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Disturbance of Arginase Activity and Nitric Oxide Levels in Iraqi Type 2 Diabetes Mellitus

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Abstract:

This study is an attempt to find whether arginine metabolism dysregulation by arginase activity is related to hyperglycemia, followed by changes in nitric oxide (NO) generation in type 2 diabetic patients. This study includes 42 control subjects (Group I), and 92 Iraqi patients with type 2 diabetes mellitus (T2DM). The patient group was subdivided into two groups: Group II (54) with T2DM only and Group III (38) with T2DM and dyslipidemia (who were treating with atorvastatin along with diabetes treatment). The samples were obtained to measure arginase activity and NO levels. Serum arginase activity increased significantly in patients(groupII and groupIII) compared to control group. While serum NO level was significantly lower in diabetic patients as compared to control group, three significant correlations appeared in this study between glucose and arginase activity, glucose and NO levels, and between arginase activity and NO levels. The results also show that treatment with atorvastatin affects arginase activity and NO levels. Increasing in levels of arginase activity can be considered as an indicator of diabetic status. Endothelial dysfunctions accompanied with diabetes mellitus reverses correlation between arginase and NO in diabetic.

Keywords: Type II diabetes mellitus**,** Arginase, Nitric oxide, Dyslipidemia.

Introduction:

Type 2 diabetes mellitus (T2DM) is a major global health problem. It is an insulin resistant (IR) state characterized by, hyperglycemia, inflammation, oxidative stress(1) and several vascular complications, such as atherosclerosis, pulmonary hypertension, coronary heart disease, and hypertension(2). Many of conditions are associated with (T2DM) diabetes mellitus including insulin resistance (IR), hyperglycemia, and dyslipidemia, all of these are mediators in endothelial dysfunction(3). Dyslipidemia is a disorder of lipoprotein metabolism that accompanies diabetes mellitus; it is manifested by hypertriglyceridemia, increased total cholesterol, and low-density lipoprotein (LDL), also decreased high density lipoprotein (HDL). Dyslipidemia is the main risk factor for cardiovascular disorders in diabetic patients(4),(5).

Endothelial dysfunction is characterized by impaired production of nitric oxide (NO) and/or decreased NO bioavailability in endothelial cells. NO is a vasodilator, anti-inflammatory molecule, and it can be a neurotransmitter.

It is synthesized by the action of nitric oxide synthase (NOS) enzyme on its substrate (L-arginine) (6).

Arginase is the final enzyme in the urea cycle that is responsible for the detoxification of ammonia in mammals, by hydrolyzing arginine to ornithine and urea(7). The arginase is constitutively expressed in cells and tissues, and indirectly regulates NO generation from NOS(8). Increasing arginase activity is found in dividing the cells of tissues to enhance polyamines biosynthesis. Such situations are prostatic carcinoma, gastric cancer, colorectal cancer, and breast cancer(9), where it is upregulated by factors such as oxidised low-density lipoprotein, glucose, hypoxia, and reactive oxygen species(10).

Thus, diabetes-induced increased in arginase activity was reported in plasma from diabetic animals and patients (11),(12), and in vascular tissue of streptozotocin diabetic rats(11). Endothelial dysfunction in diabetic patients is a major cause of morbidity and mortality which is closely associated with increasing in arginase activity(13) that leads to decrease NO production, and thus, increased arginase activity in diabetes contributes to vascular endothelial dysfunction in diabetic rats that is reported by Maritza et al.(14).

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The aim of this work is to measure arginase activity and NO level in serum of patients with T2DM and matched non-diabetic controls. Ultimately, we are trying to determine if arginase activity can be considered as indicator for T2DM, the relation between arginase and NO in T2DM, and the effect of atorvastatin on serum arginase activity, and NO level.

Materials and Methods:

A control group consisted of 42 subjects(24 males, and 18 females) with an age range of (30-65)years and will be referred to as Group I. These subjects have had no history of DM, hypertension, endocrine metabolic disorders, kidney diseases, acute illness or infection and ischemic heart disease. In addition, 92 Iraqi patients with T2DM with an age range of (30-65) years were collected from National Diabetes Center for Treatment and Research at Al-Mustansiriya University/ Iraq during the period from February to April 2016. The Scientific Committee in the College of Science for Women (at the University of Baghdad) approved this study. A verbal consent form was obtained from each participant enrolled in the study. these patients were subdivided into two groups: Group II included 54 subjects(25 males, and 29 females) who have had T2DM only, and Group III included 38 (21 males, and 17 females)subjects of T2DM with dyslipidemia and who were treated with atorvastatin with dose of 10, 20, and 40mg, one tablet each day along with diabetes treatment. The included diabetic patients were without heart or kidney diseases, and free of acute illness or infection at the time of the study.

Whole blood was collected after overnight fasting for 10-12 hours. The serum was separated from the whole blood to measure glucose level, lipid profile, arginase activity, and NO level. The cholesterol, triglyceride, and high density lipoprotein kits were obtained from Spinreact /Spain, while glucose kit was obtained from Cromatest/ Spain. Manganese chloride, Ninhydin, Ornithine, tri-chloro acetic acid, sulfunalic acid, hydrochloric acid, phosphoric acid from BDH/ England, L-arginine monochloride, Sodium barbitone from Rediel dehien/ Germany. Arginase activity was measured as described by Zofia and Maria (15). Briefly the reaction was done in a system containing 0.5ml of 0.1 M Sodium barbitone(pH 9.5), 0.1 ml of 200 mM substrate solution L-arginine monochloride (pH 7.5), 0.1 ml of 50 mM Manganese chloride solution, and 0.5ml of the serum. The incubation was carried out for 30min at 37°C. The reaction was stopped with 1.3ml Trichloroacetic acid 20%. The ornithine concentration formed was determined by adding 2ml of ninhydrin reagent(250 mg of ninhydrin dissolved in 4 ml of 6 M H_3PO_4, and 6 ml of concentrated acetic acid), and 1ml concentrated acetic acid added to the incubation medium, and to a boiling water bath for 60 minutes. The colored product was determined spectrophotometrically at λmax 515 nm. Ornithine concentration was calculated from calibration standard curve. The arginase activity was expressed as μg of ornithine/L.

Nitric oxide level was determined according to Griess method as described by Newaz (16). Briefly, 0.5 ml serum was added to 200 μl HCl (6.5M) and 200 μl sulfunalic acid (37.5mM). After incubation for 10 min, 50 μl naphthylethylenediamine dihydrochloride (12.5mM) was added and incubated for further 30 min, centrifuged for 10 min at 4000rpm. The absorbance at λmax 540 nm was immediately recorded.

The serum glucose was determined by enzymatic colorimetric method by Trinder(17) , using the kit supplied by Coromatest, Spain. Serum triglyceride was determined by the enzymatic colorimetric reaction according to Fassati and Prencipe method (18) using the kit supplied by Spinreact, Spain. Serum total cholesterol was determined by the enzymatic colorimetric method(19) using the kit supplied by Spinreact, Spain. Serum HDL cholesterol fraction was determined using the total cholesterol enzymatic reagent after precipitation of the very low density (VLDL) and LDL lipoproteins in serum by phosphotungstate in the presence of magnesium ions(20) .

The statistical analysis System SPSS program was used to effect of the difference factors in study parameters. The results were expressed as mean ± SD. P value was calculated by using one way ANOVA with post hoc Tuckey test between groups, $P<0.001$ is significant. Pearson correlation was calculated for the correlation between variables.

Results

Table 1 summarizes the study participants' characteristics, including fasting blood glucose (FBS), lipid profile in all groups. Data showed that the majority of diabetic patients had higher levels of glucose, Cholesterol, Triglyceride, Non-HDL-c, and low levels of HDL-c than the healthy subjects, and they differ significantly $P<0.001$ except cholesterol, and triglyceride whose differences are non significant, while the differences are non significant between the two patients groups (II, and III) where they have approximately the same levels for these parameters.

Table 1. Biochemical features in control, T2DM, and T2DM with dyslipidemia

Variables	Group I (n=42)	Group II (n=54)	Group III (n=38)	*P*
Age (year)	49.0±11.3	49.3±10.3 1 - 8 year	49.1±10.1 1 - 6 year	0.988
FBS (mg/dl)	80.0±12.1	186.1±77.6	162.0±50.1	a<0.001;b<0.001;c0.114
Cholesterol(mg/dl)	174.8±37.1	191.0±44.0	193.0±130.5	a0.143;b0.127;c0.974
Triglyceride(mg/dl)	118.7±25.9	151.6±65.2	192.8±130.5	a0.133;b<0.001;c0.053
HDL-c(mg/dl)	60.9±14.8	39.8±9.8	37.3±8.1	a<0.001;b<0.001;c0.539
Non-HDL-c (mg/dl)	113.9±33.2	151.2±41.3	120.6±41.1	a<0.001;b<0.001;c0.852

P value is calculated between Group I (healthy subjects) and Group II (Diabetic patients) represented by the symbol (a), and Group III (Diabetic patients with dyslipidemia) represented by the symbol (b), and between Group II and III represented by the symbol (c).

Table (2) shows that the arginase activity increases significantly in the two diabetic patients groups(30.26±9.75,and 20.78±8.97) compared with controls (4.21±3.01) where p<0.001 between all groups, while NO levels decreased significantly in patients groups(48.67±15.88,and 68.18±19.18) compared with controls(104.54±15.88) where p<0.001 between all groups.

Table 2. Serum levels of the arginase activity, NO levels in the participants included in the study

Variables	Group I (n=42)	Group II (n=57)	Group III (n=38	*P*
Arginase activity (U/L)	4.21±3.01	30.26±9.75	20.78±8.97	a<0.001;b<0.001; c<0.001
Nitric oxide (µM)	104.54±15.88	48.67±15.88	68.18±19.18	a<0.001;b<0.001; c<0.001

P value is calculated Group I (healthy subjects) and Group II (Diabetic patients)represented by the symbol (a) and Group III (Diabetic patients with dyslipidemia) represented by the symbol (b), and between Group II and III represented by the symbol (c).

Table (3) shows the Pearson correlation of FBS. In diabetic individuals with arginase activity, there was a significant positive correlation ($r_=$ 0.61), while the correlation between F.B.S and NO was significant negative correlation with ($r_=$-0.55). The analysis shows a significant negative correlation ($r_=$-0.80), between arginase activity and NO levels, as shown in Fig.1.

Table 3. Correlation coefficient between (Arginase activity and Nitric oxide), and between FBS. and (Arginase activity and NO).

Parameters	Correlation Coefficient-r	*P*
Arginase activity and NO	-0.80	**
FBS and Arginase	0.61	**
FBS and NO	-0.55	**
	** (P<0.01)	

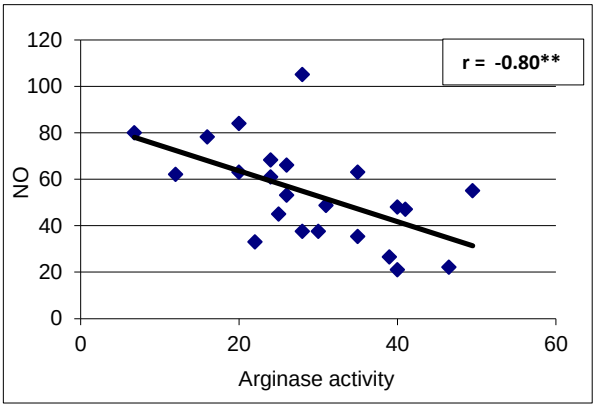

Figure 1. Correlation between serum arginase activity and serum NO in diabetic patients

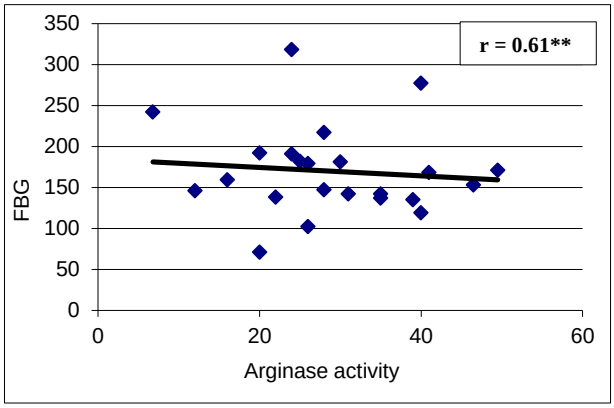

Figure 2. Correlation between FBS and Arginase activity in diabetic patients

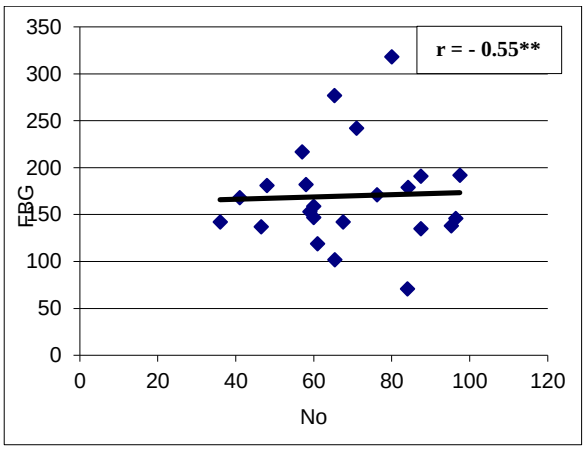

Figure 3. Correlation between FBS and NO in diabetic patients

Discussion:

In this study, arginase activity and NO levels were determined in healthy subjects (Group I), patients with diabetes (Group II), and diabetes with dyslipidemia (Group III). The results indicated that there was a significant increase in arginase activity in the two groups of diabetic patients (Group II, and Group III) in compared to Group I, and this increase is attributed to the high glucose levels in diabetic patients, where this study indicated a positive, and significant correlation between FBS and arginase activity. This is compatible with the results of Kashyap *et al*.(1), who studied 12 diabetic patients and found that arginase activity increases with T2DM and there is a correlation between FBS and arginase. our results are also compatible with the result of Shatanawi *et al*.(2) who found that arginase activity was elevated in 62 patients with T2DM as compared to age-matched healthy volunteers. Diabetes is accompanied by increased food consumption, amino acid metabolism and ratio of blood glucagon to insulin, all of which would tend to increase the activity of the urea cycle as well as arginase activity (21).

Nitric oxide levels showed a significant decrease in Group II and III which is in agreement with Amrita *et al*. (22) and Paolo *et al*.(23). They indicated that a low NO concentration was observed in type 2 diabetic patients without complications, compared to healthy controls. The mechanism by which NO decreased is a reduction in substrate concentration that results from accelerating the enzymatic conversion of arginine to ornithine by arginase. NOS and arginase are competing for the same substrate (L-arginine), and reduction in availability and production of NO by NOS (1) accors, and this can be noted in Figure (1) which shows that there is a significant negative correlation between arginase and NO. A significant negative correlation between NO and FBS is reported by Syed *et al*.(24). A significant negative correlation was found when serum NO was correlated with serum glucose levels in diabetic normotensive patients.

The increasing arginase activity in Group III was less than Group II, and NO level in the same group was decreasing but less than the reduction in Group II. This can be attributed to the effect of the atorvastatin given to the patients in Group III which gives the inhibiting effect on the enzyme activity, and also enhances the levels of NO. This finding is in agreement with Lacy *et al*. (25), who observed lower arginase activity after oral statin intervention, and with the finding of Wassman *et al*.(26) , which use statins (such as Fluvastatin) for treatment in hypercholesterolaemic rabbits, which results in improved NO availability in arteries.

Conclusion:

From this study, it can be concluded that the increasing in levels of arginase activity can be considered as indicator of T2DM. In T2DM there is a negative correlation between arginase, and NO explains the consequence of endothelial dysfunction accompanied with T2DM since their serum NO levels are low as a result of high arginase activity. Atorvastatin may have therapeutic benefits in diabetic patients where it can prevent endothelial dysfunction.

Conflicts of Interest: None.

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اضطراب نشاط ألارجينيز ومستويات أوكسيد النيتريك في المرضى العراقيين بداء السكري النوع الثاني

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الخلاصة:

في هذه الدراسه تمت محاولة ايجاد فيما اذا كان الخلل الحاصل في تنضيم ايض الحامض الاميني الارجينين (بواسة انزيم الارجنيز) له علاقه بأرتفاع مستوى السكر بالدم متبوعا بتغيرات تخليق اوكسيد النايتريك(NO). تضمنت الدراسه (42) عينه من الاصحاء (مجموعه I) و (92) عينه من المرضى العراقيين بداء السكري النوع الثاني.تم تقسيم عينات المرضى الى مجموعتين : مجموعه (II)تتضمن (54) عينه من المرضى المصابين بالسكري فقط ، و المجموعه الثانيه (III) تتضمن (38) عينه من المرضى المصابين بداء السكري و اضراب مستويات الدهون بالدم (dyslipidemia) (و الذين يخضعون لعلاج الستاتين اضافه الى علاج السكري). تم قياس مستويات فعالية انزيم الارجنيز و اوكسيد النايتريك في السيرم لجميع العينات. اظهرت النتائج ارتفاعا معنويا لقيم فعالية الانزيم في مجاميع المرضى مقارنة بمجموعة الاصحاء بينما اظهرت انخفاضا معنويا بمستويات اوكسيد النايتريك بمجاميع المرضى بالنسبة الى مجموعة الاصحاء. في هذه الدراسه ظهرت ثلاث علاقات معنويه تربط بين مستوى السكر و فعالية الانزيم، مستوى السكر و مستوى اوكسيد النايتريك، و بين فعالية الانزيم و مستوى اوكسيد النايتريك، كما اظهرت النتائج ان الستاتين له تأثير على على مستويات كل من فعالية الانزيم و اوكسيد النايتريك.

الكلمات المفتاحية: داء السكري من النوع الثاني،انزيم الارجينيز، اوكسيد النايتريك ، اضراب مستويات الدهون.