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Research Article

Assessment of Serum Levels of Malondialdehyde, Antioxidant Vitamins (A, E, C), and Lipid profile in Sudanese With type 2 Diabetes Mellitus

Abd Elgadir A. Altoum[1]*, Isam M. Sadig[2]
[1]Lecturer, Department of Clinical Chemistry, University of Sciences and Technology.
[2]Associated Professor, Omdurman Islamic University, Sudan

***Corresponding author**
Abd Elgadir A. Altoum
Email: gadoora1977@live.com

Abstract: A cross sectional study conducted during the period from May 2013 to December 2015 to assessment the serum levels of malondialdehyde (as a marker of lipid per oxidation), antioxidant vitamins (A, E, C), and zinc in addition to lipid profile in Sudanese with type 2 diabetes mellitus compared to healthy volunteers. Three hundred of diabetic patients who attended ADC centre for routine follow up and a control group of 100 healthy subjects (non-diabetic). Blood specimens were collected from both groups, and plasma levels of Malondialdehyde, antioxidant vitamins (A, E, C), in addition to fasting plasma glucose and glycetaed hemoglobin (HbA_{1c}) were determined. Age and gender of the test group were matched with the control group. The results of the study indicate a significant raised in the means of the plasma levels of malondialdehyde, fasting plasma glucose, cholesterol, LDL, triglyceride, and HbA_{1c} of the test group when compared with healthy control group subjects, whereas the means of the plasma levels of antioxidant vitamins (A, E, C),HDL and zinc showed significant reduction when compared with that of control group In conclusion, the present study indicate that the plasma levels of MDA, antioxidant vitamins (A, E, C) and ,FPG, lipid profile and HbA_{1c} are important markers for evaluation of oxidative stress, antioxidant status, and glycemic control of diabetic patients respectively.
Keywords: Diabetes mellitus, Oxidative stress, Malondialdehyde, vitamin C, E, A.

INTRODUCTION

Reactive oxygen species (ROS), in particularly free radical induced lipid per oxidation, cause tissue damage that has been implicated in the pathogenesis of various diseases including diabetes [1, 2]. Diabetes mellitus is a disorder with many complications including, cardiovascular disease, nephropathy, retinopathy which affects severely the quality of life [3]. Diabetes mellitus is an important risk factor for atherosclerosis, and coronary- heart disease, is nowadays the most frequent cause of mortality in these patients [4]. One of the pathogenic mechanisms that can explain this increased risk in diabetes is the imbalance between oxidants and antioxidants, which results in oxidative stress (os) [5].

Hyperglycemia result in-glucose auto-oxidation, non enzymatic glycation and monocyte dysfunction, which lead to increased production of free radicals [6]. This is further aggravated by the decreased levels of antioxidants and leads to oxidative damage, illustrated by the high levels of lipid and DNA per oxidation products found in these patients" [7]. All these diabetes-related abnormalities can intensify the endothelial dysfunctions, oxidation of LDL and foam cell formation, which ultimately lead to atherorma plague [8, 9]. Although of these several complication mechanisms of diabetes, the patho physiology of these complications are still needed to be investigated. Recent reports indicate that free radicals have important roles in the pathogenesis of diabetes and a relationship between oxidative stress and secondary complications of diabetes exists [10, 11].

Subsequently, free radicals change lipid/protein ratio of membranes by affecting poly unsaturated fatty acids and lipid per oxidation causes functional irregularities of several cellular organelles [12, 13]. Lipid peroxides are disintegrated quickly and form reactive carbon compounds, among these, malondialdehyde (MDA) is an important reactive carbon compound which is used commonly as an indicator of lipid per oxidation, and has become one of widely reported analytes for the purpose of estimating oxidative stress effects on lipids [14, 15]. Since free radical production is increased whereas capacity of antioxidant system is reduced in diabetes, it has been proposed that diabetic patients may require more antioxidants compare to healthy individuals [16, 17]. Since effect of free radicals in diabetes is now

documented, it has been proposed to use antioxidant vitamins to block formation of free radicals and hence prevent development of diabetes complication [18, 19]. While superoxide radicals are cleaned by enzymatic dismutation, compounds known as antioxidants clean free radicals in organisms. Glutathione is a very important non enzymatic antioxidant together with antioxidants vitamins. Vitamin A, E and C are among these important non enzymatic antioxidants [20, 21]. It has been proposed that in diabetic patients several abnormalities related with absorption develop in the absence of antioxidants vitamins [22]. Vitamin A functions as catalyzer of removal of singlet oxygen and as a result vitamin A inhibits singlet oxygen dependant reactions [23, 24]. Vitamin C is also has a role in activating vitamin E when it loses its antioxidant capacity by turning it into tocopherol.

MATERIALS AND METHODS

Subjects:

a) Control subject:

One hundred (100) healthy subject were control group with mean FBS 5.61= m mol/L. The age ranged from 22 to 78 years old. The mean age average was 50.1years.

b) Type 2 diabetic patients:

Type 2 diabetic patients were 300, the ages ranged from 30 to 80 years old. The mean age average was 51.2 years. All samples were in a state of fasting for 12 hours before drawing blood were obtained on these samples Advanced Diagnostic Center in Bahri from the period May 2013 until August 2015.

MDA assay in serum:

MDA in serum performed as described by Muslih *et al.;* [25] In brief, serum was mixed with 20% TCA and allowed to stand for 10 minutes. After that 0.05m H2So4 and TBA were added. The mixture was mixed and place in 70 c° water bath for 30 min. The resulting chromogen was extracted with n-butanol and centrifuged at 2000 rpm / min, and measured against butanol blank at 532 nm excitation and 553 nm emissions by spectrophotometer.

Determination of vitamin C

Chromatography measurements were made using Hewllet-Packard (wald born Germany) model 1050 pump system, water 717 plus Auto Sampler (Mil Ford, MA, USA), auv-vis detector, SPD-10 AV VP (Shimadzu Kyoto, Japan) and an HP-3365 series II chemistation. The analytical column used was a tracer Spherisob OD52 C18 (250x4.6mm 1.D, 5µm particle size) protected with a guard cartridge (tracer, C18, 5µm), both from Tracer Analitica Technokroma, Barcelona, Spain. The frozen specimens preserved with meta phosphoric acid (5%), were thawed to around 22°C in water bath, protected from light, and then mixed. The acidified samples with 5% meta phophoric acid (iced at -80°C) were thawed to around 22°C in water bath, protected from light, mixed and then centrifuged at 10°C (10 min, 3000 xg). 50µl of filtrate sample was directly injected into the HPLC system. Also 50µl of vitamin C standard was directly injected into the HPLC system. Isocratic chromatographic separation was carried using mobile phase of methanol and water 95:5 (v/v) respectively. The eluent flow-rate was 0.7 ml/min and the column temperature was 25°C. The vitamin C was identified by comparing the retention time of the sample peak with that of the vitamin C standard at 245 nm [26].

Vitamin A, E assay

The antioxidant vitamins (A and E) were assayed by HPLC. 100µl of the sample (plasma) was added to 120µl of spectrograde hexane mixed vigorously on a vortex – type mixture for 45 seconds, until the bottom layer was thoroughly extracted. All contents were centrifuged (2200 rpm, for 5 min) for phase separation. 75µl of the upper hexane layer was transferred to 3 ml conical centrifuge tube. The solvent was evaporated under water stream in a 60°C water bath. Immediately the lipid residue was dissolved in a 25µl diethyl ether followed by 75µl methanol, then gently swirled and taped to enhance solubility of the lipid residue. 90µl of the solution was injected into the column. [27]. A standard curve was fashioned in which the ratio of peak heights (areas) was plotted against the retinol concentration in plasma. In the samples, the peak height (area) ratio was determined and the appropriate plasma retinol (vitamin A) and the appropriate plasma tocopherol (vitamin E) concentrations were determined from the standard curve (regression line formula, Vitamin A and E were detected at 290 nm.

Statistical analysis:

Statistical Package for Social Science SPSS (version 13) computer software was used for data analysis. The means and standard deviations of varible calculated and T-test was used for comparison (significant level was set at $P \leq 0.05$).

RESULTS

Results were Table (1) shows the baseline characteristics of the test group and control group. There is no significant difference in age between the two groups Age Mean±SD was 50.1±14.0 for the control group versus 51.2±11.1 for the test group (P = 0.06). Weight, height and BMI showed significant differences between the test and control group. Weight Mean±SD was 74.5±12.2 kg for control group and 79.7±22.8 kg for the test group (P = 0.032). Height Mean±SD was 171±10.0 cm for the control group and was 164±10.0 for the test group (P = 0.0006). Body Mass Index (BMI) Mean±SD was 25.2±3.2 for control group and 29.5±8.1 for test group (P = 0.0004). Table (2) shows significant increase cholesterol, LDL, triglyceride, and HbA_{1c} of the test group when compared with healthy control group subjects, whereas the means of the plasma levels of antioxidant vitamins

(A, E), HDL and zinc showed significant reduction when compared with that of control group in the means of the plasma levels of malondialdehyde, fasting plasma glucose, significant difference when compared with healthy subjects.

Table 1: Baseline characteristics of the respondents

Variables	Control (none-diabetics) (n=100)	Test Group (diabetics) (n=300)	P value
Age (years) (Max-Min)	50.1±14.0 (22.0-78.0)	51.2±11.1 (30.0-80.0)	0.06**
Weight (kg) (Max-Min)	74.5±12.2 (52.0-105.0)	79.7±22.8 (50.0-180.0)	0.032*
Height (m) (Max-Min)	1.71±0.1 (1.52-1.96)	1.1.64±0.1 (1.35-1.90)	0.0006*
BMI (w/h^2) (Max-Min)	25.2±3.2 (19.1-34.6)	29.5±8.1 (19.6-63.0)	0.0004*

* Significant differences in Weight, Height and BMI between control and test group (P value < 0.05). ** No significant differences.

Table 2: Comparison of the means of Blood Parameters between diabetics and none diabetics

Variables	None-diabetics (n=100)	Diabetics (n=300)	P value
Vitamin A (Max-Min)	81.2±21.8 (10.0-133.0)	50.3±20.0 (14.0-94.0)	0.0009*
Vitamin E (Max-Min)	15.6±4.8 (3.5-24.0)	5.2±1.8 (1.0-9.0)	0.0005*
MDA (Max-Min)	2.4±1.1 (1.0-12.0)	6.7±6.2 (1.0-35.0)	0.0002*
HbAc1% (Max-Min)	4.9±0.3 (4.2-5.5)	7.5±1.4 (6.0-13.3)	0.0007*
Triglycerides (Max-Min)	107.1±20.1 (60.0-150.0)	124.2±79.4 (18.0-497.0)	0.033*
Total Cholesterol (Max-Min)	117.3±20.9 80.0-165.0	164.8±46.0 (37.0-526.0)	0.0006*
LDL (Max-Min)	86.6±20.6 (47.0-133.0)	104.4±405 (20.0-290.0)	0.0003*
HDL (Max-Min)	51.9±6.2 (41.0-65.0)	41.8±11.9 (20.0-88.0)	0.0008*
FBS (Max-Min)	101.5±11.9 (70.0-120.0)	160.4±65.5 (75.0-480.0)	0.0002*

* Significant differences in all blood parameters between control and test group (P value < 0.05).

DISSCUSSION

To date, many investigations have focused on the antioxidant status and oxidative stress in diabetes mellitus. It has been shown that an increase in free radicals production in type2 diabetes mellitus occurs due to lipid per oxidation or non enzymatic glycosylation of proteins or antioxidation of glucose. Poor glycaemic control in type2 diabetes mellitus also has been associated with the depletion of protective serum antioxidant activity in these cases. The current study revealed weight and BMI were significantly higher among the test and control group than apparently healthy control group. Body mass index of diabetic subjects found to be higher than that of non-diabetics. The mean BMI of control group was found to be 25.2 kg/m^2, which is slightly above the normal range of recommended BMI by WHO for healthy individuals, on the other hand the mean of BMI of test group was found to be 29.5 kg/m^2, indicating overweight and this agree with the study among Nepalese subjects by Shah *et al*.[28] and also with study done in USA which found that obesity and overweight strongly correlated with diabetes mellitus among American subjects. In this study, the weight of test group significantly raised compared to weights of the control group and this agreed with the study of Shah [28] in Nepal and Jackson in the USA who reported significant increase of weight among diabetic subjects in contrast to non-diabetic subjects.

The current study shows highly significant decrease of plasma levels of vitamin A among test group compared to control group. Merzouk *et al.;* [28] investigated plasma vitamin A, C and E levels and erythrocyte antioxidant enzyme activities in type I and type II diabetic subjects with and without complications. Results of serum vitamin E in this study showed a highly significant difference between the

means of serum vitamin E of the test group and the control group. These results agreed with Karatas [29, 30], who found that vitamin E levels in blood, plasma and serum levels of patients with type 2 diabetes mellitus were contradictory. In addition another studies reported that there are increases in vitamin E levels compared to controls [32, 33]. The results of current study revealed that a highly significant difference between the means of plasma MDA of the test group and the control group was found. This agreed with study showed that [33, 34], there is significant increase in MDA levels among Diabetic patients in comparison to the controls. Chavan *et al.;* have also observed similar results among a study population from Gujarat [35] Rama Srivatsan et al.; have reported increased MDA levels in diabetics among a Southern Karnataka population [36]. The significant direct relationship between MDA and lipid profile was reported in previous studies [37]. Hyper triglyceridemia and hypercholesterolemia were associated with oxidative modification of LDL-C, protein glycation and glucose auto oxidation, thus leading to excess production of lipid per oxidation products which may cause elevation of oxidative stress in higher lipid and hyper lipidemic subjects [38]. Enhanced oxidative stress was indicated by increased free radicals production [39], lipid per oxidation and reduced antioxidant status [40]. Several studies have reported an increased susceptibility to lipid per oxidation in patients with diabetes mellitus [41]. The generation of free radicals may lead to lipid per oxidation and the formation of several types of damage in diabetes mellitus. Al-Rawi [42] observed that MDA levels, and lipid per oxidation product and a marker of oxidative stress, were elevated significantly in diabetic patients [43].

CONCLUSION

The present study indicate that the plasma levels of MDA, antioxidant vitamins (A, E, C) and zinc, FPG, lipid profile and HbA_{1c} are important markers for evaluation of oxidative stress, antioxidant status, and glycemic control of diabetic patients respectively. Accordingly, these indicators can be used as prognostic markers for prediction of oxidative stress, antioxidant stats, and glycemic control of diabetic patients especially in patients with complications such as hypertension, ischemic heart disease, lipid abnormalities and uncontrolled diabetes.

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