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Evaluation of Malondialdehyde (MDA) in type 2 diabetic Patients with Coronary Artery Disease (CAD)

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ABSTRACT

In this study, we evaluate Malondialdehyde plasma in type two diabetic Patients with Coronary Artery Disease compared with type 2 diabetic patients. This case - control study was conducted using random sampling. Study was carried out on blood from samples of 30 type 2 diabetic patients and 30 diabetic patients with CAD of age and sex matched subjects. Heparinized blood samples were taken from the patients. The separated plasma was used for biochemical tests and lipid peroxidation. Malondialdehyde level were significantly (P = 0.000) higher in type 2 diabetic Patients with Coronary Artery Disease compared with type 2 diabetic patients. The significant difference in the increase of MDA in diabetic patients with CAD may play a significant role in the development of dangerous complications in these patients because increase in the Malondialdehyde levels and decrease in antioxidant defense system in the body can lead to cell damage.

Key words: Malondialdehyde, Coronary Artery Disease, type 2 diabetic, lipid peroxidation

1. INTRODUCTION

The purpose of this study is to research the oxidative stress indicated by plasma Malondialdehyde (MDA) associated with the prevalence of Coronary Artery Disease (CAD) after controlling the effects of confounding factors. Antibodies to Malondialdehyde (MDA) adducts macromolecules have been detected in the serum of patients with Coronary Artery Disease (CAD) and linked with the progression of this disease. Researches have shown that MDA is formed because of lipid peroxidation and its ability to be connected to various macromolecules. Proteins/lipoproteins have been in communication with the development and progression of atherosclerotic disease, these modified proteins/lipoproteins have been found in the flow and atherosclerotic waste of patients with atherosclerotic disease. Also there are a number of risk factors that affect the development progression of atherosclerosis which include; age, gender, obesity, hypertension, diabetes mellitus and serum cholesterol. Free radicals are molecules that are chemically very active and the production of these radicals is a natural process in the cell metabolism of the body. These radicals may be generated in the course of diseases such as diabetes, which are removed from the body through a combination of antioxidants. Important known antioxidant enzymes include superoxide dismutase, glutathione peroxidase and catalase. Unstable compounds of free radicals on lipid and carbohydrate affect cells and proteins and among these, fats have the most sensitivity compared to free radicals that leads to oxidative damage

and the effect of these radicals is normally neutralized by the antioxidant immune system. Indeed Oxidative stress and free radicals are factors that can influence the pathogenesis of diabetes and waste seems that these compounds have a role in the destruction of beta cells. The defensive effect of antioxidant is created by a series of enzymatic and non-enzymatic antioxidant Glutathione vitamin C. Increased production of free radicals or reduction of antioxidant levels may damage cellular oxidation of polyunsaturated fatty acids with polyunsaturated fatty acids in cell membrane structure and is also known as lipid peroxidation. Oxidation damage starts, it starts like a chain, and Malondialdehyde is produced. This situation may eventually cause cell death associated with massive disease symptoms. Imbalance between antioxidant defenses and production increase of free radicals can lead to situations called oxidative stress. Oxidative stress is created due to increases in production of reactive oxidants such as oxygen, a situation that may cause cell damage and may play a role in the incidence of certain diseases. Studies have shown that diabetes complications may be partly related to oxidative stress. Diabetes is a major disease in developed countries. The mortality rate shows an increase in patients with type 2 diabetes compared to healthy people, particularly in relation to cardiovascular diseases. The possible cause of this condition is the existence of oxidative stress in these patients which acts as as a contributing factor to heart disease-cardiovascular, kidney, and eye diseases. There are conflicting results in connection with the changes in lipid peroxidation levels in patients with type 2 diabetes so that some of them show an increase. Other studies have shown reduction. The present study examined the changes in plasma lipid peroxidation (expressed as of MDA) type 2 diabetic patients who had referred to diabetes clinic of Yazd and its comparison with the control group so that these changes are investigated in type 2 diabetic patients.

2. MATERIALS AND METHODS

This case-control study was conducted using random sampling. Sample and control cases were matched in terms of sex and age and selected for the study. Diabetic patients having cardiovascular disease were the focus of the study. Cardiovascular patients taking a statin drug. As a result, these patients have reduced Triglyceride and Cholesterol particles. Heparinized blood samples were obtained from patients. Prepared plasma was isolated from blood samples with the help of red blood cells by centrifuging (3000-rpm min to 10 min). The isolated plasma was used for biochemical tests (FBS, TG, CHO, HDL-T, LDL _T and A1C) (Table 1) and lipid peroxidation (which is expressed as Malondialdehyde). Measurement of Malondialdehyde (MDA) is done under acidic condition and temperature of 95 ° C. A molecule of MDA and reacts with two molecules of thiobarbituric acid and produce a pink complex. The measurement of Malondialdehyde was done by thiobarbituric. To perform the MDA test, initially add 10µm hydroxyl toluene with 100 µm, and then add 500µm trichloroacetic acid and centrifuge around 300 for 10 minutes. Remove 500µm liquid and mix with 400µm Thiobarbituric acid and place it in water bath at 95 ° C for 1 hour and keep it in the refrigerator temperature for 15 min and centrifuge for 10min and at 4000 RPM and read in 532nm and 573nm wavelength and calculate the differences of two readings absorbance. In this study, we have achieved wavelength by using spectrophotometer Model EPOCH-Bio Tek. We used T. Test was used to analyze the data. Values of P <0/05 was considered significant. MDA concentration according to the following Chart 1. Inhibition = (OD1 – OD2) – 0.0112 / 0.0137

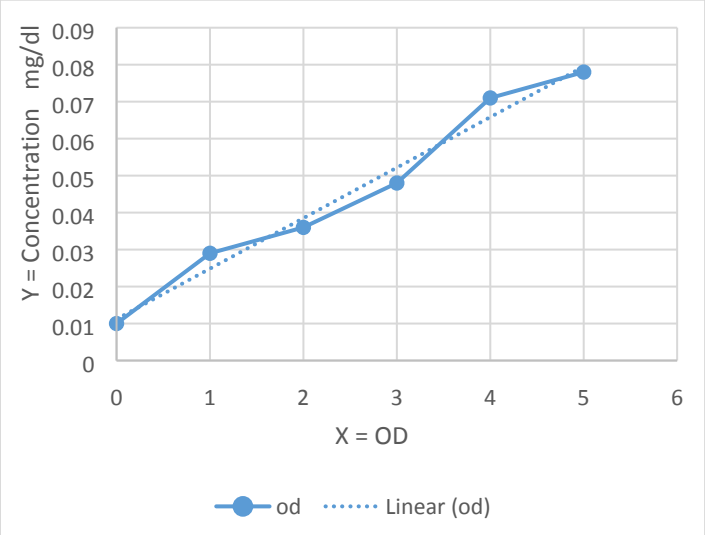

Chart 1. MDA standard concentration curve

Table 1. Clinical characteristics of studied subjects

P.Val	*Mean±SD in Diabetic*	*Mean±SD in CAD*	*Row*
0.8	209.2 ± 9.8 mmol/L	212.6 ±51.3 mmol/L	F.B.S
0.1	27.5 ± 0.3 kg/m2	26.7 ± 0.3 kg/m2	B.M .I
0.0 ***	252.4 ± 64.8 mmol/L	175.0 ± 26.9 mmol/L	Cho
0.09	35.7 ± 1.0 mmol/L	38.1 ± 0.8 mmol/L	HDL – C
0.0 ***	161.5 ± 9.7 mmol/L	93.9 ± 3.9 mmol/L	LDL – C
0.3	9.5 ± 0.1 mmol/L	9.2 ± 0.2 mmol/L	A1C
0.9	62.2 ± 06 years	62.1 ± 0.6 years	Age
0.0 ***	284.2 ± 14.2 mmol/L	213.9 ± 7.2 mmol/L	T.G
0.3	8.2 ± 0.3 years	8.6 ± 0.2 years	Duration of illness

Results are expressed as mean ± SD. Statistical significance was achieved if P values were less than 0.05. All statistical analysis was performed using the SPSS (version 18) independent – samples T. Test.

3. RESULTS AND DISCUSSION

We observed a significant (P=0.000) increase in level of Malondialdehyde (MDA) Thiobarbituric acid (TBARS) method in type 2 diabetic patients with Coronary Artery Disease (CAD) in comparison to type 2 diabetic patients control group (0.452 ± 0.270 vs. 0.405 ± 0.261) (Chart 2)

(Table 2).

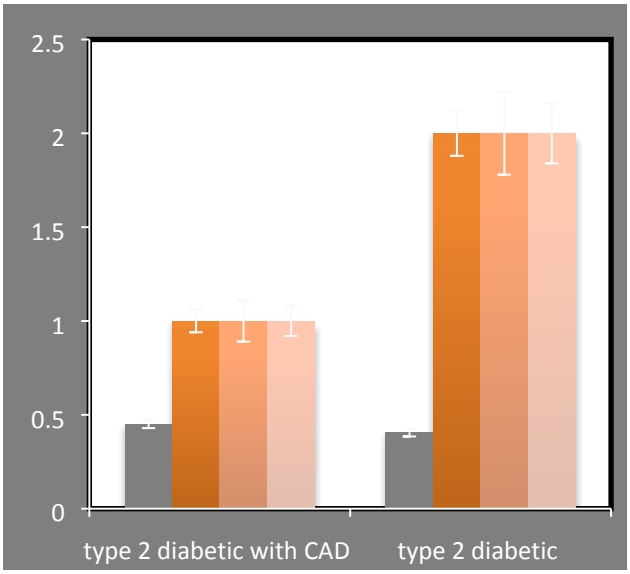

Chart 2. MDA level in type 2 diabetic with CAD patients and type 2 diabetic patients. *P<0.05. The value is mean±SD

Table 2. Lipid peroxidation

P.vale	mean±SD type 2 diabetic	mean±SD type 2 diabetic with CAD	
P=0.000	0.405 ± 0.261	0.452 ± 0.270	Malondialdehyde (MDA)

Our data further support the MDA involved in diabetic and Coronary Artery Disease (CAD). According to previous studies and the current findings, it is considered that the high concentration of Malondialdehyde in serum is a risk factor for CAD patients. Considering that in our study, serum MDA concentrations were significantly higher in patients with CAD than diabetics were, Malondialdehyde can play a role as an important factor in atherosclerosis in the population of our study. The present study showed that the significant difference in lipid peroxidation increase might be an optimal factor in development of serious secondary complications in diabetic patients. Changes of proteins or lipoproteins have many adverse effects in a number of diseases including atherosclerosis and diabetic. Plasma protein changes result in their binding to scavenger receptors, reaction of pro-inflammatory cytokines and become immunogenic, elicit autoantibody formation and the generation of T cells responses. MDA have long been thought to play an active role in the onset and/or progression of atherosclerosis. Regulating blood sugar is probably a very important factor in decreasing lipid peroxidation in patients with type 2 diabetes. Preventing the formation of lipid peroxidation and may help to delay the progression of diabetes complications. Lipoprotein abnormalities in lipid metabolism and atherosclerosis were evaluated more than other risk factors and their role has been proved. It has been demonstrated that serum Malondialdehyde is an independent risk factor for coronary heart diseases. Studies Kavocas, Dincer, kostner Mc Murray has also confirmed increase in the level of Malondialdehyde in patients with coronary artery disease. Evaluation of the rate of ion oxidase lipids, lipid oxidation determination to determine their sensitivity to oxidation and the amount of products obtained from these processes are among the methods of determining the level of oxidation lipids, which is one of the MDA products. Disorder of lipoprotein and lipid metabolism than other risk factors for atherosclerosis and its role has been investigated. Several studies have shown a significant effect on LDL oxidation in atherosclerosis is exacerbated. According to previous studies and the current findings is a perception that the silt concentration of serum Malondialdehyde is a risk factor for coronary artery disease. Considering that in our study, serum Malondialdehyde concentrations in patients with coronary artery disease was significantly higher than controls, In our study population, as well as Malondialdehyde (MDA) as an important factor in atherosclerosis may play a role.

4. CONCLUSION

The present study showed that the changes of proteins or lipoproteins have many adverse effects in a number of diseases including atherosclerosis and diabetes considering that in our study too. The significant difference in the increase of MDA in diabetic patients with CAD may play a significant role in the development of dangerous complications in these patients because increase in the Malondialdehyde levels and decrease in antioxidant defense system in the body can lead to cell damage.

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AUTHORS CONTRIBUTION

This work was carried out in collaboration between all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

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