Levels of Arginase Isoenzymes in Sera of Iraqi Patients with Atherosclerosis and Type 2 Diabetes Mellitus

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Abstract

The purpose of the current study was to assess the level of human serum arginase I (Arg I) and arginase II (Arg II) in atherosclerosis patients with a type 2 diabetes mellitus and to correlate their values with the levels of fasting serum glucose (F.S.G) and lipid profile. Another part of this study sought to find the effect of differences in the level of these parameters between genders. This prospective study comprised of 128 male and female patients who underwent percutaneous coronary intervention (PCI) and diagnostic catheterization (DIG) for atherosclerosis symptoms and 64 healthy control (C) with matched ages ranged between 45-70 years. The result illustrated that Arg I and Arg II levels were elevated significantly (P< 0.05) in the two patient groups as compared to healthy controls. These include F.S.G, TC, TG, LDL and VLDL in sera of the both patient groups when compared with the control group.

Meanwhile, high-density lipoprotein (HDL) level was increased slightly, but non-significantly, in patient groups. On the other hand, there were weekly correlations, yet non-significant, between Arg I and Arg II levels and a levels of the F.S.G and lipid profile. When it comes to gender, there was a significant increase (P< 0.05) in the level of serum Arg I, TC, HDL, LDL and VLDL in both female-patient groups when compared with male-patient groups. Furthermore, there was a non-significant, slight increase in the level of Arg II and triglyceride in patient groups as compared to healthy controls. This study concluded that the increase of the levels of Arg I and ArgII was associated with the incidence of atherosclerosis patients with T2DM. Additionally, a non-significant correlation existed between Arg I and the Arg II levels with the levels of F.S.G and lipid profile. Finally, women with T2DM are more susceptible to atherosclerosis than men.

Keywords: Atherosclerosis, Arginase I, Arginase II, fasting serum glucose and lipid profile.

Introduction

Acute atherosclerosis (AS) is known to result from the chronic inflammatory condition which affects the size of the large and medium arteries and is characterized by the development and progress of the pests that are composed of fibrosis, fat, inflammatory cells infiltrating the inner layers[1]. Above factors may be considered as causes of the disrupted lipid homeostasis[2]. Despite the remarkable progress being made towards understanding the therapeutic modalities and mechanisms in recent decades, the ASCAD remains the leading cause of death in the industrialized countries[3].

T2DM is one of the causes of AS acceleration because it is an insulin-resistant condition and is characterized by mixing inflammation and oxidative stress.[4] T2DM with AS shares almost all the traditionally recognized main common risk factors: high blood pressure, obesity, dyslipidemia, age, sedentary lifestyle and dietary factors. AS in diabetic patients tends to develop earlier and has a more extensive and diffuse presentation than AS in non-diabetic subjects. Early lesions in several arterial plaques can be identified in AS and there is an increased burden of AS plaques in patients with T2DM well before the appearance of cardiovascular events[5].

Arginase (EC 3.5.3.1) enzyme is often found in the liver, kidneys and erythrocytes. It converts the amino acid L-arginine to L-ornithine with urea. This enzyme will reduce the substrate availability of the other NOS competitors which produce nitric oxide[6]. Arginase occurs as two distinct isoforms and both are present in human plasma Arg I and Arg II, these two isoforms are in mammalian tissues. The ODC catalyses the following conversion of ornithine to the polyamines such as putrescine, spermidine and spermine, subsequently which are required components for progression of the cell cycle and play an important a role in cell proliferation[6].

Material and Methods

This study's protocol was approved by the committee ethics of College of Science at the University of Baghdad. During the period from the beginning of June 2017 until January, 2018, blood samples were collected from both patient groups attending the department of Clinical Chemistry, Coronary care unit and Catheterization unit in AL- Sheikh Zayed and Ibn Al-Nafees Hospital in Baghdad. 128 male and female patients who underwent PCI n=64 with (Cardiac Troponin-I (cTnI) positive (+ve)) and DIG n=64 with (Cardiac Troponin-I (cTnI) Negative (-ve)). Among participants, there were healthy individual n=64 as controls (C) groups who had no previous history of diabetes or cardiovascular disease.

Blood collection: Fasting blood samples (10 ml) were collected from each case study by venipuncture using

disposable syringes. After clotting, blood samples were centrifuged for 10 min at 4000 rpm. Serum was separated and divided into proportions of 500µl, put and stored in Eppendorf tube at -80°C for examination.

Methods: Cardiac Troponin I (cTn I) was measured qualitatively by using Combo Rapid Test – Cassette. Schiffgraben 41, 30175 Hannover, Germany. Arg I was measured in serum according to Hycult Biotech / Germany kit and Arg II was measured in serum according to Cusabio/Germany Kit by using an enzyme-linked immunosorbent assay (ELISA). F.S.G, lipid profile (total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) and low-density lipoprotein (LDL)) were measured by using Cobas C111 auto analyzer system/ Germany.

Statistical Analysis: The statistical assessment was resolved by SPSS of version 23 and the program Windows, using ANOVA one-way analysis of variance. The relation of correlation for ArgI and Arg II with other parameters was performed by Pearson correlation test, (r value as a coefficient). The study data were presented as a mean± standard error. The P value < 0.05 was statistically significantly considered.

Results and Discussion

This study was conducted on 192 subjects divided into three groups. The demographic data and baseline characteristics of the patients are presented in table 1.

Non-significant differences were between the study groups in terms of body mass index (BMI), waist, hip circumference and heart rate. Systolic blood pressure and diastolic blood pressure of both groups of PCI patients and DIG patients showed significant increase (P<0.05) when compared with C group. The duration of DM was more than 5 years. The obesity in the abdomen is related with the occurrence of AS in patients with T2DM shown by the measurement of body mass index in predicting atherosclerosis. Therefore, it is important to control abdominal obesity and insulin resistance in patients with T2DM [7]. However, a few studies have compared the validity of different simple adiposity indices with measured body fat in the discerning people at the particular risk of subclinical AS and lipids profiles abnormal[8].

At rest, high heart rate is associated with increased risk of all-cause mortality and events of CV in healthy individuals as well as those with pre-existing cardiovascular disease including hypertension, acute myocardial infarction, heart failure or dysfunction of the left ventricle through many epidemiological studies. In another study, resting heart rate could be a predictor of major cardiovascular events, progression of diabetic kidney disease and all-cause mortality in a cohort of T2DM patients[9]. The blood pressure seems to have a continuous and consistent relationship with the risk of cardiovascular events, the higher is the BP, the better is the chance of CVD[10]. 90% of the patients with hypertension were from the good socio-economic status and those who adopt the stable life style[11].

The systolic pressure indicates the highest pressure exerted as blood pushes through the heart. Diastolic pressure on the other hand, is maintained by the arteries when the vessels are relaxed between heartbeats. A spread of the arterial stiffness and hypertension increases with age, thus, there is an increase in systolic blood pressure with ageing that may be because an increase in vascular- stiffness of the arteries in combination with AS change in a vessel wall[12]. Also, the cause of left ventricular hypertrophy (LVH) is hypertension[13] which is considered to be a cause of AS, MI, CAD, arrhythmia, cardiac death, or heart failure.

Masahiko Harada et al[14] found that decreased early diastolic mitral annular velocity relates to the parameter reflecting carotid AS. Therefore, the presence of severe carotid AS may affect LV diastolic dysfunction. Both increased systolic pressure and pulse pressure will contribute to endothelial dysfunction, making it easier for low -density lipid cholesterol to enter the wall of the blood vessel, leading to atherosclerotic[16].

On the other hand, the elevation in ArgI levels in PCI group and DIG group with mean 236.66 ^{A} ± 29.12, 135.90 ^{B} ± 44.49 ng/ml, respectively, compared to control group with mean 194.70^{AB} ± 26.07ng/ml showed a significant increase (P<0.05). The difference of levels between PCI group and DIG group was noticed. These levels had a higher value than the LSD value which was 92.869. The elevation in mean levels of Arg II was higher in group DIG 12.58 ^{A} ± 0.87 mU/ml than mean in groups PCI 10.77 ^{B} ± 0.36 mU/ml and the healthy control group was 10.43 ^{B} ± 0.31 mU/ml with a significant increase of (P<0.05). Meanwhile, the level difference between PCI group and DIG group was noticed and it was higher than the LSD value which was 1.422 as shown in table 2.

Recent study failed to demonstrate a beneficial effect of arginine amino acid in reducing atherothrombotic events in the development of acute myocardial infarction but was able to explain that the accelerated precipitation of arginine is secondary to increased arginase activity. There are several potential mechanisms by which Arg II affects leading to impaired endothelial function and the development of AS[17]. Accelerated worsening endothelial dysfunction, nitric oxide consumption, decreased the NO bioavailability and increased oxidative stress demonstrated in individuals with T2DM. Hyperglycaemia in increased arginase activity and ROS further inhibits NOS activity especially in T2DM patients[18]. The Arg I enzyme works to stabilize the plaque in the non-segregated plaque. As promotes the proliferation of aortic smooth aortic muscle cells and therefore, can be associated with increased intracellular polyamines production helping to stabilize plaque AS by stimulating the proliferation of smooth muscle cells and inhibiting the expression of inflammatory cytokines[19].

Upon the comparison between both patient groups and the control sample in table 3, the results indicated that there is significant increases (P<0.05) of TC, TG, VLDL, LDL in DIG group in each serum sample, while there is a significant decrease (P< 0.05) of LDL in PCI group when compared with the healthy control group, as the difference of the mean value for both groups (patients and control) is higher than LSD for TC, TG, VLDL and LDL which is 16.255, 41.04, 9.106 and 12.821 respectively. Meanwhile, the HDL level slightly increased, but not significantly, by p>0.05. The difference of the mean value for both groups (patients and control) is less than LSD which is 3.69.

TG/HDL ratio showed highly significant increase (P <0.05) in patient groups compared to the control group. In table 3, no significant differences were found in the serum in terms of the TC, TG, VLDL-C, HDL-C and LDL / HDL ratio between group PCI and group DIG patients. It means that whenever AS with T2DM becomes severe, the bulge in artery increases (group PCI and DIG)) and the TG/HDL ratio will increase. Therefore, the deposition of cholesterol in the intima-media of vessels supplying cardiac leads to lipid-driven inflammatory disorder of the arterial wall and causes AS [20]. HDL-C may be protective.

While Blood lipid disorders plays the role in endothelial dysfunction which is essential in causing of AS, the insulin resistance, coagulation, as well as high blood pressure. It has been shown that lipoproteins rich in triglycerides and low – density lipoprotein cholesterol are toxic to endothelial cells[21]**.** Since epidemiologic evidence indicates that CAD risk factors frequently cluster, it should be expected that many patients have multiple risk factors. The association between high serum cholesterol levels, especially high LDL-cholesterol and CAD is independent of other risk factors [22].

LDL-C can damage endothelial cells and smooth muscle cells by oxidation, modification and glycation. The modified LDL-C eventually transforms macrophagocytes in intima into foam cells after they phagocytosed LDL-C. It plays an important role in the development of diabetic macrovascular disease, because accumulation and deposition of cholesteryl ester can increase the content of lipid plaque closely related to its stability[23.]

Tables 4, 5 and 6 show compression between males and females in the three studied groups (PCI, DIG, C respectively). Some major biological variable between male and female subjects bind to variation size of the arteries. The carotid arteries are junior in women[24] with the more apparent stenosis but less plaque [25]. In the diagnosis of acute coronary syndrome, the size of the small coronary arteries in women, explains the difference between the arteries of women and men. Gender-related variables may help in explaining health-related gender variations, smoking or excessive alcohol consumption. The higher of CVD is in male than in female addition. The reason why men are at an increased risk may partly be explained by their gender-based propensity to engage in risk taking behaviours[27].

Testosterone hormone would probably progress the quality of life and reduce cardiovascular risk. A previous study points out that the primary data in the Vigen study actually showed fifty percentage reduction of cardiovascular risk with the testosterone replacement before adjustment for some fifty variables[28]. In another study estrogen hormone is responsible for the protection of female from the CVD. Interestingly, preponderance of vascular and connective tissue disorders in women also points to an inherent role of hormones and tissue factors in maintenance of vascular endothelial function.

On the other hand, hormone replacement therapy has failed to decrease CVD events in clinical studies pointing to the complexity of the relationship between vascular biology and estrogen hormones[29]. The mechanisms of this called coronary micro-vascular dysfunction are varied and may be associated with endothelial reactivity, low levels of the endogenous oestrogen, also coagulation disorders and abnormal inflammatory reactions and its manifestation can have a substantial variability among subjects[30].

Table 1
Baseline clinical characteristics between group patients PCI, DIG and healthy controls.

Parameter	PCI catheterization	DIG catheterization	C	p-value
Age(year)	58.32 ± 0.78	58.39 ± 0.83	56.43 ± 0.81	0.135
BMI (kg/m^2)	27.81 ± 0.23	28.03±0.19	27.85±0.23	0.786
Circumference (m)	102.62 ± 1.60	106.08 ±1.50	101.81 ± 2.00	0.182
Hip Circumference (cm)	102.62 ± 1.60	106.08 ±1.50	101.81 ± 2.00	0.182
WHR waist to Hip ratio	0.96 ± 0.01	0.97 ±0.01	0.95 ± 0.02	0.646
WHtR waist to height ratio	0.59 ±0.01	0.62±0.01	0.58±0.01	0.093
Waist (m)	98.69 ± 1.68	103.29 ± 1.51	98.55 ± 3.13	0.226
Heart rate (beat / minute)	80.00 ±1.41	81.79 ±1.54	79.77 ± 1.38	0.546
SBP mmHg	169.30 ± 15.37	154.95 ± 13.49	139.14± 2.97	0.047*
DBP mmHg	99.23± 10.11	82.28± 2.26	76.37± 2.68	0.037*

*: Mean ± standard error; **: significant (P <0.05); NS: Non- Significant.

Table 2
The mean ± standard error of serum Arg I and Arg II in all studied groups

Group	Mean ± SE	
	Arginase-1(ng /ml)	**Arginase-II (mU/ml)**
PCI	236.66 ^{A} ± 29.12	10.77 ^{B} ± 0.36
DIG	135.90 ^{B} ± 44.49	12.58 ^{A} ± 0.87
Control	194.70^{AB} ± 26.07	10.43 ^{B} ± 0.31
LSD value	92.869 *	1.422 *
p-value	0.0474 *****	0.0398*

* (p <0.05), ** (p<0.01). Means having with the different letters in same column differed significantly

Table 3
Comparison of fasting serum glucose and lipid profile between both patient groups and healthy controls.

Group	Mean ± SE							
	TC (mg/dl)	**TG (mg / dl)**	**HDL-C (mg/dl)**	**VLDL-C (mg/dl)**	**LDL-C (mg/dl)**	**Tri/HDL ratio**	**LDL/HDL ratio**	**F.S.G (mg/dl)**
PCI	181.85^{AB} ± 6.23	226.59^{A} ± 17.08	40.45^{A} ± 1.37	45.31^{A} ± 3.41	96.01^{B} ± 4.90	5.95 ^{A} ±0.49	2.59 ^{A} ±0.18	201.60^{A} ± 10.35
DIG	193.51^{A} ± 5.76	201.27^{A} ± 15.53	40.39^{A} ± 1.19	43.06^{A} ± 3.96	112.81^{A} ± 4.32	5.40 ^{A} ±0.51	2.91 ^{A} ±0.13	144.06^{B} ± 10.24
Control	173.52^{B} ± 5.14	137.39^{B} ± 8.66	40.18^{A} ± 1.36	27.84^{B} ± 1.75	106.89^{AB} ± 4.36	3.71 ^{B} ±0.26	2.90 ^{A} ±0.16	96.30^{C} ± 1.55
LSD value	16.255 *****	41.04 **	3.69 NS	9.106 **	12.821 *	-----	-----	24.575 **
P-value	0.050 *	0.0001**	0.989	0.0004**	0.0289*	0.001*	0.295	0.0001**

* (P <0.05), ** (P <0.01), NS: Non-Significant. A, B, C that Means having with the different letters in same column differed significantly

Table 4
Comparison between male and female PCI patients with atherosclerosis T2DM

Parameters	Mean ± SE		P-value
	Male	**Female**	
F.S.G	186.78 ± 13.22	↑ 215.89 ± 17.45	0.198 NS
TC	177.19 ± 10.03	↑ 191.39 ± 8.82	0.278 NS
TG	↑ 214.06 ± 22.60	210.78 ± 16.07	0.897 NS
HDL	39.25 ± 2.01	↑ 43.33 ± 2.12	0.132 NS
TRI/HDL ratio	↑ 6.10 ± 0.82	5.47 ± 0.59	0.522 NS
VLDL	↑ 48.43 ± 6.63	42.15 ± 3.21	0.414 NS
LDL	89.44 ± 7.78	↑ 105.29 ± 7.00	0.085 NS
LDL/HDLratio	2.50 ± 0.25	↑ 2.68±0.30	0.625NS
ArgI	213.65 ± 38.46	↑ 241.82 ± 23.97	0.474 NS
ArgII	↑ 10.87 ± 0.50	10.50 ± 0.42	0.527 NS

* (P<0.05), ** (P<0.01), NS: Non-Significant.(↑) Represents increased value

Table 5
Comparison between male and female DIG patients with T2DM

Parameters	Mean ± SE		P-value
	Male	Female	
F.S.G	135.85±14.49	↑ 152.28±14.56	0.441NS
TC	193.42±8.43	↑ 193.59±8.07	0.986NS
TG	↑ 223.52±25.56	179.03±17.18	0.186NS
HDL	39.25±1.22	↑ 41.54±2.05	0.379NS
TRI/HDL	↑ 5.98±0.83	4.84±0.59	0.330NS
VLDL	↑ 50.33±6.98	35.80±3.43	0.078NS
LDL	109.48±5.67	↑ 116.156±6.57	0.413NS
LDL/HDL	2.91±0.19	2.91±0.191	0.983NS
Ratio A/G	↑ 1.97±0.11	1.77±0.11	0.211NS
ArgI	146.36±30.73	↑ 301.02±94.35	0.207NS
ArgII	10.22±.0.56	↑ 13.36±1.55	0.072NS

* (P<0.05), ** (P<0.01), NS: Non-Significant. (↑) Represents increased value

Table 6
Comparison between male and female healthy control

Parameters	Mean ± SE		P-value
	Male	Female	
F.S.G	94.44 ± 2.37	↑ 97.25 ± 1.87	0.311NS
TC	164.41 ± 6.50	↑ 181.37 ± 7.73	0.115NS
TG	136.98 ± 10.34	↑ 137.75 ± 13.38	0.961NS
HDL	38.74 ± 1.84	↑ 41.40 ± 1.91	0.257NS
Tri/HDL	↑ 3.84±0.36	3.52±0.40	0.528NS
VLDL	↑ 29.01 ± 2.47	27.55 ± 2.67	0.684NS
LDL	99.50 ± 6.53	↑ 112.42 ± 5.48	0.170NS
LDL/HDL	2.85±0.26	↑ 2.88±0.19	0.935NS
ArgI	110.77 ± 23.60	↑ 127.49 ± 30.01	0.618NS
ArgII	↑ 11.14 ± 1.14	8.24 ± 2.11	0.293NS

* (P<0.05), ** (P<0.01), NS: Non-Significant. (↑) Represents increased value

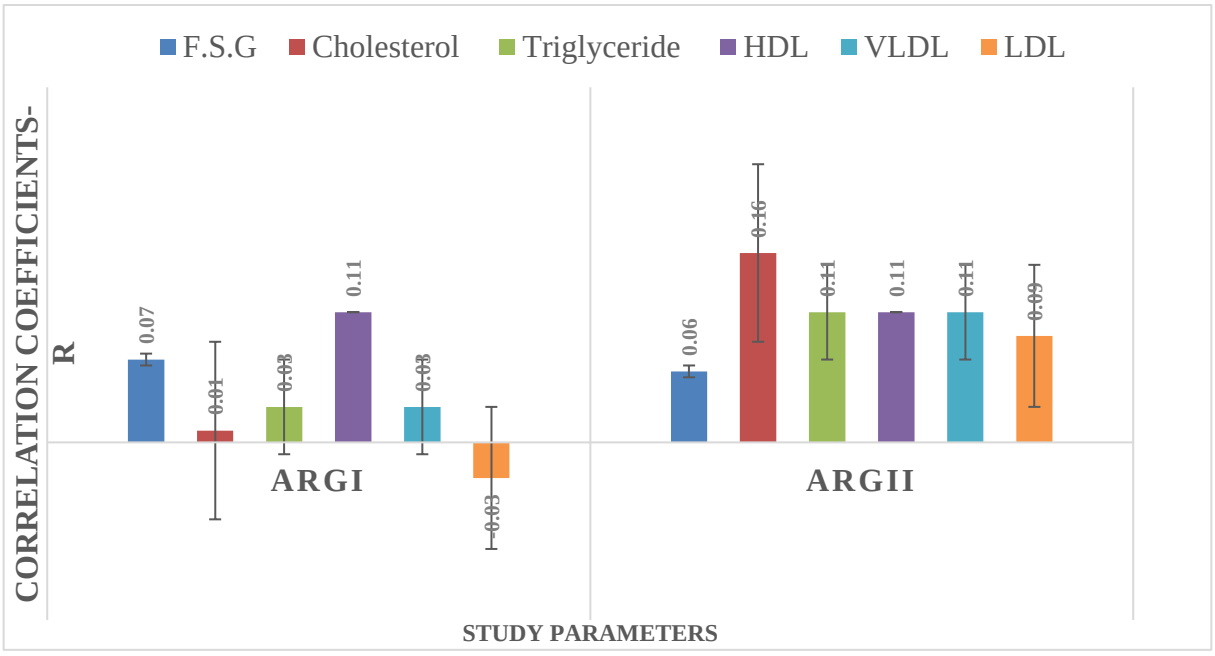

Figure 1: Correlation coefficient between Arginase I, Arginase II and other studied parameters

Female, during the menopause state, TC and LDL, the levels rise by ten to fourteen percentage respectively and lipoprotein (a) increases four to eight percentage whereas the HDL-c levels remain unchanged[32], the lipid profile after menopause means low-density lipoprotein - cholesterol is increases in the female compared to men. HDL-cholesterol levels are 0.26 to 0.36 mmol/l higher in female, while study suggested that low level of HDL- c, implicates the higher risk of CHD in women than in men[33].

Studies on animal samples have provided satisfactory evidence demonstrating the roles of vascular oxidative stress and nitric oxide in atherosclerosis. Hyper-cholesterolemia, hyper-tension, diabetes mellitus and smoking these are all established cardiovascular risk factors, decrease endothelial, NO production and enhance ROS generation. The major molecular proceedings in atherogenesis such as oxidative modification of phospholipids and lipoproteins, activation of the endothelial cell and macrophage infiltration /activation are facilitated by vascular oxidative stress and inhibited by endothelial nitric oxide. AS develops preferentially in vascular regions with disturbed blood flow (arches, branches and bifurcations).

The fact that these sites are related to enhanced OS and reduced endothelial NO production is a further indication for the roles of ROS and NO in atherosclerosis. Therefore, prevention of vascular OS and improvement of endothelial NO production represent reasonable therapeutic strategies in addition to the treatment of established risk factors (hyper-cholesterolemia, hyper-tension and diabetes mellitus)[34].

In univariate correlation analysis figure (1), there was no significant correlation between Arg I, Arg II and F.S.G and cholesterol, triglyceride, high density lipoprotein and very low-density lipoprotein.

Conclusion

After completion of this study, it was found that increases in the levels of ArgI and Arg II are correlated with atherosclerosis and type 2 diabetes mellitus. It was also found that there was no correlation between ArgI and Arg II with F.S.G and lipid profile. Furthermore, women are more affected by incidences of AS and T2DM than men.

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