Serum levels of Asymmetric dimethyl arginine and Nitric oxide in patients with prediabetes and type 2 diabetes mellitus

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

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

Abstract

Background: Diabetes mellitus is a global disorder, characterized by in elevation blood glucose level (hyperglycemia) for extended period of time, resulting from absolute or relative insulin deficiency. Pre-diabetes is determined on the basis of glycemic parameters which are above normal but below diabetic value. Nitric oxide (NO) is one of the major endothelium derived vaso-active substances whose role is of prime importance in maintaining endothelial homeostasis. Low levels of NO are associated with impaired endothelial function. Asymmetric dimethyl arginine (ADMA), an analogue of L-arginine, is a naturally occurring product of metabolism found in human circulation. Elevated levels of ADMA inhibit NO synthesis therefore impair endothelial function and thus promote insulin resistance and atherosclerosis. ADMA levels are increased in people with diabetes mellitus, hypertension, chronic renal failure and dyslipidaemia.

Objective: To evaluate asymmetric dimethyl arginine (ADMA) and Nitric oxide (NO) in patients 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 asymmetric dimethyl arginine was determined by High performance liquid chromatography method. The concentration of nitric oxide was determined by quantitative colorimetric method according Griess method.

Results: The mean concentration of asymmetric dimethyl arginine was significantly higher in the diabetic and prediabetic group compared with the control group (35.89±2.79 µg/ml, 22.98±1.86 µg/ml, 12.69±1.65 µg/ml, p<0.001 respectively). The mean concentration of Nitric oxide was significantly lower in the diabetic and prediabetic group compared with the control group (12.29±8.67, 16.29±7.36, 19.48±4.72, p<0.001 respectively).

Conclusion: The asymmetric dimethyl arginine (ADMA), might be a useful prognostic marker in patients with diabetic and prediabetic and can be used as a marker showing the condition of developing diabetic complication. Nitric oxide level was significant lower in diabetic and prediabetic compared with healthy control, therefore maybe useful as a biomarker in the diagnostic.

Key words. Diabetes mellitus, prediabetes, Asymmetric dimethyl arginine (ADMA), Nitric oxide (NO)

How to cite this article: Dayir BS, Hamzah M, Khudair MSH (2020): Serum levels of asymmetric dimethyl arginine and nitric oxide in patients with prediabetes and type 2 diabetes mellitus, Ann Trop Med & Public Health; 23(IIb): S424. DOI: http://doi.org/10.36295/ASRO.2020.2322

Introduction

Diabetes mellitus is a global disorder, characterized by in elevation blood glucose level (hyperglycemia) for extended period of time, resulting from absolute or relative insulin deficiency (Marin-Penalver et al., 2016). Feature of hyperglycemia often include thirst and urination, constant hunger, a fruity breath, weight loss, extreme fatigue, and poor wound healing. If untreated, the symptoms can progress to blurred vision, deep respiration, dehydration, and coma (Lorenzi et al., 1992). Chronic complications of diabetes include micro vascular and macro vascular complications that
cause visual damage, blindness, kidney disease, nerve loss, amputation, heart illness, and stroke. Pre-diabetes is determined on the basis of glycemic parameters which are above normal but below diabetic value. It is a high risk condition for diabetes with an estimated annual conversion rate of 5%–10%; a similar proportion is converting back to normo-glycemia. Pre-diabetes is characterized by β-cell dysfunction and the presence of insulin resistance which occurs before changes in glucose level. Asymmetric dimethyl arginine is an endogenous competitive inhibitor of nitric oxide synthase (NOS). Plasma ADMA concentrations have been reported to increase in connection with diseases associated with an impaired endothelial L-arginine/NOS pathway. Patients with diabetes have an adverse cardiovascular risk profile. Elevated ADMA concentrations have been described in patients with type 2 diabetes mellitus. Hyperglycaemia per se may increase ADMA concentrations by reduced metabolism. An in vitro study showed that elevated glucose levels are capable of inhibiting DDAH activity in cultured endothelial cells. Clinical investigations in patients also indicate that ADMA is directly related to blood glucose levels. In addition, there is evidence that insulin resistance is related to elevated concentrations of ADMA. Some studies report increased ADMA levels in prediabetics. Remarkably increased level of HOMA-IR, and ADMA, was detected in both IGT and DM subjects compared with the NGT group. Production of NO is impaired in patients with T2D, especially with bad controlled glycaemia. Impaired NO metabolism, especially reduced NO production and NO bioavailability has been recognized as a risk factor for development of cardiometabolic disorders, especially vascular dysfunction, cardiovascular disease, chronic kidney disease, endocrine disorders, insulin resistance, metabolic syndrome, and type 2 diabetes. The nitric oxide (NO) and insulin resistance have a reciprocal relationship, NO suppresses insulin resistance and reduction in NO leads to increase insulin resistance. NO might act as a regulatory factor for the downstream signaling molecules linking GLUT4 translation and glucose uptake.

Materials and methods

Subjects

This study involved 50 patients with type 2 diabetes mellitus comprised of 25 males and 25 females and 50 patients with prediabetes comprised of 25 males and 25 females. The patients clinically diagnosed. A well 50 normal healthy persons, age and sex matched with the study group were selected as controls.

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 asymmetric dimethyl arginine (ADMA),nitric oxide (NO) and clinical parameters.

The concentration of asymmetric dimethyl arginine (ADMA) was determined by using high performance liquid chromatography. The concentration of nitric oxide (NO) was determined by using colorimetric method according on Griess reagent. 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 Friedewalds 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 ADMA and NO were expressed as mean ±SE, the significance of differences in mean was estimated by the student's t-test. Analysis where the p-value was <0.05 were considered to be statistically significant.
Results

The mean concentration of asymmetric dimethyl arginine was significantly higher in the diabetic and prediabetic group compared with the control group (35.89±2.79 µg/ml,22.98±1.86 µg/ml,12.69±1.65 µg/ml, p<0.001 respectively). The mean concentration of Nitric oxide was significantly lower in the diabetic and prediabetic group compared with the control group (12.29±8.67, 16.29±7.36, 19.48±4.72, 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.

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) and 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 20.4±5.25 µU/ml) than either prediabetics (110.63±21.0 mg/dl and 18.26±5.46 µU/ml) 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 prediabetics 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 (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>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>25(50%)</td>
<td>25(50%)</td>
<td>25(50%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Female</td>
<td>25(50%)</td>
<td>25(50%)</td>
<td>25(50%)</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.31±1.49a</td>
<td>27.65±4.16b</td>
<td>29.47±3.19c</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

BMI: body mass index. Different small letters indicate significant differences, p value is <0.05

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>ADMA(µg/ml)</td>
<td>12.69±1.65a</td>
<td>22.98±1.86b</td>
<td>35.89±2.79c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NO(µmol/L)</td>
<td>19.48±4.72a</td>
<td>16.29±7.36b</td>
<td>12.29±8.67c</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±57.04e</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±2.36c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin(µIU/ml)</td>
<td>8.71±2.25a</td>
<td>18.26±5.46d</td>
<td>20.4±5.25 µU/ml</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>212.96±41.82c</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

©Annals of Tropical Medicine & Public Health S424
Correlation between Different Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean Value</th>
<th>SEM</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL, mg/dl</td>
<td>48.26±6.54</td>
<td>a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>102.46±18.88</td>
<td>a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VLDL, mg/dl</td>
<td>22.66±9.86</td>
<td>a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urea, mg/dl</td>
<td>24.89±4.48</td>
<td>a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine, mg/L</td>
<td>0.76±0.11</td>
<td>a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.7±0.44</td>
<td>a</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

TG: triglycerides, HDL: high density lipoprotein, LDL: low density lipoprotein, VLDL: very low density lipoprotein.
NO: nitric oxide, Different small letters indicate significant differences, P value < 0.05 is significant.

Discussion

The present study showed a significant increase in asymmetric dimethyl arginine level in type 2 diabetes mellitus compared with either prediabetes and control, similarly several studies reported that asymmetric dimethyl arginine (ADMA) was significantly higher in diabetic (Asija et al., 2014). Asymmetric dimethyl arginine (ADMA) is also elevated in patients with cardiovascular disease and diabetes (Celik et al., 2014). There is an association between high ADMA levels and the development of type 2 diabetes mellitus (Triches et al., 2018). There are also studies indicating high asymmetric dimethyl arginine (ADMA) in patients with prediabetes. There is a significant correlation between ADMA levels and impaired fasting glucose (IFG). Plasma ADMA levels are significantly lower in the control group than the IFG, IGT (Eliana et al., 2011; Bayrak et al., 2014; Jinget al., 2016). The most reasonable explanation for this increase that the insulin resistance and hyperglycemia-induced oxidative stress upregulates the expression of the protein arginine methyltransferase (PRMT) and attenuates dimethyl arginine dimethylaminohydrolase (DDAH) activity. PRMT and DDAH are redox sensitive leading to increased ADMA production and decreased ADMA degradation under circumstances of hyperglycemic status (Jing, Z. et al., 2016). Hyperglycemia increases asymmetric dimethyl arginine (ADMA) concentration. ADMA is mainly metabolized by dimethyl arginine dimethylaminohydrolase (DDAH). Hypothesized that hyperglycemia impairs the ADMA metabolizing enzyme activity (DDAH). In other study demonstrated that elevated glucose levels are capable of inhibiting DDAH activity in cultured endothelial cells (Bayrak et al., 2014). Other mechanism Tumor necrosis factor-alpha (TNF-alpha) has been shown to suppress the insulin signaling in both adipose tissues and skeletal muscles, thus being involved in insulin resistance in patients with diabetes and prediabetes. Further, plasma level of TNF-alpha was elevated in diabetic patients and positively correlated with insulin resistance. Since TNF-alpha stimulated ADMA accumulation in human cultured ECs via suppression of DDAH insulin resistance and visceral obesity could elevate ADMA levels in diabetes via TNF-alpha overproduction (Yamagishi et al., 2008).

In this study we found nitric oxide level in the patient with prediabetes and type 2 diabetes mellitus decreases compared with the control, there are studies support this view, including the study conducted in Japan by Mchiro Shimabukuro, this study suggested that is insulin resistance leads to reduce nitric oxide availability in patient with impaired glucose fasting (Shimabukuro et al., 2013). However, some studies demonstrated increase in nitric oxide level with hyperglycemia (Schneideret al., 2013). The reason for this decline can be explained by vascular insulin resistance leads to downregulation of IRS-1 and -2 and decreased phosphorylation of Akt and NOS, in patients with prediabetes and diabetes (Surdacki et al., 2007). Hyperglycemia may also lead to increased O-GlcNAcylation of NO decreases Ser1177 phosphorylation and inhibits its activity. Thus this leads to decrease nitric oxide availability. In type 2 diabetes mellitus, insulin resistance and obesity conditions, the expression of nitric oxide synthase is reduced, which causes low nitric oxide availability (Georgescu et al., 2011; Kraus et al., 2012). Hyperglycemia may also play a role in the decreased NO production in type 2 diabetes, because high glucose per se inhibited endothelial NOS activity in the glomeruli, through a protein kinase C- associated mechanism. Moreover, high glucose and/or the associated advanced glycosylation end products decreased NOS expression (Tessari et al., 2010). Increased AGEs levels associated with hyperglycemia. Moreover, binding of AGEs to specific cell surface receptor for AGE can activate intracellular redox signaling and subsequent to activate the expression of redox-sensitive transcription factors. Superoxide reacts with nitric oxide that is cause degradation for nitric oxide molecule (Tangvarasitichai et al., 2015).

Correlation between Different Variables

©Annals of Tropical Medicine & Public Health S424
ADMA correlated with each of urea ($r = 0.351, p = 0.012$), TG ($r = 0.336, p = 0.017$), VLDL ($r = 0.349, p = 0.013$), statistically NO showed not related with FBS, HbA1c, Cholesterol, triglyceride, HDL, LDL, VLDL, urea, creatinine, insulin and HOMA-IR.

**Conclusion**

The asymmetric dimethyl arginine (ADMA) can be used as a good biomarker in patient with type 2 diabetes mellitus. Nitric oxide level was significant lower in diabetic and prediabetic compared with healthy control, therefore maybe useful as a biomarker in the diagnostic.

**References**


