele carriers of Leu554Phe polymorphism were shown to have a significantly lower repeat revascularization rate in comparison with the CC genotype. There was no significant difference between the incidence of in-stent restenosis and Leu554Phe polymorphism [51].

Srivastava et al, 2018 analysed the Leu554Phe polymorphism and expression of E-selectin gene in 250 patients with essential hypertension and 250 normal healthy controls. A significant association of E-selectin genotypes (CT + TT) with essential hypertension ($P < .0001$, Odds ratio = 2.2 [1.58-3.24] at 95% CI) was observed [52].

Marteau et al 2004, studied the association between E selectin polymorphism (Leu554Phe), blood pressure and obesity.478 men and 546 women were selected from volunteers for a free health check-up. These individuals underwent two examinations (t_0 and t_{+5}) and they were not taking medications that can affect blood pressure. At t_0, no relationship was observed between Leu554Phe polymorphism and blood pressure. However at t_{+5}, systolic blood pressure (SBP) was greater in individuals carrying the T allele, and the Leu554Phe polymorphism was associated with SBP in interaction with BMI ($P < 0.001$ in men and $P < 0.05$ in women). There was a steeper increase in SBP with BMI greater than 25 Kg/m^2 in carriers of the T allele than in CC homozygotes. Similar results were observed for diastolic blood pressure in men ($P = 0.0103$).These results suggest a BMI- specific effect of Leu554Phe polymorphism of the E-selectin gene on blood pressure [53].

CONCLUSION

The main conclusion that emerged from the present results was the following:

1. E-selectin Leu^{554}Phe polymorphism can be of value as a genetic marker to assess risk of atherosclerosis in ESRD.

2. No significant correlation could be found between E-selectin Leu^{554}Phe polymorphism and atherosclerosis markers in the studied group of ESRD patients on HD.

3. E-selectin Leu^{554}Phe polymorphism may require an underlying risk factor that predisposes to endothelial dysfunction to exert its effect and be expressed.

4. The genotype distribution of the Leu554Phe polymorphism of the E-selectin gene was identical among ESRD patients on HD and control subjects of the same population.

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