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Original Article

The effect of glycemic control on salivary lipid peroxidation in type II diabetic patients

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ABSTRACT

Background and objective: Hyperglycemia and some disturbance in antioxidant system lead to free radicals production and oxidative stress. Assessment of some products of oxidative stress could be effective in evaluation of diabetic control. This study aimed at evaluation of glycemic control on salivary lipid peroxidation in diabetic patients.

Methods: This case control study has been done on 44 diabetic (type II) and 44 healthy subjects. Unstimulated saliva was collected and correlation between malondialdehyd (MDA) as an end-product of lipid peroxidation and HbA1c was assessed.

Results: MDA and HbA1c of diabetic patients were significantly higher than control group. There was an indirect correlation between MDA and glycemic control level.

Conclusion: Evaluation of salivary MDA levels could be useful in prediction of glycemic control.

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1. Introduction

Diabetes is one of the most common chronic metabolic diseases and is the third leading cause of mortality around the world. Because of acceleration of atherosclerosis process, diabetes plays an important role in the development of cardiovascular diseases. One meta analytic study revealed that glycemic levels is an independent factor for cardiovascular diseases, even in non-diabetic subjects [1]. There is a direct relationship between poor glycemic control and diabetes complications such as retinopathy and nephropathy; therefore, proper glycemic control could postpone micro vascular complications [2].

Since, in diabetic patients, glucose joins to hemoglobin and other proteins, therefore, HbA1c is a useful indicator for assessment of long term glycemic control [3]. On the other hand, elevated blood glycemic levels leads to produce more free radicals of oxygen and peroxidation of fatty acid in the cells and affects on normal cells survive.

This elevated production of free radicals occurs in type I and II diabetes [5]. One study in Sweden showed that there is a direct

correlation between glycemic levels and oxidative stress, even in good glycemic controlled patients. In fact, poor glycemic control raises oxidative stress [6], some products of oxidative stress promotes the damages to cell regulation [7]. Malondialdehyde (MDA) is considered as a lipid peroxidation marker, which is very important in clinical evaluation of free radicals levels in diabetic patients [8].

On the base of previous studies, a scientific hypothesis is generated; if there is any correlation between diabetes control levels and products of oxidative stress.

Different studies reached to paradoxical results in evaluation of HbA1c and MDA, as a product of oxidative stress [9,10]. Aydin et al. evaluated the poor-controlled diabetic patients (HbA1c > 8%, FBS > 180 mg/dl) without any diabetic complications and concluded that thiobarbituric acid reactive substances (TBARS) of erythrocytes and plasma decreased after treatment of diabetes during a three-months follow-up [11]. A considerable enhancement in serum and salivary MDA in diabetic patients as compared to the healthy subjects was reported in other studies [12,13]. Another study showed that there was an increase in plasma TBARS in type II-diabetic patients; however, they could not find any correlation between TBARS and blood glucose and HbA1c [10]. Varashreeb's study reached to similar results; diabetic patients had higher levels of MDA, although, no direct correlation between MDA and FBS and HbA1c was found [14]. Although, a positive correlation between

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MDA levels and HbA1c, as well as, some diabetes complications were reported in the previous studies [15–17].

This present study was aimed to assess any correlation between MDA levels and glycemic control, in respect to important role of oxidative stress in pathogenesis of diabetes, and paradoxical results of the previous studies.

2. Methods and materials

Among diabetic patients (type II), who referred to Zahedan Bu Ali Hospital, 44 patients were enrolled to this study.

Inclusion criteria for case group:

- Type II diabetes for at least past 5 years.

Exclusion criteria:

- History of other systemic diseases such as Sjögren syndrome
- History of radiotherapy
- Alcoholism, smoking
- Usage of any vitamin or antioxidant supplements for past 3 months
- Periodontitis.

Among persons, who referred to Zahedan Dental School for dental treatments, 44 healthy subjects without any systemic diseases or chronic consumption of any drugs or supplements enrolled as control group. Exclusion criteria for the control group were similar to the case group.

After signing informed consent form, all participants were referred for the laboratory tests including FBS and HbA1c. Kit of Biosystem Company was used for assessment of HbA1c on base of the protocol, HbA1c separated from other blood hemoglobin through spectrophotometric analysis and measured.

After that, for evaluation of salivary MDA, saliva collected according to the standard technique at 9–11 a.m. For collection of unstimulated saliva, from the subjects asked to not eat, drink, smoke or brush at least 90 min before sample collection. Then, they were asked to spit the saliva into 50 ml laboratory tubes, every 1 min, for 2–5 min [18]. All saliva samples were sent to the lab and were stored at -80°C until test time for prevention of saliva protein degeneration.

MDA, which is a product of lipid peroxidation, was measured through the estimation of serum thiobarbituric acid species (TBARS) [9]. Salivary samples were added to a mixture of hydrochloric acid, 25% and thiobarbituric acid, 15% solution with a ratio of 2 to 1. This tube of solution was placed in boiling water for 30 min. After that, the solution was exited from the boiling water to become cold. Then, the mixture was centrifuged at 3000 rpm for 15 min. A pink color (wavelength = 532 nm) resulting from reaction of MDA with thiobarbituric acid at $\text{pH} = 1-2$ and $90-100^{\circ}\text{C}$ was quantified by spectrophotometer. TBARS was measured using fluorometric standard curve.

Glycemic control of the patients was classified as:

Good control ($\text{HbA1c} < 8.0\%$), poor control ($8.0 \geq \text{HbA1c} \leq 10.0$) and very poor control ($\text{HbA1c} > 10.0\%$) [19].

Following data collection, they were entered into SPSS software (Ver.19). The explanatory analysis was conducted by using two statistical tests: for assessment of MDA levels independent T-test was used with a confidence interval of 95%. For any correlation between study variables Pearson Correlation Coefficient and regression analysis were used. The significance level was set at 0.05 in all tests. The protocol of this study was approved by the Zahedan Ethics Panel of Zahedan University of Medical Sciences.

3. Results

In this study, which was designed to evaluate correlation between salivary lipid peroxidation and glycemic control (HbA1c), 44 diabetic patients (type II) including 21 men and 23 women and 44 healthy subjects including 21 men and 23 women were assessed.

Mean age of diabetic patients and healthy participants were 53.86 ± 10.26 and 55.04 ± 13.74 years, respectively; two groups were statistically matched. The mean of duration of diabetes was 8.4 ± 5.7 years in the case group.

The mean of HbA1c in diabetic patients ($9.3 \pm 1.96\%$) was significantly more than healthy subjects ($4.7 \pm 1.31\%$) ($p = 0.001$). Also, the diabetic patients had more salivary MDA than the control group ($p = 0.001$) (Table 1).

Also, analysis of HbA1c showed a remarkable difference between well, poor and very poor controlled-diabetic patients ($p = 0.001$).

There was a significant difference between good, poor and very poor-controlled diabetes regarding the salivary MDA (11.06 ± 3.87 , 14.19 ± 4.99 , 18.49 ± 4.89 $\mu\text{mol/L}$; respectively) ($p = 0.027$). Also there was a direct correlation between MDA levels and HbA1c ($P = 0.035$) (Fig. 1).

4. Discussion

Smoking, hypertension and high plasma cholesterol are the most important risk factors, which accelerate cardiovascular diseases, consequently, increase the rate of morbidity, in diabetic patients. There is a direct correlation between oxidative stress and some diabetes complications, especially vascular damage [20].

In diabetes, an increase in free radicals leads to oxidation of glycosylated proteins, activation of sorbitol pathway, oxidation of cell lipids and proteins and some damages to cell membrane [8]. Assessment of lipid peroxidation and lipid profile could be beneficial as screening test in evaluation of lipid status in diabetic patients [12,13]. Since free radicals are very active and they have short half-life, researchers assess their products such as MDA to evaluate their activity.

In present study, mean MDA levels in diabetic patients were higher than healthy subjects and there was a direct relationship between HbA1c and salivary MDA. It revealed that poor control of diabetes leads to increase of salivary MDA.

Some studies evaluated a correlation of HbA1c and MDA levels, as a final product of lipid peroxidation; and reported different results [9,10]. In some studies, a remarkable raising in serum and salivary MDA were reported in diabetic patients [12,13], and a positive coefficient between poor glycemic control (HbA1c levels) and MDA levels reported in some of them [15–17]. These results correspond with the findings of the present study.

Moreover, Varashreebs et al. demonstrated a higher levels of MDA in diabetic patients as compared to healthy subjects; however, they did not find any correlation between MDA levels and HbA1c or FBS. In their study, both type of diabetes enrolled in assessment, which could be explaining of different results of Varashreebs' study and the present study [14].

Table 1
Salivary MDA and HbA1c in diabetic and healthy subjects.

Parameters	Group	Amounts			P value
		The least	The most	Mean \pm SD	
HbA1c (%)	Diabetic	6.2	14.3	9.3 ± 1.96	0.001
	Healthy	3.7	5.6	4.7 ± 1.31	
Salivary MDA ($\mu\text{mol/L}$)	Diabetic	6.4	26.34	14.44 ± 5.17	0.001
	Healthy	0.76	16.22	10.23 ± 1.03	

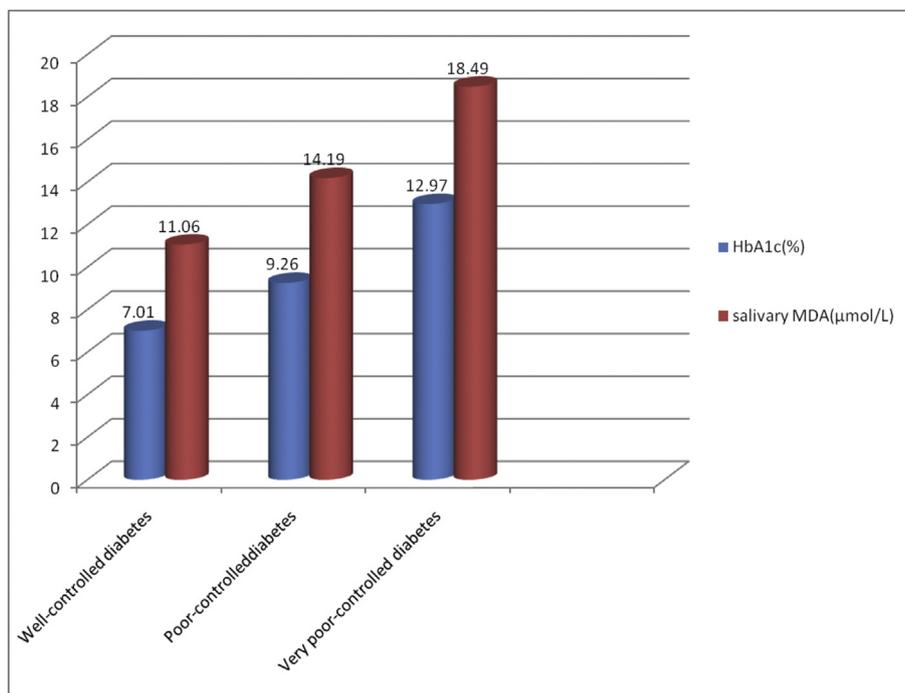


Fig. 1. Mean of salivary MDA and HbA_{1c} in diabetic patients.

Furthermore, MDA level and its correlation with diabetes complication were assessed in other studies; however, diverse results were reported according to the type of target organ [10,17].

Turk et al. revealed that diabetic patients had higher plasma TBARS levels as compared to healthy subjects; however, there was not any significant difference between diabetic patients with and without vascular disease such as nephropathy or retinopathy [10]. In contrast, Kumaunt et al. revealed that not only MDA levels were lower in healthy participates, but also MDA levels in patients complicated with nephropathy was higher than other diabetic patients without this complication [15]. Different sample size and various diabetes afflictions in the studied patients could explain contradictory results in those studies.

In Likidilid's study, the highest erythrocyte MDA levels were observed in diabetic patients with coronary artery diseases. Also, they showed that MDA in poor-controlled diabetes afflicted with CHD was higher than good-controlled diabetic patients; however, there was not any remarkable difference between poor and fair-controlled diabetic patients [21]. In contrast, present study revealed a significant direct correlation between mean salivary MDA and levels of diabetes control, which may be related to various sources of MDA in two studies.

It seems that hyperglycemia and some disturbance in anti-oxidant system causes more production of free radicals of oxygen, which accelerate nitrogen and fatty acid peroxidation, consequently, this process could damage to cell membrane and threat cells lives [2,4]. On the other hand, an increase in free radicals and decrease in antioxidants accelerates oxidative destruction of double bonds of fatty acids in the cell membrane. Consequently, this process continues serially and promotes cell death and widespread signs and symptoms and complications of diabetes. In respect to this process, Long term diabetes control is considered as protective step for inhibition of lipid peroxidation, cell death and potential complications.

5. Conclusion

Salivary MDA levels as a product of lipid peroxidation in diabetic patients is higher than healthy participants. As there is a reverse correlation between lipid peroxidation and levels of diabetes control, assessment of salivary MDA could be a non-invasive beneficial procedure for monitoring the effectiveness of treatment of diabetes.

Conflicts of interest

The authors declare that they have no conflict of interest.

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