Serum Levels of Asymmetric Dimethylarginine and Testosterone Among Egyptian Type 2 Diabetic Men with Erectile Dysfunction as Risk Markers for Coronary Artery Disease

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Abstract

Background: Erectile dysfunction (ED) is now beginning to be considered as an index of subclinical coronary artery disease (CAD). ED has been shown to precede the development of CAD in men with type 2 DM. Moreover ED may be a significant marker for diabetes. There is a strong evidence that endogenous testosterone has a protective role on the endothelium. It may be an independent risk factor for increased CAD. Asymmetric dimethylarginine (ADMA) is found to be increased in conditions associated with atherosclerosis and considered as plasma marker of endothelial function as well as endothelial dysfunction. ADMA level is usually high in case of atherogenesis.

Objective: To measure serum ADMA and testosterone levels in type 2 diabetic patients with ED for detection of their value as risk markers for Coronary artery diseases.

Patients and Methods: The study was conducted on Fifty participants; Thirty of them were diabetic with mean duration ±SD (6.97±1.6ys), mean duration of ED ±SD (3.03±1.43ys), their mean age ±SD (39.47±4.1ys) (group1). Twenty apparently healthy age matched group as controls (group 2), their mean age ±SD (37.65±2.91ys). Clinical assessment was done for all participants regarding CAD (by Electrocardiogram and Echocardiography) and DM. Penile Doppler for ED patients. Samples were taken from both group for measuring HbA1c, fasting blood sugar, fasting lipid profile (TC, TG, LDL-c, HDL-c), prolactin, ADMA, free and total testosterone (FT), (TT) as well as other routine investigations. Informed consent was taken from all participants.

Results: Non significant difference in serum prolactin level was found between group 1 and group 2. Significantly higher serum FBS, TC, TG, LDL-c, ADMA, free and total testosterone (FT), (TT) as well as other routine investigations. Informed consent was taken from all participants.

Conclusions: Serum ADMA and testosterone levels may considered as risk markers for endothelial dysfunction and atherogenesis in diabetic men with ED. Therefore ED should alert diabetic men and healthcare givers to the future risk of developing CAD. Testosterone replacement is recommended for diabetic males diagnosed as ED with low testosterone.

Key Words: Asymmetric dimethylarginine – Erectile dysfunction – Testosterone – Diabetes mellitus – Coronary artery disease – Dyslipidemia.

Introduction

ERECTIONS dysfunction is the consistent inability to achieve or maintain a penile erection sufficient for satisfactory sexual performance [1]. Vascular disease is by far the most common cause of ED. Erectile dysfunction has now assumed center stage as a readily treatable disorder and a powerful risk marker for CAD [2,3].

Erectile dysfunction is one of the most common complications of DM. Depending on the severity and duration of diabetes, the prevalence of ED ranges from 20% to 85% [4]. It is known to occur at an earlier age in men with diabetes than in those without it. In some cases ED may be a significant marker for diabetes, particularly in younger patients, it may be manifestation of previously undiagnosed DM [5]. Men 45ys old or younger with ED were more than twice as likely to have DM as men without. Thus, it may represent an early warning for the development of diabetes [6].

The pathophysiology of diabetic ED is multifactorial. Physical factors are thought to play a major role but psychologic and relationship issues often coexist. These factors include endothelial dysfunction, peripheral and autonomic neuropathy,
vasculopathy, and hypogonadism. Endothelial dysfunction is thought to be an important factor underlying diabetes-associated ED [7,8]. Pathogenic mechanisms of endothelial dysfunction in diabetes are incompletely understood [9]. Vasculogenic ED results from impairment of endothelial-dependent or independent smooth muscle relaxation (functional vascular ED, initial stages), occlusion of the cavernosal arteries by atherosclerosis (structural vascular ED, late stages) or a combination of them [10].

Testosterone has an important physiological role in male sexual response, it regulate the time of the erectile process and sexual desire, thereby coordinating penile erection with sex. It has clearly a relevant role in ED. Now a day a link between ED, hypogonadism and underlying disorder such as type 2 diabetes mellitus is well documented [11].

Low Testosterone level is positively associated with the presence and severity of atherosclerosis; reduction in plasma testosterone might contribute to increased arterial stiffness, which in turn has been associated with increased cardiovascular risk [12]. Interestingly, men with established CAD display reduced circulating testosterone levels [13]. It is often associated with a certain degree of endothelial dysfunctions independently of other vascular risk factors suggesting a protective role of endogenous testosterone on the endothelium [14].

Thompson et al. [15] pointed out that up to 46% of ED patients usually suffering from coronary disorders 5ys before the onset of cardiac events, they postulated that endothelial dysfunction occurring in the smaller arteries of the penis before the appearance of atherosclerotic changes in the larger coronary arteries.

The major cardiovascular risk factors aging, smoking, diabetes, dyslipidemia and hypertension have raised prevalence in patients with ED [16]. The prevalence of ED is also directly related to the number of cardiovascular risk factors present, being highest in individuals with more than three risk factors [17].

Asymmetric dimethylarginine (ADMA) is a competitive inhibitor of endothelial nitric oxide synthase (eNOS) that is associated with endothelial dysfunction, and is a risk marker for cardiovascular disease in type 1 diabetics suffering from erectile dysfunction [18]. It is elevated in many conditions associated with ED, such as hypertension, diabetes, hyperlipidemia, and renal failure; it is also increased in men with coronary artery disease and ED [19]. Asymmetric dimethylarginine is produced by methylation of arginine residues in intracellular proteins via protein arginine N-methyltransferases (PRMT) [20]. The major pathway of elimination for ADMA is its degredation by dimethylarginine dimethylaminohydrolases (DDAH) [21]. Whereas only a small amount is eliminated by renal excrtection [22].

Endothelial function can be modulated by several factors associated with diabetes including degree of acute hyperglycaemia, duration of diabetes, accumulation of advanced glycosylated end products and complications such as nephropathy and microalbuminuria [23]. Elevation of circulating ADMA levels leads to an increased resting vascular tone and enhances several pro-atherogenic mechanisms including platelet aggregation and adherence of monocytes, proliferation of vascular smooth muscle cell, as well as extracellular matrix formation [24]. Long term exposure to ADMA would cause atherogenesis, it elevates blood pressure, vascular resistance, reduces vessel vasodilation and increases endothelium cell adhesiveness. Plasma ADMA levels are also related to cardiovascular complications such as peripheral artery disease, stroke, and congestive heart failure [25,26].

### Material and Methods

This study was conducted on Fifty participants, during a period from November 2009-April 2010, thirty of them were complaining of ED from 1-7ys with mean duration±SD (3.03±1.43ys), their ages ranged from 32-46ys with mean age±SD (39.47±4.1ys), duration of diabetes was ranged from 5-10ys with mean±SD (6.97±1.6ys) (group 1). Twenty apparently healthy men were taken as controls (group 2), their ages ranged from 35-42ys with mean±SD (37.65±2.91ys). All patients and controls were selected from Endocrinology, Urology and Internal medicine outpatient clinics at AL-Zahraa University Hospital, and subjected to complete history taking (tobacco smoking, DM, hypertension, dyslipidemia, CAD, other endocrinial disorders as well as medication history). Personal history including job, social and sexual history as regard frequency of intercourse and morning erection. Erectile Dysfunction was assessed using a five-item version of the International Index of Erectile Function (IIEF-5) (After translation to Arabic language). Erectile Dysfunction was categoried into five grades of severity on the basis of their IIEF score. Scores between 22 and 25 (inclusive) were categorized as normal erectile function, 17-21 as mild, 12-16 as mild to moderate, 8-11as moderate, and 1-7 as severe to complete ED [27].
Through clinical evaluation for all patients and diabetes complication, genital examinations for secondary sexual characters; penile and testicular examinations as well as penile Doppler were performed for ED patients using ICI and color Doppler ultrasonography with 7.5MHz probe Toshiba SAL 270-A transducer (Toshiba Corp., Tokyo, Japan). It included measurement of peak systolic velocity (PSV), end diastolic velocity (EDV) following ICI of 0.5mL of a Trimix solution containing 30mg/mL of papaverine hydrochloride, 10lg/mL of prostaglandin E1 and 1mg/mL of phentolamine. The patients were then observed for 1hr to assess the erectile response. A PSV of >30cm/sec in the deep penile arteries and EDV of <5cm/sec are considered to prove normal penile blood supply [28]. Electrocardiography and Echocardiography were done to rule out ischemic heart diseases, only three cases (10%) from ED patients showed ischemic changes and mild diastolic dysfunction in their ECG and Echocardiography; they were excluded from the study.

**Exclusion criteria:** Both groups were non smoker, non hypertensive, non obese (have normal waist circumference), no history of CAD, liver, renal, psychiatric diseases and or medication, and no history of drug addiction, all patients were selected with normal fundus, no microalbuminuria using Micral test strips. Men who did not have any opportunity for sexual activity were excluded from the study as the IIEF-5 scale has only 5- Serum ADMA levels was done by ELISA technique supplied from immunodiagnostic AG, k7828.

**Both groups were subjected to the following investigations:**

1- Serum FBS, fasting lipid profile, renal and liver function tests all were done on Hitachi 911 autoanalyzer Using Bohringer Mannheim Company Photometric Kits West Germany.

2- HbA1c level was done by chromatographic-spectrophotometric ion exchange. Ion exchange resin (Kit supplied by Biosystems COD. 11044).

3- Serum prolactin, Total testosterone was done by Electrochemiluminescence immunoassay (ECLIA) technique on Roche Elecsys 1010.

4- Serum Free testosterone was done by ELISA dbc kit (diagnostics Biochem Canada Inc. Cat. No.: CAN-fTE-260.

5- Serum ADMA levels was done by ELISA technique supplied from immunodiagnostic AG, k7828.

**Sampling:**

6ml of venous blood were taken from each participant and divided as the following; Two ml on vacutainer EDTA for HbA1c, and the remaining on vacutainer tube with gel. Serum was separated and taken for measuring FBS, renal and liver function tests. The remaining part of serum was stored at-20 until assay of prolactin, free and total testosterone, ADMA levels. Three ml of venous blood was taken after 12 hrs fasting on vacutainer tube with gel and serum was separated and test done for lipid profile.

**Statistical analysis:**

Data was analyzed by Microsoft Office 2003 (excel) and Statistical Package for Social Science (SPSS) version 16. Parametric data was expressed as mean±SD, and non parametric data was expressed as number and percentage of the total. Comparing the mean±SD of two groups was done using the paired and unpaired student’s t-test. p-value >0.05 is considered non-significant. p-value <0.05 is considered significant p-value <0.01 is considered highly significant [30].

**Results**

According to penile Doppler our patients were divided into Mild ED was reported by 18 (60%), moderate by 9 (30%), and severe ED by 3 (10%) patients. The result of the present study are presented in (Table 1) and Figs. (1-3).

There were no significant difference in serum prolactin between group 1 and group 2 p>0.05. Significantly higher HbA1c, serum FBS, ADMA, TC, TG, LDL-c levels in group1 as compared to group 2 p<0.01. Moreover significantly lower serum HDL-c, TT, FT levels in group 1 as compared to group 2 (p<0.01) Table (1).

Table (1): Demographic and laboratory data of patients with ED (group 1) and controls (group 2) expressed as mean values±SD.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group1 ED patients</th>
<th>Group 2 control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADMA umol/L</td>
<td>0.97±0.62</td>
<td>0.38±0.12</td>
<td>0.0000*</td>
</tr>
<tr>
<td>TT mg/ml</td>
<td>4.73±1.56</td>
<td>6.26±0.82</td>
<td>0.0000*</td>
</tr>
<tr>
<td>FT mg/ml</td>
<td>2.61±1.42</td>
<td>5.56±0.72</td>
<td>0.0000*</td>
</tr>
<tr>
<td>PRO mg/ml</td>
<td>12.72±2.77</td>
<td>13.50±4.97</td>
<td>0.5310*</td>
</tr>
<tr>
<td>FBS mg/dl</td>
<td>146.63±23.70</td>
<td>94.90±18.16</td>
<td>0.0000*</td>
</tr>
<tr>
<td>Hb A1c %</td>
<td>8.41±2.10</td>
<td>5.46±0.48</td>
<td>0.0000*</td>
</tr>
<tr>
<td>TC mg/dl</td>
<td>208.13±42.20</td>
<td>165.40±9.05</td>
<td>0.0000*</td>
</tr>
<tr>
<td>TG mg/dl</td>
<td>163.20±46.72</td>
<td>90.55±17.88</td>
<td>0.0000*</td>
</tr>
<tr>
<td>HDL-c mg/dl</td>
<td>45.87±8.13</td>
<td>64.30±10.96</td>
<td>0.0000*</td>
</tr>
<tr>
<td>LDL-c mg/dl</td>
<td>125.37±36.59</td>
<td>84.50±13.54</td>
<td>0.0000*</td>
</tr>
</tbody>
</table>

* Insignificant.
* Significant.
Non significant correlation between ADMA level and duration of diabetes as well as duration of ED ($r = 0.26, p>0.05$), ($r = 0.055, p>0.05$) respectively. A significant negative correlation between ADMA level and total testosterone ($r = -0.408, p<0.05$) Fig. (1). Non significant negative correlation between ADMA level and free testosterone ($r = -0.279, p>0.05$). A significant positive correlation between ADMA level and LDL-c ($r = 0.397, p<0.05$). However a significant negative correlation between ADMA level and HDL-c ($r = -0.446, p<0.05$) was noted respectively Figs. (2,3).

**Discussion**

Erectile function is increasingly being recognized as an indicator of the overall health of men [31]. It is not only effects men’s sex life, but also effects their overall satisfaction with life [32].

Erectile Dysfunction and CAD are worsened by dyslipidemia [33]. The study done by [10] pointed out a strong association between ED and clinical atherosclerosis. Furthermore, there is a high incidence of CAD in men with ED. Data suggested that ED may be an early manifestation of endothelial dysfunctions in the presence or absence of cardiovascular risk factor [34].

In our study there were significantly increased serum TC, TG and LDL-c levels and significantly decreased HDL-c levels in group 1 as compared to group 2 $p<0.01$, this is in agreement with [35] which found up to 42.4% of men with ED had hyperlipidemia and elevated levels of TC and LDL-c were associated with moderate to severe ED. Ponholzer et al. [36] pointed out that elevation of serum lipid levels were the most important risk factors for ED in healthy population. The study of men with and without ED by [33] showed that the prevalence of hyperlipidemia was 70.6% in men with ED vs 52.0% in men without. HDL-c and TC/HDL-c ratio were predictors of ED in that population, thus aggressive treatment of both ED and dyslipidemia may result in a decrease in cardiovascular events and improvement in erectile function [37]. We found a significant positive correlation between ADMA level and LDL-c ($r = 0.397, p<0.05$), this result in agreement with [38] which reported a positive correlation between plasma LDL-c and ADMA concentrations and between plasma TC and ADMA concentrations $p<0.01$. However a significant negative correlation between ADMA level and HDL-c ($r = -0.446, p<0.05$), as usually dyslipidemia associated with type 2 DM.

Asymmetric dimethylarginine has emerged as an independent predictor of cardiovascular disease [1]. It is also a marker of endothelial dysfunction in hypercholesterolemia, hypertension, type 2 diabetes, insulin resistance, and smoking [39,40].

Highly significant increased ADMA level showed in group 1 as compared to group 2 $p<0.01$. The study done by [41] showed that elevated ADMA concentrations have been found in type 1DM patients, and by [42] in type 2DM patients; in particular in those patients presenting with diabetic nephropathy or micro-and macroalbuminuria. Moreover; elevated ADMA levels have also been found in Type 2DM patients with diabetic retinopathy as
reported by [43]. The study done by [44] found that higher ADMA plasma level was found in men with endothelial dysfunction as compared to men with normal endothelial function and increased level may be a potential mechanism of endothelial dysfunction in ED patients. Blumentals et al. [48] told that ED patients had two folds increase in risk of Acute Myocardial Infarction when compared to non ED patients. Non significant correlation between ADMA level and duration of diabetes as well as duration of ED \( (r=0.265, p>0.05), (r=0.055, p>0.05) \) respectively; This may be due to short duration of DM and ED; also no apparent diabetic complications.

Montorsi et al. [46] found that men with symptomatic CAD had ED prevalence of 49\%, and patients noticed ED on average 39 months before the onset of angina [34], showed high rate of ED in men with diabetes and angiographically documented overt and silent CAD. [47] demonstrated a strong correlation between ED and burden of disease in men with CAD. Asymptomatic middle aged men with ED and no cardiovascular disease should be considered as having a 10ys cardiovascular risk or diabetes [15].

In our study serum free and total testosterone levels were significantly decreased in group1 as compared to group 2 \( p<0.01 \); this in accordance with [48] they found that serum levels of total and free testosterone are lower in men with type 2 DM than in normal subjects. Isidori et al. [49] explained the lower levels of testosterone usually due to its conversion into estradiol by the effect of aromatase which formed by metabolically active visceral fat resulting into more fat deposition, activation of lipoprotein lipase and increased insulin resistance.

A significant negative correlation between ADMA level and total testosterone \( (r=-0.408, p<0.05) \); this is mostly due to early andropause and hypogonadism associated type 2 DM. Non significant negative correlation between ADMA level and free testosterone \( (r=-0.279, p>0.05) \), this may be due to low sex hormone binding globulin. The study done by [50] reported that low testosterone shown to be an independent risk factor for increased cardiovascular events by about 40\% (independent of their lifestyle and dyslipidemia), also he told that low testosterone either free or total mostly found in men with type 2 diabetes which may be due to secondary reduction in sex hormone binding globulin.

Geoffrey Hackett [51] reported that at least 75\% of type 2 diabetic males suffer from ED and low testosterone, both of them are considered as additional risk for CAD in a group of patients with multiple risk factors.

The dynamic penile colour Doppler ultrasound is considered the gold standard for the evaluation of penile vascular damage. ADMA level is independently associated with ultrasonographically documented poor penile arterial inflow. This finding underlines the important role of ADMA as a marker of penile arterial damage and implies a contribution of this compound to the pathophysiology of generalised vascular disease associated with ED [19].

**Conclusion and recommendation:**

Measurement of ADMA and testosterone and levels may be of important value in diabetic men with newly diagnosed ED. Testosterone replacement therapy is recommended in diabetic patients with ED to improve endothelial function. Screening for diabetes mellitus is mandatory for every young men presented with ED. We need more study on large scale of diabetic populations with early ED and follow-up for long period.

**References**


