Lipid peroxidation and antioxidant enzymes activity in controlled and uncontrolled Type 2 diabetic patients

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Original Article

Abstract

BACKGROUND: This study was designed to compare lipid peroxidation and antioxidant enzymes activity in Type 2 diabetes patients with good or weak glycemic control.

METHODS: In this case-control study, 62 Type 2 diabetic patients with glycated hemoglobin (HbA1c) between 6 and 8 were enrolled as the controlled group and 55 patients with HbA1c > 8 were selected as an uncontrolled group. Patients were all referred to Iranian Diabetes Association in Tehran, Iran, from 2010 onward. Groups were chosen by convenience sampling and were matched based on age, sex and duration of disease. Demographic questionnaire, two 24-hour food recall, HbA1c, insulin, malondialdehyde (MDA), superoxide dismutase (SOD), and catalase were measured in blood samples. Data were analyzed by Food Processor II and SPSS software.

RESULTS: A mean daily consumption of energy, carbohydrate, protein, and fat was not significantly different between two groups. MDA in the uncontrolled group was significantly higher than controlled group (2.03 ± 0.88 vs. 1.65 ± 1.01 nmol/ml; P = 0.030). A mean SOD was slightly higher in the uncontrolled group comparing to the control group (843.3 ± 101.9 vs. 828.0 ± 127.3 U/g Hb; P = 0.400).

CONCLUSION: These data suggest that MDA as a lipid peroxidation indicator is higher in uncontrolled diabetes probably due to chronic high blood sugar followed by higher oxidative stress.

Keywords: Antioxidant, Lipid Peroxidation, Diabetes Mellitus

Introduction

Diabetes mellitus, one of the most common endocrine disorders, is known as high fasting blood sugar (FBS) more than 126 mg/dl threshold.[1] Hyperglycemia is a major factor in the development of diabetic complications.[2-4] However, the mechanism by which these changes occur is not clear. Glycosylated hemoglobin (HbA1c), which reflects long-term (2-3 months) control of blood glucose, is more reliable and valid compared with the FBS.[5]

HbA1c < 7% is considered as well diabetes control according to the American Diabetes Association. The association has advised physicians and patients that when the HbA1c levels exceed 8% may be related to developing of diabetes micro- and macro-vascular complications,[6] and additional medical therapies are needed.[7] Hence, the HbA1c levels of 8% were considered in this study as cut-off point for allocating patients into two groups (controlled and uncontrolled). Chronically high blood sugar

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can be seen in patients with diabetes, and this may increase glycosylation and peroxidation processes which may lead to oxidative stress and resulting changes in structure and function of proteins and lipids.[8] In comparison with other cells, red blood cells are more prone to oxidative damage due to the high levels of iron and poly-unsaturated fatty acids and also their role in oxygen transportation.[9] Lipid hydroperoxides are degraded into the harmful aldehydes, such as malondialdehyde (MDA), in the presence of iron and copper ions. Therefore, MDA is considered as an indicator of lipid peroxidation and its value is used as indirect method of measuring free radicals.[10]

Superoxide dismutase (SOD) and catalase are important antioxidant enzymes which play a role in oxidant defense of body and often as the first line of defense against oxidative crisis.[11]

Some studies have previously evaluated the status of MDA and antioxidant enzymes in diabetic and healthy individuals.[12-14] However, this study was designed and performed for the first time to compare lipid peroxidation and antioxidant enzymes in Type 2 diabetes patients with good or weak control.

Materials and Methods

In this study, 62 Type 2 diabetic patients with HbA1c between 6 and 8 were enrolled as the controlled group and 55 patients with HbA1c > 8 were selected as uncontrolled group. Patients were all referred to Iranian Diabetes Association in Tehran, Iran, from 2010 onward. Groups were chosen by convenience sampling and were matched based on age, sex and duration of disease. A sample size of at least 50 patients in each group was estimated according to previous reports on leptin and adiponectin with the assumption of 80% power, and a 0.05 level of significance. The level of body mass index (BMI) was not different between groups. Inclusion criteria included fasting blood glucose above 126 mg/dl, HbA1c levels more than 6, the minimum age of 40 years, at least 3 years of diagnosed diabetes, consumption of oral hypoglycemic drugs, and consent for cooperation in this research study. Meanwhile, the exclusion criteria

were insulin injections, supplementation with vitamins A, C, E and omega-3, history of hepatic, renal, cardiovascular, thyroid, or respiratory diseases. All the process was in the patient privacy and in accordance with Declaration of Helsinki-Ethical Principles for Medical Research.

Demographic data, including age, sex, and duration of diabetes, were recorded. Weight was measured using a digital scale (803, Seca Clara, Germany) with an accuracy of 100 g, in light clothes and without shoes. Height was measured without shoes using a stadiometer (206, Seca, Germany) with an accuracy of 0.1 cm. Hip and waist circumferences were measured using a measuring tape (201, Seca, Germany) with an accuracy of 0.1 cm. BMI was calculated using these recorded values [weight (kg)/height^2 (m)]. In addition, two 24-hour food recall questionnaires were completed by an expert nutritionist. Then, the average daily intakes of calorie, protein, carbohydrate, and fat were calculated. After recording the necessary information and before taking oral hypoglycemic drugs, 10 ml of venous blood was taken in 12-hours fasting state by a laboratory expert. Samples were centrifuged in 3000 rpm for 10 minutes. Blood glucose, HbA1c, insulin, MDA, SOD, and catalase were then measured in blood samples. HbA1c was measured by an ion exchange chromatography using a NycoCard® Reader II instrument (Catalog ref 1042184, Axis-Shield poC AS, Oslo, Norway) on ethylenediaminetetraacetic acid anticoagulated samples; insulin was measured using insulin kits and serum MDA values were determined by the spectrophotometric method described by Satoh using thiobarbituric acid. This measurement is based on the reaction of MDA with thiobarbituric acid to form 1:2 adduct, which has a stable pink color that absorbs maximally at 532 nm.[15] Measurements of SOD activity in erythrocytes were done spectrophotometrically using Randox Kit (Cat # SD125, Crumlin, UK). Catalase activity was determined using spectrophotometric method and measuring the decrease in hydrogen peroxide absorbance at 240 nm wavelength.[16]

Table 1. Demographic characteristics and hematological indices in controlled and non-controlled Type 2 diabetes groups

| Variable | Group | | Independent sample t-test |
| | Non-controlled Type 2 diabetes (n = 55) | Controlled Type 2 diabetes (n = 62) | |
	Mean ± SD	Mean ± SD	P
Age (year)	54.27 ± 7.53	56.82 ± 7.61	0.080
Weight (kg)	74.16 ± 10.94	72.34 ± 10.61	0.360
BMI (kg/m^2)	29.49 ± 4.34	27.65 ± 3.89	0.080
Duration of diabetes (year)	10.89 ± 6.41	9.94 ± 6.75	0.430
Insulin (μU/ml)	13.53 ± 9.74	8.76 ± 7.17	0.510
Insulin resistance	6.96 ± 5.67	2.95 ± 2.64	< 0.001
FBS (mg/dl)	214.36 ± 68.22	136.15 ± 40.59	< 0.001
HbA1c (%)	8.88 ± 0.64	7.09 ± 0.58	< 0.001

The controlled group (62 patients with 6 < HbA1c ≤ 8) and uncontrolled group (55 patients with HbA1c > 8); BMI: Body mass index; FBS: Fasting blood sugar; HbA1c: Glycated hemoglobin; SD: Standard deviation

A SPSS software for Windows (version 13.0, SPSS Inc., Chicago, IL, USA) was used for statistical analysis of data. After normalizing the distribution of data by log transformation, independent t-test was used for comparing the means of quantitative variables in two groups. Furthermore, the nutritional data were analyzed by Food Processor FP II (version 2, Esha Research, salem, OR). P = 0.050 or less were considered as the statistically significant difference.

Results

In this study, 117 non-insulin dependent Type 2 diabetic patients of both sexes that had passed at least 3 years of diabetes onset from Iran Diabetes Association were involved. Participants were divided into the well-controlled (62 patients: 27 female, 35 male) and uncontrolled (55 patients: 34 female, 21 male) group. Of all participants, 52.1% (61 patients) were female and 47.9% (56 patients) were male. Patients' characteristics are shown in table 1. Mean MDA, catalase, and SOD values in the two groups are given in table 2. The table 2 indicates that MDA in the uncontrolled diabetes group was

significantly higher than controlled diabetes group (P = 0.030). Mean SOD was slightly higher in the uncontrolled group comparing to the control group but was not statistically significant (P = 0.480). Catalase was not much different between the two groups (P = 0.940). According to table 3, the most important variable affecting the level of catalase is protein intake. However, no variable had statistically significant effect on the level of SOD. Mean daily consumption of energy, carbohydrate, protein, and fat was not significantly different between well controlled and uncontrolled diabetes groups.

Discussion

Results obtained in this study suggest that MDA in uncontrolled diabetes group was significantly higher than the control group. In some studies, elevated HbA1c in patients with diabetes was associated with increased lipid peroxidation, while such a relationship has not been seen in some other studies.[17-19] Ahmed et al.[20] have been found that high blood glucose levels lead to increased oxidative stress, and consequently, MDA levels may increase.

Table 2. Comparison of mean malondialdehyde (MDA), catalase and superoxide dismutase between controlled and non-controlled Type 2 diabetes groups

| Variable | Group | | Independent sample t-test |
| | Non-controlled Type 2 diabetes (n = 55) | Controlled Type 2 diabetes (n = 62) | |
	Mean ± SD	Mean ± SD	P
MDA (nmol/ml)	2.01 ± 0.88	1.63 ± 1.01	0.030
Catalase (k/gHb)	205.12 ± 47.25	206.11 ± 80.49	0.940
SOD (U/gHb)	843.30 ± 101.90	828.00 ± 127.30	0.480

MDA: Malondialdehyde; SOD: Superoxide dismutase, Hb: Hemoglobin; SD: Standard deviation

Table 3. Comparison of mean energy, quantity and percent of carbohydrate, protein and fat intakes between controlled and non-controlled Type 2 diabetes groups

Variable	Group		Independent sample t-test
	Non-controlled Type 2 diabetes (n = 55)	Controlled Type 2 diabetes (n = 62)	
	Mean ± SD	Mean ± SD	P
Energy (kcal)	1330.22 ± 442.37	1416.21 ± 447.26	0.300
Carbohydrate (g)	196.37 ± 72.62	211.96 ± 80.02	0.270
Carbohydrate (%)	58.88 ± 8.16	59.56 ± 8.39	0.660
Protein (g)	53.08 ± 17.74	59.03 ± 19.72	0.090
Protein (%)	16.23 ± 3.12	16.97 ± 3.82	0.260
Fat (g)	40.71 ± 19.92	41.37 ± 17.16	0.850
Fat (%)	27.48 ± 8.72	26.39 ± 7.07	0.460

SD: Standard deviation

Seghrouchni et al.[21] have found that patients with Type 2 diabetes had a higher thiobarbituric acid reactive substances value than those with Type 1 diabetes. They have claimed that patients with Type 2 diabetes are more extendedly exposed to oxidative stress. In this study, the rate of catalase activity was not much different in the two groups (206.11 ± 80.49 vs. 205.12 ± 45.25). In Ahmed et al.[20] study the activity of catalase and SOD were significantly higher in the diabetic group than the healthy group.

Colak et al.[22] showed that anti-oxidative defense reduces in Type 2 diabetics, which negatively correlates with glucose concentrations and duration of diabetes and cardiovascular complications. In another study aiming to evaluate the effect of blood glucose control on catalase, catalase levels in patients with diabetes (HbA1c more than 8%), did not differ from healthy controls. However, after 3 months of hypoglycemic drugs consumption, catalase levels in patients with diabetes was less than the control group.[20]

In this study, subjects in both groups (controlled and uncontrolled diabetes) have been taking hypoglycemic agents (metformin or glibenclamide) and the strong sweeper effects of these drugs may have diminished the difference of catalase amount between the two groups.

Increase of free radicals in diabetes may increase antioxidant enzyme activities. In addition, high blood glucose can combine with the protein enzymes so that in patients with diabetes extracellular SOD is highly glycosylated comparing to healthy subjects.[23] Although, in some studies antioxidant enzymes such as SOD were inversely correlated with HbA1c, SOD in the present study was directly correlated with the level of FBS, HbA1c, and insulin resistance. However, these correlations were not statistically significant. In some other studies, no correlation was seen between SOD, FBS, and HbA1c. In present study higher SOD in uncontrolled diabetes group was an indicator of higher oxidative stress.

Other studies have evaluated the antioxidant status and SOD activity in patients with diabetes compared with healthy controls, but they did not found any significant differences between groups in terms of SOD activity.[22]

Furthermore, no significant relationship was observed between nutritional factors, MDA and SOD. It was previously reported that intake of antioxidant supplements can reduce reactive oxygen species and free radicals and may result in reducing lipid peroxidation.[24] In this study, vitamin supplements-such as vitamin A, C, E and omega-3 consumption-were among our exclusion criteria, so we had removed the effect of these confounding factors on the measurements and study results.[25] In this study, a mean percentage of protein intake was about 17% in both groups. The protein intake was the only nutritional factor which has affected the catalase level. The equal amount of protein intake in both groups was possibly one of the reasons that catalase was not so different between groups. This study encountered with a limited budget and sample

size. In future study, we can examine this variable with large sample size or with more sensitive indicator in diabetic patients.

Conclusion

MDA (lipid peroxidation indicator) is higher in uncontrolled diabetes (HbA1c > 8) probably due to chronic high blood sugar, followed by long and high oxidative stress. Furthermore, SOD is also higher in uncontrolled diabetes which might be an indicator of greater vascular damage in this group.

Acknowledgments

Conflict of Interests

Authors have no conflict of interests.

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