ABSTRACT

Objective: The objective of the current study is to compare the levels of oxidative stress markers malondialdehyde (MDA), zinc, and antioxidant Vitamins (A, E, and C) in ischemic heart disease (IHD) and non-IHD patients with diabetes mellitus.

Method: This is cross-sectional study group, conducted in the advanced diagnostic center (Khartoum- Sudan) from the period May 2013 to August 2015. Comprised 100 healthy subjects were, control group with mean (fasting blood sugar) 5.61=m mol/L, the age ranged from 22 to 78 years old, the average of the age was 50.1 years. 300 patients (78 IHD and 222 without) as cases groups, the ages ranged from 30 to 80 years. The age average was 51.2 years, all samples were in a state of fasting for 12 h, and the data were collected using a structured questionnaire and direct interview to collect information. Blood specimens were collected from both groups, and plasma levels of MDA, zinc, and antioxidant Vitamins (A, E, and C) were determined.

Results: There was a significant difference between the level of serum Vitamin A, E, Zinc and MDA in diabetic patients with IHD and those diabetic without IHD (p<0.05). No significant differences in serum Vitamin C level between both groups (p>0.05).

Conclusion: Due to the significant differences in serum Vitamin A, E, Zinc, and MDA between diabetics with IHD and diabetics without IHD, these parameters can be used as prognostic markers for prediction of oxidative stress and antioxidant stress of diabetic patients with IHD complications.

Keywords: Diabetes mellitus, Ischemic heart disease, Malondialdehyde, Zinc, Vitamin C, E, and A.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder manifested in the form of hyperglycemia as a consequence of a defect in insulin secretion and/or insulin action along with an imbalance in the metabolism of carbohydrates, fats, and proteins [1]. Hyperglycemia or raised blood sugar is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels [2]. Type two DM diabetes is a major risk factor for developing macrovascular complications, including atherosclerosis, myocardial infarction, stroke, and peripheral vascular disease. In adults with diabetes, the risk of death from heart disease and stroke is 2-4 times higher than in adults without diabetes’s [3]. Oxidative stress is closely associated with the pathogenesis of diabetes mellitus and results from overproduction of reactive oxygen species and overproduction is associated with hyperglycemia and metabolic disorders, such as impaired antioxidant function in conjunction with impaired antioxidant activity [4]. Apart from the traditional risk factors such as arterial hypertension, dyslipidemia, and obesity, hyperglycemia is an independent risk factor for the development of ischemic heart disease (IHD). The course of IHD in diabetic patients is usually more complicated [5]. Oxidative stress alters normal endothelial function, supporting pro-inflammatory, prothrombosis, proliferative, and vasoconstrictor mechanism that favors the development of atherosclerosis and vascular tissue injury. The pathogenesis of atherosclerosis involves damage to the capillary endothelium caused by various factors including oxidized low-density lipoprotein [6]. The aim of the current study was to evaluate and compare the levels of oxidative stress markers malondialdehyde (MDA), zinc, and antioxidant Vitamins (A, E, and C) in IHD and non-IHD patients with DM.

METHOD

This study was conducted in Khartoum state in the advanced diagnostic center from the period May 2013 to August 2015. 100 healthy subjects were control group with mean fasting blood sugar (FBS) 5.61=m mol/L. The age ranged from 22 to 78 years old. The age average was 50.1 years. Type 2 diabetic patients were 300 (78 IHD and 222 without), the ages ranged from 30 to 80 years old. The age average was 51.2 years. All samples were collected after 12 h fasting. MDA in serum performed by mixing with 20% trichloroacetic acid and incubated for 10 min. After that 0.05 ml of H2SO4 and thioarbituric acid 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 emission by spectrophotometer. The antioxidant Vitamins (A, C, E, and Zinc) were assayed by high-performance liquid chromatography. 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. The Vitamin C was identified by comparing the retention time of the sample peak with that of the Vitamin C standard at 245 nm. Zinc present in the sample is chelated by 5-Br-PAPS 2-(5-bromo-2-pyridyldiaz)-5-(N-propyl-N-sulfopropylamino)-phenol in the reagent. The formation of this complex is measured at a wavelength of 560 nm [7]. The study was approved by in advanced diagnostic center, in Khartoum. All samples were taken from the participants after they agreed and fill the questionnaire. Statistical Package for the Social Sciences (version 13) computer software was used for data analysis. The means and standard deviations of a variable calculated and t-test were used for comparison (significant level was set at ps0.05).
RESULTS

The general characteristics of the subjects (case and control) included in the study are listed in Table 1. There is no significant difference in age between the two groups as mean±standard deviation (SD) was 50±11.4 for the control group versus 51.2±11.1 for the test group (p=0.06). Weight and height showed significant differences between the test and control group. Weight Mean±SD was 74.5±12.2 kg for the 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 164±10.0 cm for the test group (p=0.0006). Shows a highly significant difference between the mean of test group (n=300) and the mean of control group (n=100) in FBS Mean±SD: (160±65.5) versus (101±52.1) mg/dl respectively (p=0.001), serum Vitamin A Mean±SD: (50.3±20.0) versus (81.2±21.8) µg/ml respectively (p = 0.001), serum vitamin E Mean±SD: (5.2±1.8) versus (4.6±1.0) µg/ml respectively (p = 0.001), serum vitamin C Mean±SD: (3.9±1.3) versus (4.5±2.4) µg/ml respectively (p = 0.0003) and plasma MDA Mean±SD: (6.7±6.2) versus (2.4±1.1) n mol/L respectively (p = 0.001) and serum zinc Mean±SD: (77.2±9.8) versus (100±12.9) µg/L, respectively (p = 0.001).

78 of the test group (26%) have IHD, whereas 222 of them 74% have no heart disease. A significant difference according to the means of serum Vitamin A of the test group with IHD (n=78) and those without IHD (n=222). Mean±SD: 45.8±21.6 versus 51.9±19.2 µg/ml, respectively (p = 0.002). A significant difference between the means of serum Vitamin E in the test group with IHD (n=78) and those without IHD (n=222). Mean±SD: 4.8±1.8 versus 5.3±1.8 µg/ml, respectively (p = 0.023). Insignificant difference between the means of serum MDA of the test group with IHD (n=78) and those without IHD (n=222). Mean±SD: 12.2±8.5 versus 6.7±6.2 µmol/L, respectively (p = 0.04). A significant difference between the means of serum zinc of the test group with IHD (n=78) and those without IHD (n=222). Mean±SD: 75.4±10.3 versus 77.9±9.5 µg/L, respectively (p=0.05) (Table 2).

**DISCUSSION**

In the present study, we have determined the main oxidative stress markers among diabetic patients with IHD complication and non-IHD. Our results showed that serum MDA is a highly significant difference between the means of serum MDA of patients with IHD and the patient’s group without heart disease. This concept is analogous to that described by Shabana and Steve, observed significantly different blood levels of MDA among patients with chronic heart disease which were indicating overall oxidative stress in those patients [8]. This result also is consistent with another study conducted by Khan, and Abdul Baseer reported that the elevated serum levels of MDA indicate an increase in the level of production of oxygen free radicals, suggesting their possible role in atherogenesis, leading to coronary heart disease [9,10]. The results of the study also indicated a highly significant increase in the levels of serum antioxidant Vitamins A and E of patients with IHD and the patient’s group without heart disease. Our findings are in agreement with those of other authors who showed serum Vitamin A and Vitamin E levels in patients with chronic heart disease were found to be lower than without heart disease [11-13]. On the other hand, some reports indicate that no significant changes occur in patients with heart disease when compare to patients without heart disease [14,15]. Reduced levels of Vitamin E may also be due to the increased requirement of Vitamin E in pro-oxidant milieu with enhanced free radical status, leading to the increased lipid peroxidation, a resultant depletion of free radical scavenger, and antioxidant reserves of the body [16]. The findings showed that there is no significant change between the means of serum Vitamin C in the test group with IHD and those without IHD. Our finding supported by some cohort studies and randomized trials have shown no relationship between Vitamin C intake and heart disease risk [17,18]. The results disagreed with many studies reported that **Table 1**: Baseline characteristics of the respondents and comparison of the means of blood parameters between diabetics and none diabetics

| Variables      | Control (none-diabetics) (n=100) | Test group (diabetics) (n=300) | p     |
|----------------|----------------------------------|---------------------------------|-------|
| Age [years]    | 50.1±14.0                        | 51.2±11.1                       | 0.06**|
| Weight [kg]    | (22.0–78.0)                      | (23.0–80.0)                     |       |
| Height [m]     | 171±10.0                         | 164±10.0                        | 0.006*|
| FBS (mg/dl)    | (152–196)                        | (135–190)                       |       |
| Vitamin A [µg/ml] | 81.2±21.8                       | 50.3±20.0                       | 0.001*|
| Vitamin E [µg/ml] | 10.0–133.0                | 14.0–95.0                       |       |
| Vitamin C [µg/ml] | 6.7±6.2                        | 4.0±1.3                         | 0.023*|
| MDA [µg/ml]    | 2.4±1.1                          | 1.0–12.0                        | 0.001*|
| Serum zinc [µg/L] | (100±12.9)                    | (77.2±9.8)                      |       |

*Significant differences in weight, height, serum Vitamin A, C, E, FBS, Zinc, and MDA between control and test group (p<0.05). **No significant differences in age.

MDA: Malondialdehyde, FBS: Fasting blood sugar

**Table 2**: Comparison of blood parameters among test group according to IHD

| Variables | IHD N (78) | Non-IHD N (222) | p     |
|-----------|------------|-----------------|-------|
| FBS [mg/dl] | 159.0±54.2 | 160.9±69.2 | 0.822**|
| Vitamin A [µg/ml] | 45.8±21.6     | 51.9±19.2 | 0.022* |
| Vitamin E [µg/ml] | 4.8±1.8       | 5.3±1.8   | 0.023* |
| Vitamin C [µg/ml] | 3.8±1.3       | 4.0±1.3   | 0.234**|
| MDA [µg/ml] | 12.2±8.5    | 4.8±3.6     | 0.04* |
| Zinc [µg/ml] | 75.4±10.3    | 77.9±9.5   | 0.05*  |
| (Max-Min) | (55.0–95.0) | (55.0–95.0) |       |

*Significant differences in Serum Vitamin A and E, Zinc, MDA between diabetics with ischemic heart disease and diabetics without ischemic heart disease (p<0.05). **No significant differences in Serum Vitamin C and FBS (p>0.05). MDA: Malondialdehyde, FBS: Fasting blood sugar, IHD: Ischemic heart disease
CONCLUSION
This study shows that antioxidant status is impaired in diabetic patients with IHD compared to diabetic patients without IHD. Significant differences in serum Vitamin A, E, Zinc, and MDA between diabetic patients with IHD and diabetics without IHD can be used (A, E, Zinc, and MDA) as prognostic markers for prediction of oxidative stress and antioxidant stress of diabetic patients with IHD complications.

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AUTHOR’S CONTRIBUTION
There are no conflicts of interests between authors; all three authors have made substantial contributions to conception and design, analysis, and interpretation of data. All authors have participated in drafting and revising the article and have given their final approval for submission.

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