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Vitamin C and Some Antioxidants Role in Endothelial Dysfunction in Type 2 Diabetic Patients

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ABSTRACT

Objective: *Endothelial dysfunction (ED) in type-2 diabetes mellitus (DM) plays a crucial role in angiopathies development and progression. The most frequent cause of endothelial dysfunction in DM is oxidative stress.*

Aim of the study: *To investigate the relationship between ascorbic acid level in serum and some anti-oxidant parameters in blood with clinical, and duplex findings of brachial artery and aorta in patients with type 2 -DM.*

Patients and methods: *Case-Control prospective study included sixty patients with type 2-DM, and 20 healthy volunteers were included. History of peripheral ischemia, diabetic retinopathy, frequency of metabolic syndrome was analyzed. Measurements of plasma levels of ascorbate, super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GTx) and lipid peroxidase (LPO) were studied. Estimation of HOMA-IR was also done. Duplex study in brachial artery and aorta to measure flow mediated vasodilatation (FMD) reactive hyperemia and intima media thickness (IMT) of aorta was done. Echocardiography was done to measure ejection fraction (EF%) and left ventricular dimensions.*

Results: *Patients' group showed highly significantly decreased plasma insulin (P=0.009), plasma ascorbate, SOD, CAT and glutathione (P=0.001, each) than control, while showed highly significant increased plasma level of fasting sugar, T-Cholesterol, LDL and lipid peroxidation (P=0.001 each) than control. Urinary ACR was highly significantly also increased in patient' group (P=0.001) than control. Patients' group showed highly significant decreased brachial volume blood flow (P=0.008), mean velocity (P= 0.001) and increase IMT of aorta (P=0.001) and LV diameter in diastole (P=0.034) than control. Brachial artery diameter (P=0.001), EF% of LT ventricle (P=0.004), plasma ascorbate (P=0.005) and BMI (P=0.007) were highly significantly predict FMD. While plasma cholesterol (P=0.012), LDL (P=0.018), IMT (P=0.021) and reactive hyperemia (P=0.026) showed less significant prediction to FMD. Plasma ascorbate, SOD, Catalase and glutathione peroxidase showed (100%) diagnostic accuracy for endothelial dysfunction while IMT and lipid peroxidase showed less value (74.4% &89.7%) respectively.*

Conclusion: *Vitamin C, plasm levels of SOD, CAT, glutathione and Lipid peroxidase are decreased in type-2 DM and showed strong relevance to micro and macrovascular complications.*

Keywords: Tooth root, Dentin, Dental cement, Nanoindentation, Micromechanical properties

INTRODUCTION

Uncontrolled DM created pro-atherogenic environment characterized by hyperglycemia, dyslipidemia, systemic inflammation, endothelial dysfunction, and oxidative stress that resulted in the development of early aortic atherosclerotic

lesions. Endothelial dysfunction is one of the initial key steps in athero-sclerogenesis in type 2-DM. Reduced expression of endothelial nitric oxide synthase (eNOS) is a hallmark of endothelial dysfunction in diabetes, which predisposes diabetic patients to cardiovascular (CV) complications including blunted angiogenesis. Several risk factors, such as hypertension, dyslipidaemia, inflammation, oxidative stress, and advanced glycation end products (AGEs), are associated with micro- and macro-vasculopathies [1]. Among these risk factors, oxidative stress contributes to the formation of atherosclerotic plaques and increased risk of CVD. Nitric oxide synthases (NOSs), which are expressed in endothelium, contribute to the mitigation of vascular endothelium-dependent dysfunction in prediabetes mellitus.

Aim of the study

To study the relationship between ascorbic acid level and serum SOD, CAT, GTX and lipid peroxidase in blood with clinical, duplex findings of brachial artery and aorta and echocardiographic data in patients with type 2 -DM.

Patients and methods

Case-Control prospective study included sixty patients with type 2 -DM were selected among those attained to the MINIA University hospital out clinic of diabetes and endocrinology.

Criteria for inclusion

Criteria of type 2 DM are according to American Diabetes Association (ADA 2018) that is, patients with fasting glucose levels ≥ 126 mg/dL or 2-h plasma glucose ≥ 200 mg/dL, after a 75-g oral glucose loading test or a patient with HbA1C ≥ 6.5%.

Excluded patients

Patients with other chronic medical illnesses affecting cardiovascular system, excessive alcohol consumption, pregnant, lactating women, those with 2ry diabetes or other endocrinal pathologies, mental diseases (senile dementia and Alzheimer's disease) will be excluded [2-4]. The control group (25 volunteers) will be selected as healthy participants of matched. Age and gender without history of any chronic medical illness.

Clinical history data were collected for information on:

- Age; and Family history of ischemic diseases.
- Smoking index, Use of medications, Duration of diabetes,
- History of peripheral ischemia or angina attacks or transient ischemic attacks or visual disturbances was taken.

Anthropometric data

Body mass index (BMI) was estimated. Arterial blood pressure measurement was obtained before blood collection. The frequency of metabolic syndrome (MS) will be assessed according to the criteria of the National Cholesterol Education Program's Adult Treatment Panel III (defined by 3 or more of the following five abnormalities: Waist circumference > 102 cm in men or > 88 cm in women, Serum triglyceride level > 150 mg/dL, HDL level of < 40 mg/dL in men or < 50 mg/dL in women, blood pressure of > 130/ 85 mm Hg, and Serum glucose level > 110 mg/dL (Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol 2001). Blood Collection and Biochemical Analysis (Patients and control). Blood samples were collected after 12 to 14 hours fasting (overnight) from the ante-cubital vein before duplex study.

- A lipid panel was obtained by standard techniques
- Fasting serum insulin: Assayed through use of a double-antibody immunochemi-luminometric method completed on an Access automated platform. Serum Insulin: Adult (Normal): 0.7-9.0 µU/ml, Diabetic (Type II): 0.7-25µU/ml. Fasting blood sugar: Assayed using fully automated clinical chemistry auto-analyzer system Konelab 60i (Thermo Electron Incorporation, Finland).
- Calculation of HOMA- IR: (Homeostatic Model Assessment of Insulin Resistance): Healthy Range: 1.0 (0.5–1.4). Less than 1.0 means optimal insulin-sensitivity. Above 1.9 indicates early insulin resistance. Above 2.9 indicates significant insulin resistance. HOMA-IR was calculated according to the formula: fasting insulin (micro/L) x fasting glucose (mmol/L)/22.5. Duplex study measurements on brachial artery: Parameters of vascular dysfunction [5].

The following parameters of vascular wall function were calculated

Brachial artery FMD, with the measurement of changes in internal brachial artery systolic diameter after 5 min of arm compression using a cuff at a pressure that is 30 mm/ Hg greater than systolic Bp; this was calculated according to the following formula: FMD (%) = 100% × (systolic diameter after arm ischemia – systolic diameter before arm ischemia)/

systolic diameter before arm ischemia. This parameter reflects endothelium-dependent vasodilatation;

Flow-mediated vasodilation (FMD)

Method of Flow Mediated Dilatation procedures have been described by Hamburg al., (2011) and Maruhashi, A Bp cuff is placed on the right arm proximal to the elbow and distal to the placement of the ultrasound Doppler probe on the brachial artery (BA). The BA is insonated midway between the antecubital and axillary regions, and measurements of BA diameter and blood velocity measurements will be obtained continuously at rest and for 2 minutes following cuff deflation. (FMD): is quantified as the maximal (%) change in BA diameter following cuff release. Shear rate is calculated as: Shear Rate (s−1) = Vmean • 8 /vessel diameter. Blood flow is calculated as: Blood flow (ml/min) = (Vmean•π• [Vessel Diameter/2]2•60). Reactive hyperemia is quantified as cumulative BA blood flow for two minutes (AUC) following cuff occlusion. Normalized FMD is calculated by dividing FMD (%) by the cumulative shear rate AUC at the time of peak BA vasodilation. ABI was calculated as a quotient of the systolic Bp measured at the ankle and in the upper arm, presented as the lower of two values from both sides.

Echocardiography

Was done to all patients and control groups with determination of intima-media thickness of aorta (IMT) and ejection fraction of left ventricle.

Bioethics

The study protocol was approved by the local Bioethical Committee, and the study was conducted in compliance with the Declaration of Helsinki for medical research. All study participants signed informed consent for participation in the study.

Statistical analysis

Statistical analysis was conducted using licensed versions of statistical software Statistica (a data analysis software system), Stat-Soft, Inc. (2015), version 12. The results were mainly presented as the mean ± standard deviation or n, %. The statistical significance of the differences between groups was verified using Student's t-test and the chi2 test. The statistical significance level was set at a p-value < 0.05. Pearson's correlations were determined [6-8]. Stepwise backward multiple regression (using the General Regression Models module of Statistica) was applied to evaluate the relationships between the values of the respective parameters of vascular wall function and the parameters of oxido-reductive balance.

RESULTS

Showed significant increase in waist circumference (P=0.02), DBP (P=0.04) and SBP (P=0.009) in patients' group than control (**Table 1**). Significant increase frequency of metabolic syndrome in patients' group (P=0.04) than in control. Metabolic syndrome was present in 30% of patients significantly than control (P=0.05). Highly significant decreased brachial volume blood flow (P=0.008), mean velocity (P= 0.001) and increase IMT of aorta (P=0.001) and LV diameter in diastole (P=0.034) in patients' group than control. Highly significantly decreased plasma insulin (P=0.009), plasma ascorbate, SOD, CAT and glutathione (P=0.001, each) than control, while showed highly significant increased plasma level of fasting sugar, T-Cholesterol, LDL and lipid peroxidation (P=0.001 each) than control. Urinary ACR was highly significantly also increased in patient' group (P=0.001) than control (**Table 2**). BMI (P=0.007), brachial artery diameter (P=0.001), EF% of LT ventricle (P=0.004), plasma ascorbate (P=0.001) highly significant correlation with FMD. Meanwhile; Brachial volume blood flow (P=0.046), Reactive hyperemia (P=0.026), IMT (P=0.021) and T-cholesterol (P=0.012) showed significant correlation with FMD. As regarding the simple linear regression analysis of variables predicting the FMD percent, the most predicting variable is FMD with R2 = 0.814 as it can explain 81.4% of the results, followed by brachial artery diameter R2 = 0.219. Otherwise, the other variables that predict the FMD percent has weak effect size (R2) EF, Vitamin C level, BMI, T-Ch level LDL level, aortic intimal -media thickness, reactive hyperemia and brachial volume blood flow respectively. Subjects' age, gender, and the values of Laboratory data were selected as the independent variables, and the above-listed parameters of vascular wall function were used as the dependent variables. Discussion Persistent hyperglycaemia in type-2 DM increases the production of reactive oxygen species (ROS), activates mediators of inflammation and suppresses antioxidant defense mechanisms, ultimately leads to endothelial dysfunction (ED) in diabetes. Furthermore, ROS, inflammation and fibrosis promote each other and are part of a vicious connection leading to development and progression of CVD and kidney disease in diabetes. In the current study; 35.8% of diabetic patients showed manifestations of peripheral ischemia and 34% of them showed diabetic retinopathy (P <0.001). Patients group showed metabolic syndrome in 30% of cases. Endothelial dysfunction (ED) is the initial key steps in atherosclero-genesis in diabetic subjects. Several risk factors, such as hypertension, dyslipidemia, inflammation, oxidative stress, and AGEs, are associated with micro- and macro-vasculopathies. The mechanism of (ED) in type 2-DM may be due to increased inactivation of endothelium-derived NO by oxygen-derived free radicals study, showed that vitamin C may be a potential prognostic indicator of diabetic microangiopathy. All patients with long-standing diabetes

showed the highest imbalance between the antioxidant status and increased concentrations of oxidative damage markers. In the current study; plasma levels of ascorbate, SOD, catalase and glutathione were highly significantly decreased, while plasma lipid peroxidation levels were highly significantly raised in patients' group than control (P= 0.01). Urinary ACR, plasma T- cholesterol and LDL were highly significantly raised in patients' group than control (P= 0.001). These results agreed with those of Chou & Tseng (2017) who showed that T2-DM patients with the highest Urinary ACR (≥ 300 mg/g) had the lowest levels of vitamin C. They found that vitamin C levels correlated negatively with serum creatinine, urine(2016) study found that vitamin C was significantly lower in patients with metabolic syndrome (MS) than in control group. In Type-2 DM, reduced serum antioxidant activity correlates with worsened glycemic control. Increased oxidative stress and low vitamin C levels were correlated with severity of diabetic neuropathy. SOD and vitamin C prevent the rapid inactivation of NO by superoxide anion found that (53.8 %) of diabetic patients had low levels of vitamin C concentration and there was a significant difference in vitamin C concentrations between diabetic patients with CV diseases and those without CV ones (p = 0.036). In the study of Zhao (2018); they found that serum SOD activity was an independent risk factor for diabetic retinopathy (P < 0.001) and was associated with the progression of its severity. Hence it might be a biomarker for diabetic retinopathy screening and evaluation of the clinical severity. This decrease in SOD activity may be resulted from glycation of the enzyme, in diabetes with poor glycemic control. A critical biomarker of oxidative stress and atherosclerosis is Lipid peroxidation. Lipid peroxidation of unsaturated fatty acids has been an index of increased oxidative stress and cytotoxicity. Lipid peroxidation correlated significantly with endothelial dysfunction and arterial stiffness in diabetic patients. Glycemic variability control, in addition to long-term glycaemic control, is important in diabetic patients to prevent micro- and macro-vasculopathies. While HbA1c measures the mean glycemic exposure during the preceding 2 to 3 months, it does not reflect the degree of glycemic variability (the frequency and magnitude of glucose excursions) that a patient may experience during a given day. Therefore, HbA1c % cannot be used to determine whether abnormal glycemic levels are primarily due to high fasting plasma glucose (FPG) levels or high post-prandial plasma glucose (PPG) levels. PPG is associated more with CVD than FPG. Monocyte adhesion to the endothelium leads to endothelial dysfunction and progression of atherosclerosis. Repetitive postprandial fluctuation in glucose concentration evokes monocyte adhesion to endothelial cells. Therefore, glycemic variability has been proposed as one of the risk factors for T2-DM patients. In the current study, fasting plasma insulin was highly significantly decreased in diabetic patients than control (P= 0.001) and did not correlate with FMD. Hyperinsulinemia may represent a novel mechanism for negatively regulating eNOS expression and help to explain for the T2-DM-related endothelial dysfunction at the transcriptional level [9]. Endothelial cells most abundantly express the insulin-independent glucose transporter type 1 (GLUT-1), which means that they cannot regulate the glucose uptake through insulin action. This probably makes the endothelial cell more susceptible to the effects of hyperglycemia than the SMCs in the circulation that can down-regulate GLUT-1 in response to increasing glucose concentrations. Compared with controls, Type-2 DM patients had significantly lipid peroxidation and Glut-1 expression whereas declined levels of plasma and cellular antioxidants. Correlation studies revealed positive association of peroxidation whereas negative association with glutathione and catalase, the hyperglycemia and hyper-oxidation that characterize diabetes lead to reduced vitamin C in diabetic patients with albuminuria [10].

Table 1 Demographic data and patient's characteristics comparative analysis

		Control	**Patients**	**P value**
		N0=25	**N0=60**	
Age by years	Range	(41-67)	(42-72)	0.551
	Mean ± SD	52.1±7.3	53.2±7.7	
Sex	Male	7(28%)	15(25%)	0.774
	Female	18(72%)	45(75%)	
BMI	Range	(22-46)	(18-51)	0.663
	Mean ± SD	30.2±5.6	30.9±7	
Waist circumference	Range	(75-130)	(76-155)	0.025*
	Mean ± SD	100.3±17.4	110.2±18.6	
Smoking	Yes	0(0%)	7(11.7%)	0.1
	No	25(100%)	53(88.3%)	

Anti-diabetic drugs	No		1(1.7%)	----------
	Oral	----------	32(53.3%)	
	insulin		16(26.7%)	
	Both		11(18.3%)	
History of peripheral ischemia	Yes	0(0%)	21(35%)	----------
	No	25(100%)	39(65%)	
History of angina attacks	Yes	0(0%)	7(11.7%)	----------
	No	25(100%)	53(88.3%)	
History of TIA	Yes		6(10%)	---------
	No		54(90%)	
History of retinopathy	Yes	0(0%)	18(30%)	----------
	No	25(100%)	42(70%)	
Duration of diabetes	Range	----------	(1-240)	-------
	Mean ± SD		74.2±64.8	
	Median		60	
Systolic Bp	Range	(110-180)	(90-190)	0.009*
	Mean ± SD	124.8±16.4	136.8±19.7	
Diastolic Bp	Range	(70-110)	(60-120)	0.042*
	Mean ± SD	81.2±8.8	86.2±10.6	
Metabolic syndrome		5/25 (20%)	20/60 (30%)	0.05*

Table 2 Simple linear regression analysis of variables that predict FMD percent

Variables	Unstandardized coefficients		P value	Adjusted R^2
	Constant	B		
Brachial volume blood flow	30.23	5.11	0.046*	0.051
Brachial artery diameter	59.75	-102.96	<0.001*	0.219
Intimal media thickness of the aorta	6.36	6.15	0.021*	0.072
FMD	0.26	283.39	<0.001*	0.814
Ejection fraction of left ventricle	95.02	-1.15	0.004*	0.118
Reactive hyperemia	149	0.07	0.026*	0.067
Plasma ascorbate level	-67.63	98	0.005*	0.115
Plasma TC level	40.13	-0.11	0.012*	0.088
Plasma LDL level	38.41	0.11	0.018*	0.078
BMI (Kg/Ht^2)	0.18	0.65	0.007*	0.104

In the current study; simple logistic analysis predicting ED in diabetic patients showed that increased FBS (P<0.001), T. cholesterol (P<0.003) and LDL (P<0.002) had highly significant predictive value in diagnosis of ED. Meanwhile; in simple discriminant functional analysis; plasma SOD Catalase, Glutathione and ascorbate showed (100%) diagnostic accuracy but lipid peroxidation and IMT showed diagnostic accuracies (89.7% and 74.4% respectively) for ED. Vitamin C protects from oxidative stress, prevents non-enzymatic glycosylation of proteins and enhances arterial dilation through its effect on NO release. It also decreases lipid peroxidation and alleviates inflammation. The anti-inflammatory property of vitamin C could be attributed to ability to modulate the NF-kB DNA binding activity and down-regulation in the hepatic mRNA expression for the interleukins and TNF. In endothelial cells, intracellular ascorbate can tighten the endothelial barrier by increasing intracellular (NO) and cyclic GMP. Another way; ascorbate protects the endothelium is by reducing pro-oxidant molecules. However, during inflammatory states including sepsis and critical illness, plasma ascorbate levels decline potentially leading to intracellular ascorbate depletion and increased vascular permeability. So, diabetic subjects may require higher

doses of Ascorbic acid than that recommended dietary allowance (RDA). Research confirmed that subjects with T2-DM after three months supplementation of vitamin C demonstrated significantly low level of hypertension, decrease levels of blood glucose, HbA1c% and increase SOD and GSH enzyme activity hence reduce insulin resistance by enhanced lowering oxidative stress parameters. Vitamin C disrupted glucose tolerance by attenuating up-stream hepatic insulin action through impairing the phosphorylation and activation of insulin receptor and its subsequent substrates. Moreover, vitamin C showed antioxidant capabilities [nuclear factor-erythroid-2-related factor 2 (Nrf2), total and reduced glutathione] and negative effect on Wnt signaling pathway [phosphorylated glycogen synthase kinase-3β (p-GSK-3β)], by altering the previously mentioned parameters, inevitably leading to severe reduction of (ROS) below the physiological levels. Chou & Tseng (2017) concluded that lipid peroxidation may play a role in diabetic nephropathy and various oxidative stress markers are directly associated with altered renal function in diabetic patients and cross-associated with each other, but the precise mechanism remains to be explained. Oxidative stress itself leads to the accumulation of altered proteins, lipids and nucleic acids that, in turn, damage tissues of major organ systems. The inflammatory response in the pathophysiology of diabetic nephropathy is contributed to by inflammatory mediators such as interleukin-1 (IL-1), IL-6, IL-18, and TNF-α, and macrophage chemotactic protein-1; however, the role of IL-18 seems to be more specific than other cytokines in the inflammatory process. IL-18 is expressed in renal tissue and is up regulated by several stimuli including hyperglycemia. The expression/urinary level of IL-18 are positively correlated with the progression of diabetic nephropathy and the urinary ACR. The glycemic control itself partially normalizes the redox state and well-controlled diabetes is associated with lower oxidative damage. Besides their glucose normalizing effects, drugs exhibit antioxidative effects and this can be considered as another protective property against diabetes-induced vascular complications. Inhibition of ROS production can be a potential target for diabetic nephropathy treatment.

RECOMMENDATION

Further prospective studies with larger samples of T2-DM patients with and without diabetic nephropathy are necessary to confirm the results of this study and to identify a marker (or combination of markers) able to predict the progression of type-2 DM angiopathies. The association between oxidative stress and diabetic angiopathies also requires further examination and prospective follow up.

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