Serum level of Arginine and Symmetric dimethyl arginine in patients with prediabetes and type 2 diabetes mellitus.

Basheer Sultan Dayir¹*, Mohammed I. Hamzah¹, Mahmood S.H.Khudair²

¹Department of Chemistry and Biochemistry, Collage of Medicine, AL-Nahrain University, Iraq
²Department of Internal medicine, Collage of Medicine, AL-Nahrain University, Iraq

*Corresponding author: Basheer Sultan

Abstract

Background. Diabetes mellitus is defined as a metabolic disorder of different reasons characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Prediabetes is defined as a medium state with plasma glucose levels ranging between normoglycemia and diabetes. L-arginine, a functional amino acid, the precursor of nitric oxide, plays a crucial role in homeostasis of glucose and lipid metabolism. Symmetric dimethyl arginine (SDMA), an endogenous inhibitor of nitric oxide synthase activity, has been recognized as risk factor for endothelial dysfunction, diabetes and cardiovascular disease. Objective. To estimation symmetric dimethyl arginine (SDMA) and arginine in patient with type 2 diabetes and prediabetes. Subject and Methods. One hundred (100) subjects were included, fifty (50) with type 2 diabetes mellitus and fifty (50) with prediabetes compared with fifty (50) healthy controls matched age and gender. The concentration of symmetric dimethyl arginine and arginine were determined by High performance liquid chromatography method. Results. The mean concentration of symmetric dimethyl arginine was significantly higher in the diabetic and prediabetic group compared with the control group (46.73± 2.79 µg/ml, 33.47±1.41 µg/ml, 21.95±1.97 µg/ml, respectively). However, Controls demonstrated much higher level of arginine compared with the prediabetics and diabetics (214.53±2.16 µg/ml, 146.73±3.28 µg/ml and 92.3±2.2 µg/ml respectively) with highly significant differences between the three groups. Conclusion. Arginine levels are lower in patient with type 2 diabetes mellitus and prediabetes compared with the healthy control and can be used as a diagnostic biomarker and predict the incidence of microvascular complications and the symmetric dimethyl arginine (SDMA) might be a useful prognostic marker in patients with diabetic and prediabetic and level of SDMA can help us to prevent the complications in patient with T2DM.

Key words. Diabetes mellitus, prediabetes, Arginine, symmetric dimethylarginine (SDMA).

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Introduction.

Diabetes mellitus is a complex metabolic disorder that is associated with insulin resistance (IR), abnormal glucose levels, impaired insulin signalling, β-cell dysfunction, altered lipid metabolism, sub-clinical inflammation and increased oxidative (Testa et al., 2016). Prediabetes is defined as an intermediate state with plasma glucose levels ranging between normoglycemia and diabetes. It is a high risk condition for diabetes with an estimated annual conversion rate of 5%–10%; a similar proportion is converting back to normoglycemia (Bansal et al., 2015). Pre-diabetes is characterized by β-cell dysfunction and the presence of insulin resistance which occurs before changes in glucose level (Burnset et al., 2016). Arginine (2-amino-5-guanidinovaleric acid) is one of the 20 amino acids that are coded for as part of ribosomal protein synthesis in humans (Pernow et al., 2013). L-arginine is a conditionally or semi-essential amino acid for humans and serves as a precursor in the biosynthesis of nitric oxide (NO), an important messenger molecule with several physiological roles; the family of enzymes, nitric oxide synthases (NOS), are responsible for catalysing this formation of NO from L-arginine (Bode-Böger et al., 1998). Type 2 diabetes patients suffer a serious factor of cardiovascular disease after endothelial dysfunction (Hoang et al., 2013). Patients with diabetes have a lower level of arginine bioavailability compared to healthy people, which reduces NO production and increases the risk of cardiovascular disease (Romero et al., 2008). L-arginine can improve vascular function through the endothelium-derived relaxing factor of the catabolism product NO, Therefore it has vastly accepted. Orally 3 g of L-arginine per hour for 10 hours in a diabetic patient’s treatment can decrease systolic and diastolic medium arterial blood pressure significantly in a clinical experiment (Huynhet al., 2002). Other studies have shown that 9 g / day of L-arginine supplementation improved the endothelial function and increase blood flow in type 2 diabetic patients (Regensteiner et al., 2003). Moreover, some studies demonstrated that there is a relationship between insulin resistance and the endothelial response to the inhibition of NO synthesis. L-arginine is able to improve the vasodilatory response by NO to decrease insulin-resistant individuals, and it can promote glucokinase activity in cultured rat hepatocytes to improve peripheral and hepatic insulin sensitivity in patients with type 2 diabetic (Xue et al., 2013). Other research has demonstrated that the potential relation between a decreased NO bioavailability and the increase in insulin resistance, means that L-arginine--NO may be an underlying strategy for the reduction of insulin resistance (Sydow et al., 2005). Symmetrical dimethyl arginine (SDMA) is the structural isomer of the endogenous nitric oxide synthase (NOS) inhibitor asymmetric dimethyl arginine (ADMA). Both ADMA and SDMA derive from intranuclear methylation of l-arginine residuals and are released into the cytoplasm after proteolysis (Cooke et al., 2004). Compared to healthy subjects, the concentrations of circulating ADMA and SDMA are higher in many cardiovascular and renal diseases including diabetes mellitus. Free ADMA was first identified as a cardiovascular risk factor. Free SDMA was only recently identified as a cardiovascular risk factor with some studies revealing SDMA even as a more significant cardiovascular and renal risk factor than free ADMA and MMA (Emrich et al., 2017). Elevated SDMA levels have been reported in clinical conditions related to endothelial dysfunction such as hypertension, coronary artery disease (Hermenegildo et al., 2005) diabetes mellitus, preeclampsia (Schulze et al., 2010) and polycystic ovary syndrome (Lakhaniet al., 2011).
Materials and methods

Subjects

One hundred (100) subjects were included fifty (50) with type 2 Diabetes mellitus and, fifty (50) with prediabetes compared with fifty (50) healthy controls matched age and gender.

Sample collection

Ten (10) ml of blood was collected from each subject (patients and controls) and put in sterile tubes, then centrifuged and serum separated, aliquoted and stored at -20°C until analyzed.

Determination of symmetric dimethyl arginine (SDMA), arginine and clinical parameters.

Serum symmetric dimethyl arginine (SDMA) and arginine were determined by using high performance liquid chromatography. Fasting blood sugar, Total cholesterol, triglyceride, High density lipoprotein cholesterol (HDL), urea, creatinine were determined by enzymatic methods using commercial Kits (linear chemicals. S.L Company Spain). Serum low density lipoprotein cholesterol (LDL-C) was calculated using Friedewald's formula. Fasting serum insulin was estimated with immunochemiluminescent by Cobas technique. Body mass index (BMI) was calculated as weight of individuals divided by the square of their height (Kg/h2). Hemoglobin A1c was analyzed by High performance liquid chromatography (HPLC), Homeostatic model assessment of IR (HOMA-IR) was calculated by the formula: Fasting insulin (µIU/ml) × fasting glucose (mg/dl)/405.

Statistical analyses

Statistical analyses were done using SPSSv19. The serum symmetric dimethyl arginine (SDMA) and arginine were expressed as mean ± SE, the significance of differences in mean was estimated by the student's t-test. Analyses where the p-value was <0.05 were considered to be statistically significant.

Results

The mean concentration of Arginine was significantly lower in the diabetic and prediabetic group compared with control group (92.3±2.2 µg/ml, 146.73±3.28 µg/ml, 214.53±2.16 µg/ml respectively). In contrast, the mean concentration of symmetric dimethyl arginine was significantly higher in the diabetic and prediabetic group compared with the control group (46.73±2.79 µg/ml, 33.47±1.41 µg/ml, 21.95±1.97 µg/ml, p<0.001 respectively). Mean age of diabetic patients was 47.08±9.23 years which was significantly higher than either prediabetics (44.1±6.74 years) or controls (42.3±5.52 years). However, mean BMI in diabetics, prediabetics and controls was 29.47±3.19 kg/m², 27.65±4.16 kg/m² and 22.31±1.49 kg/m², respectively with significant differences between the three groups Table (1).

Table (1). Clinical characteristics of patients and controls group.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls (n=50)</th>
<th>Prediabetic (n=50)</th>
<th>Diabetic (n=50)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>42.3±5.52ᵃ</td>
<td>44.1±6.74ᵃ</td>
<td>47.08±9.23ᵇ</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Fasting blood sugar and HbA1c, *per se*, were significantly higher in diabetics (199.64±57.04 mg/dl and 9.65±2.36% respectively) than prediabetics (108.46±9.27 mg/dl and 6.12±2.92% respectively) which was in turn significantly higher than controls (81.87±8.87 mg/dl and 5.16±0.24% respectively) as shown in table (2). Mean serum insulin was slightly higher in diabetics than prediabetics (20.4±5.25 µU/ml vs. 18.26±5.46 µU/ml) with no significant difference. However, both groups were much higher than controls (8.71±2.25 µU/ml) with a highly significant difference. The lipid profile in the three groups. Mean serum cholesterol in controls, prediabetics and diabetics was 174.12±19.03 mg/dl, 186.02±23.13 mg/dl and 212.96±41.82 mg/dl, respectively with significant differences between the three groups. Diabetics and prediabetics showed comparable levels of TG (212.96±41.82 mg/dl and 186.02±23.13 mg/dl, respectively) and differed significantly from controls (111.9±48.56 mg/dl). In contrast, controls showed significantly higher HDL level (48.26±6.54 mg/dl) than either prediabetics (40.51±5.12 mg/dl) or diabetics (35.61±8.51 mg/dl). For LDL and vLDL, diabetic patients had significantly higher level (136.32±37.5 mg/dl and 36.51±11.86 mg/dl, respectively) than either prediabetics (110.63±21.0 mg/dl and 34.81±13.32 mg/dl, respectively) or controls (102.46±18.88 mg/dl and 22.66±9.86 mg/dl, respectively) with no significant difference between controls and prediabetics. Mean serum level of urea in diabetics and prediabetics was 29.86±6.7 mg/dl and 28.6±5.34 mg/dl, respectively with no significant difference. However, both groups differed significantly from controls (24.89±4.48 mg/dl). On the other hand, serum level of creatinine in controls and prediabetic was comparable (0.76±0.11 mg/dl and 0.78±0.18 mg/dl respectively) and lower significantly from that in diabetics (0.92±0.17 mg/dl) as shown in table (2). Diabetic patients showed higher HOMA-IR than prediabetics (9.7±3.71 vs. 4.94±1.62) with a highly significant difference. Again both groups were much higher than controls (1.7±0.44) with a highly significant difference *table (2)*.

Table (2). Laboratory characteristics of patients and controls.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls (n=50)</th>
<th>Prediabetic (n=50)</th>
<th>Diabetic (n=50)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine(µg/ml)</td>
<td>214.53±2.16a</td>
<td>146.73±3.28b</td>
<td>92.3±2.2c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SDMA (µg/ml)</td>
<td>46.73±2.79a</td>
<td>33.47±1.41b</td>
<td>21.95±1.97c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FBS, mg/dl</td>
<td>81.87±8.87a</td>
<td>108.46±9.27b</td>
<td>199.64±57.04c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c%</td>
<td>5.16±0.24a</td>
<td>6.12±2.92b</td>
<td>9.65±2.36e</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin(µIU/ml)</td>
<td>8.71±2.25a</td>
<td>18.26±5.46b</td>
<td>20.4±5.25b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>174.12±19.03a</td>
<td>186.02±23.13b</td>
<td>212.96±41.82c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>111.9±48.56a</td>
<td>174.32±26.74b</td>
<td>180.53±28.51a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>48.26±6.54a</td>
<td>40.51±5.12b</td>
<td>35.61±8.51f</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>102.46±18.88a</td>
<td>110.63±21.0b</td>
<td>136.32±37.5b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VLDL, mg/dl</td>
<td>22.66±9.86a</td>
<td>34.81±13.32a</td>
<td>36.51±11.86b</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Urea, mg/dl</th>
<th>24.89±4.48&lt;sup&gt;a&lt;/sup&gt;</th>
<th>28.6±5.34&lt;sup&gt;b&lt;/sup&gt;</th>
<th>29.86±6.7&lt;sup&gt;b&lt;/sup&gt;</th>
<th>&lt;0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine, mg/dl</td>
<td>0.76±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.78±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.92±0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.7±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.94±1.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.7±3.71&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

TG: triglycerides, HDL: high density lipoprotein, LDL: low density lipoprotein, VLDL: very low density lipoprotein.
Different small letters indicate significant differences, p value < 0.05 is significant.

**Discussion**

The present study revealed a significant decrease in arginine concentration in patients with type 2 diabetes mellitus and prediabetes compared with the control. This study is consistent with the studies that suggest the serum arginine levels are lower in individuals with T2DM compared with control (Ganzet al., 2016; Osaro et al., 2018). Also, there are studies revealed that arginine levels are reduced in individuals with prediabetes; this study demonstrates serum levels of arginine were negatively correlated with HOMA-IR (Ernandez-Garcia et al., 2019; Monti et al., 2016). However, some studies revealed that arginine levels are elevated with diabetes (Angela et al., 2012). The most reasonable explanation for this low concentration of arginine in diabetic and prediabetic patients, the hyperglycemia increases arginase activity that is used arginine substrate therefore serum arginine is low with hyperglycemia (Kashyap et al., 2007). There is reason binding with insulin action that is insulin increases L-arginine transport by increasing the expression of cationic amino acid transporter-1 (CAT-1), which is the predominant L-arginine transporter expressed in endothelial cells (González et al., 2011). In hyperglycemia and insulin resistance, the insulin mediated regulation of L-arginine transport is abnormal and impaired (Rajapakse, N. W et al., 2013). There is other reason that it explain decrease arginine, the presence of increased oxidative stress is well established in diabetes and hyperglycemia (Angvarasitichai et al., 2015) and since reactive oxygen species rapidly inactivate NO, increased vascular tissue consumption of NO can increase local requirement for Arginine (Ganzet al., 2016). Symmetric dimethyl arginine (SDMA) is another methylated analogue of L-arginine found in humans ADMA and SDMA are generated from proteins, which are methylated on arginine residues by protein arginine N-methyltransferases (PRMTs). PRMTs utilize SAM (S-adenosylmethionine) as a methyl donor and generate SAH (S-adenosyl homocysteine) and, ultimately, homocysteine as a byproduct (Sasser et al., 2010). ADMA is either eliminated by renal excretion or degraded by dimethylargininedimethylaminohydrolase (DDAH). In contrast to its stereoisomer, SDMA is almost completely eliminated with urine. Both SDMA and ADMA may reduce NO synthesis indirectly by inhibiting the cellular uptake of L-arginine (Maas et al., 2009). The current study showed significantly increased in serum SDMA in patients with prediabetes and diabetes compared with control; that is similar some studies, elevated SDMA levels have been reported in clinical conditions related to endothelial dysfunction such as hypertension and diabetes mellitus (Hermenegildo et al., 2002; Krzyzanowska et al., 2007). Symmetric dimethyl arginine (SDMA) has been elevated with diabetes by a study completed in Australia (Sotoodeh et al., 2009). The most reasonable explanation for this elevation, the protein arginine methyltransferase activity increase with hyperglycemia that is lead to increase synthesis SDMA (Tsikaset al., 2018). Also, elevated levels of glucose, oxidized LDL-cholesterol and oxidative stress are associated with decreased levels of DDAH and because the symmetric dimethyl arginine
SDMA can be metabolized by the enzyme dimethyl argininedimethylaminohydrolase (DDAH) to L-citrulline and dimethylamine (Lin et al., 2002).

**Correlation between Different Variables.**

SDMA demonstrated a negative correlation with cholesterol ($r = -0.314$, $p = 0.026$). Statistically arginine showed not related with FBS, HbA1c, Cholesterol, triglyceride, HDL, LDL, VLDL, urea, creatinine, insulin and HOMA-IR.

**Conclusion**

Arginine levels show significant decrease in patients with type 2 diabetes mellitus and prediabetes compared with the healthy control and can be used as a diagnostic biomarker in type 2 diabetes mellitus and prediabetes patients. The symmetric dimethyl arginine (SDMA) might be a useful prognostic marker in patients with diabetic and prediabetic.

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