The Myanmar Health Sciences Research Journal, Vol. 26, No. 1, 2014

Oxidative Stress Marker and Antioxidant Status in Patients with Type 2 Diabetes Mellitus

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The persistence of hyperglycemia in diabetic patients leads to the generation of free radicals. Free radicals in the cells play important roles in the pathogenesis of type 2 diabetes mellitus and in the development of diabetes complications. Alterations in lipid peroxidation and antioxidant defense have been investigated as related with diabetes mellitus. Therefore, this study was aimed to investigate oxidative stress marker and antioxidant status in patients with type 2 diabetes mellitus and controls. For this purpose, 30 type 2 diabetes mellitus patients from diabetic clinic at Mandalay General Hospital and 30 apparently healthy controls were studied. The subjects were females, 35-50 years of age. The plasma malondialdehyde level was represented as oxidative stress marker. The plasma ascorbic acid level was determined as antioxidant vitamin. The mean plasma malondialdehyde level (9.7±4.88 μmol/L) in patients was significantly higher than that of controls (4.09±2.17 μmol/L) ($p<0.001$). The mean plasma ascorbic acid level in patients (1.08±0.24 mg/dl) was found to be significantly lower than that of controls (1.21±0.27 mg/dl) ($p<0.05$). Negative correlation was observed between these two parameters in patients ($r=-0.47$) ($p<0.01$). Therefore, this study indicated that type 2 diabetes mellitus is associated with enhanced lipid peroxidation and it may be due to impaired antioxidant system.

INTRODUCTION

Diabetes mellitus is not a disease, but rather is a heterogeneous group of syndromes characterized by an elevation of fasting blood glucose caused by relative or absolute deficiency in insulin.[1] Type 2 diabetes mellitus usually appears in adults, often in middle age. Family history of diabetes can significantly increase risk of developing it. It is the most common form of diabetes mellitus and usually accounts for 90-95% of total diabetics.[2]

The persistence of hyperglycemia in diabetic patients leads to the generation of Reactive Oxygen Species (ROS) which causes oxidative damage to carbohydrate, protein, lipid, DNA and is efficiently neutralized by cellular antioxidant defense mechanisms. When antioxidant defenses are not efficient or when free radical formation in the body is increased, this imbalance creates the situation of oxidative stress.[3] The possible causes of oxidative stress in diabetes mellitus include free radicals generated by auto-oxidation of sugar and sugar adducts to proteins and by auto-oxidation of unsaturated lipids in plasma and membrane proteins.[4] Glycation of protein, formation of advanced glycation end-products by ROS and resultant oxidative stress can initiate auto-catalytic cycle of delerious reactions in tissues.[5] The metabolism of glucose produces reducing equivalents during fuel oxidative phosphor-

rylation in mitochondria, the by-products of which include free radicals, that seemed to be first and key event in the activation of other metabolic pathways (eg., polyol pathway activation) involved in the pathogenesis of diabetes complications.[6]

Diabetes mellitus is associated with increased lipid peroxidation which has been implicated in the pathogenesis of diabetic complications.[7] Lipid peroxidation is an auto-catalytic free radical mediated destructive process whereby polyunsaturated fatty acids (PUFAs) in cell membrane undergo degradation to form lipid hydroperoxides.[8] Within biological system, the evidence of oxidative stress is usually determined by malondialdehyde (MDA) formation from lipid peroxidation.[9]

Malondialdehyde (MDA) arises from peroxidation of PUFAs, oxidation of arachidonic acid in cell membrane, and enzymatically during eicosanoid metabolism and is readily metabolized in mammalian tissues. Antioxidant defense is impaired in diabetic condition and further exacerbate oxidative stress shift the homeostatic balance in favor of tissue destruction.[10] Ascorbic acid is the most effective aqueous phase antioxidant in human blood plasma and major important antioxidant defense against diseases and degenerative processes caused by oxidative stress.[11] It can regenerate active tocopherol so that it can also restore the antioxidant properties of oxidized tocopherol, and it offers most effective protection against plasma lipid peroxidation. Therefore, it is important to assess the serum MDA level as the oxidative stress marker and vitamin C level as antioxidant parameter in type 2 diabetes mellitus.

In Myanmar, one study found the relationship between fasting blood sugar (FBS), hemoglobin A_{1c}, serum lipid profile and plasma thiobarbituric acid reactive substances (TBAR-S) level with severity of periodontal disease in non-insulin dependent diabetes mellitus subjects.[12] Moreover, another study reported about lipid profile and lipid peroxidation in diabetes mellitus and its complication (microalbuminuria).[13] However, oxidative stress marker and vitamin C level in type 2 diabetes mellitus and controls had not yet been investigated.

Therefore, this study was aimed to investigate plasma malondialdehyde as oxidative stress marker and plasma ascorbic acid as antioxidant status in patients with type 2 diabetes mellitus in comparison to controls.

MATERIALS AND METHODS

Study design

Laboratory-based, comparative study

Study site

Biochemistry Department, University of Medicine (Mandalay)

Study period

From June to December, 2007

Subject selection

Cases: 30 patients with type 2 diabetes mellitus

Inclusion criteria

- Between 35-50 years of age
- Female patients
- Patients diagnosed as type 2 diabetes mellitus (FBS ≥126 mg/dl) and were regularly visiting the diabetic clinic in Mandalay General Hospital
- Patients who had blood pressure ≤140/90 mmHg
- Non-smokers

Exclusion criteria

- Patients taking regular vitamins supplements such as vitamin E and C
- Patients with pregnancy
- Patients with other chronic inflammatory diseases

Controls: 30 apparently healthy subjects

Inclusion criteria

- Between 35-50 years of age
- Female subjects

- No history of diseases and physical examinations revealed nothing abnormal
- Subjects who had normal FBS level (80-100 mg/dl)
- Subjects who had blood pressure ≤140/90 mmHg
- Non-smokers

Exclusion criteria

- Subjects taking regular vitamins supplements such as vitamin E and C
- Pregnant women

Operational definition

Non-smoker: one who does not smoke at anytime

Sample size calculation

The number of subjects required to provide optimal ability to detect changes in plasma MDA and plasma ascorbic acid were calculated by following formula for a study using unpaired 't' test. Two-sided significance level (1-alpha) was 0.05 and power (1-beta, % chance of detecting) was 80.

Standard difference= δ/σ

σ=the assumed equal standard deviation of the observations in each group

δ=the smallest difference in means that is clinically important

Using Altman's normogram, the line connecting a standard difference and power of 80% cut the sample size axis (N) approximately and the required sample size in each group was obtained by N/2.[14] So, sample size in this study was chosen as 30 subjects for each group (cases and controls).

After taking written consent, subjects were interviewed, and history taking and clinical examinations were done.

Blood sampling

After overnight fasting, about 7 ml of blood were collected into two clean and dry test tubes from each subject. For FBS determination, 2 cc of blood were collected in the test tube containing sodium fluoride and potassium oxalate as anticoagulant. For plasma MDA and ascorbic acid determinations, 5 cc of blood were collected in the test tube containing double oxalate as anticoagulant.

Biochemical methods

Plasma MDA level was determined by using thiobarbituric acid reaction test.[15] Plasma ascorbic acid level was determined by using phosphotungstate method.[16] FBS level was determined by using direct O'toluidine method.[17]

Data collection and analysis

Data were recorded according to the proforma. Results were reported as mean ±SD. Student 't' test (unpaired) was used to observe the significance of difference between above parameters (MDA and ascorbic acid) in type 2 diabetes mellitus patients and controls. The Pearson correlation was determined. Data were analyzed by using Microsoft excel and SPSS 11.0 version.

RESULTS

Thirty type 2 diabetes mellitus patients from diabetic clinic at MGH and thirty apparently healthy controls were studied. The subjects were females aged between 35-50 years. The plasma malondialdehyde level and erythrocytes catalase activity in both diabetes mellitus patients and controls were determined. The values were expressed as mean±SD. Table 1 shows the physical data of type 2 diabetes mellitus patients and controls.

Table 1. The physical data of type 2 diabetes mellitus patients and controls (mean±SD)

	Age in years	FBS (mg/dl)	Blood pressure (mmHg)	
			Systolic	Diastolic
Diabetes mellitus	45±3	175.64 ±14.29	131.67 ±10.91	80.67 ±6.71
Controls	42±3	75.68 ±11.94	115.67 ±9.66	76.55 ±8.82

The mean±SD of the plasma MDA levels in type 2 diabetes mellitus and controls were 9.7±4.88 μmol/L and 4.09±2.17 μmol/L,

respectively. The mean±SD of the plasma ascorbic acid levels in type 2 diabetes mellitus and controls were 1.08±0.24 mg/dl and 1.21±0.27 mg/dl, respectively. Type 2 diabetes mellitus subjects were found to have significantly higher plasma MDA level than that of controls ($p<0.001$). The plasma ascorbic acid level in type 2 diabetes mellitus was found to be significantly lower than that of controls ($p<0.05$), considered as significant.

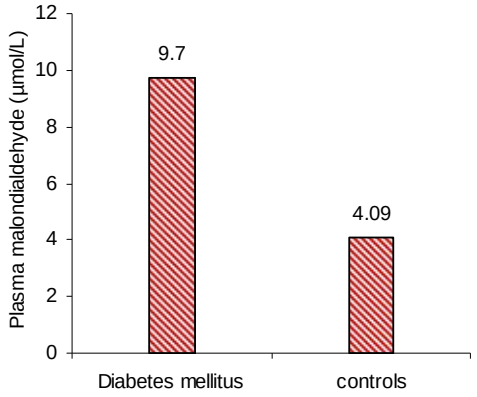

Fig. 1. The mean plasma MDA in diabetes mellitus and controls

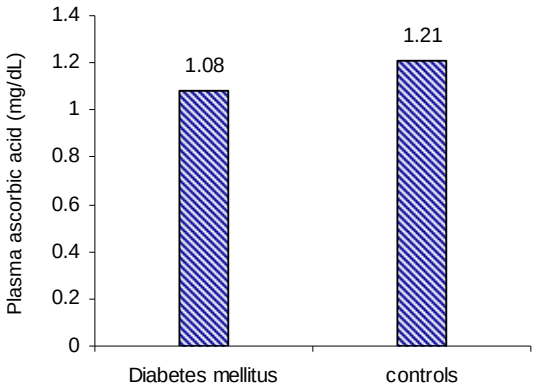

Fig. 2. The mean plasma ascorbic acid in diabetes mellitus and controls

Figure 1 shows the mean plasma MDA levels in patients with type 2 diabetes mellitus and controls. The mean plasma MDA level in patients with type 2 diabetes mellitus was found to be significantly higher than that of controls ($p<0.001$).

Figure 2 shows the mean plasma ascorbic acid levels in patients with type 2 diabetes mellitus and controls. The mean plasma ascorbic acid level in patients with type 2 diabetes mellitus was found to be significantly lower than that of controls ($p<0.05$).

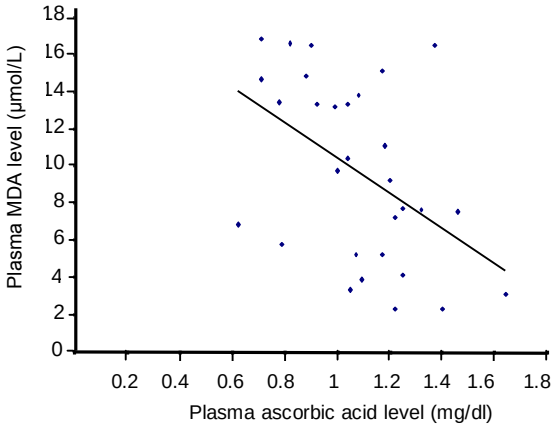

Fig. 3. The correlation between plasma MDA and ascorbic acid level in type 2 diabetes mellitus

Figure 3 shows the negative correlation between plasma MDA and ascorbic acid levels in type 2 diabetes mellitus patients ($r=-0.47$) ($r^2=0.22$). The correlation was significant at $p<0.01$ level ($y=-9.54x+19.98$).

DISCUSSION

Diabetes mellitus has been known to be a state of excessive generation of free radicals contributed by several mechanisms, including hyperglycemia and impaired antioxidant status, causing oxidative stress. This oxidative stress exacerbates the development and progress of diabetes and its complications. Oxidative stress can be measured by several blood markers that typically reflect the tissue peroxidation. Lipid peroxidation is one of the important oxidative stresses induced by the reactivity of oxygen free radicals. Many methods have been described to assess some of the chemical stages of the oxidative degradation of an unsaturated fatty acid including measurement of MDA.[18]

In the present study, the mean plasma MDA level of controls was found to be 4.09±2.2 µmol/L. It was comparable with

the values mentioned in the other studies in which the same method was used (3.92± 0.66 and 3.11±0.17 μmol/L).[19, 20] Mean plasma MDA level of type 2 diabetic patients was 9.7±4.9 μmol/L. It was comparable with the values mentioned in the other studies (11.39±1.78 and 10.28± 0.41 μmol/L).[12, 13]

In the present study, the mean plasma MDA level in patients with type 2 diabetes mellitus was found to be significantly higher than that of controls ($p<0.001$). In the study from Germany, it was found that the generation of ROS is increased in both types of diabetes and that the onset of diabetes is closely associated with oxidative stress.[21]

A group of researchers from France mentioned that type 2 diabetes mellitus patients had significantly higher plasma oxidative stress marker than control subjects ($p<0.001$).[22] In Myanmar, a study found that mean plasma MDA level was significantly higher in diabetes mellitus ($p<0.01$). There was correlation between lipid peroxidation and diabetes nephropathy.[13]

These studies strongly suggested that enhanced oxidative stress is present in type 2 diabetes mellitus. Variation of values in these studies and in the present study may reflect different methods and procedures. In the present study, duration and complications of diabetes, and the physiological variations like life style, stress, and exercise were not taken into account.

The present study revealed that there is highly significant association of oxidative stress with type 2 diabetes mellitus probably due to increase production of ROS. Therefore, there is enhanced lipid peroxidation indicating increased tissue oxidative stress and it may contribute to diabetic complications.

There are efficient and sophisticated antioxidant systems in living cells. The non-enzymatic antioxidants and enzymatic defenses can offer an indication of the antioxidant status of an individual.[23] In the present study, the mean plasma ascorbic acid level in controls was 1.21±0.27 mg/dl. It was comparable with the values mentioned in other studies (1.23±0.26 and 1.38± 0.13 mg/dl).[24, 25] Mean plasma ascorbic acid in patients with type 2 diabetes mellitus was 1.08±0.3 mg/dl. It was comparable with the value mentioned in one study from Nigeria (1.03±0.09 mg/dl).[25]

In the present study, the mean plasma ascorbic acid level was significantly lower in diabetic patients than controls ($p<0.05$). The observed value in the present study was in agreement with reports of other investigators. A group of researchers from United Kingdom reported that lower plasma ascorbate levels in patients with type 2 diabetes mellitus who consumed adequate dietary vitamin C than controls were found ($p<0.001$).[26]

In the study from India, the mean plasma ascorbic acid level in all diabetic patients, i.e., those with and without retinopathy was markedly lower than normal controls ($p<0.001$).[27] So, they suggested that low ascorbate levels in diabetes appear to be a consequence of the disease itself and not due to inadequate dietary intake of vitamin C. Therefore, lower plasma ascorbic acid level in diabetic patients may be caused by increased urinary excretion of the vitamin, defective transport across the cell membrane, and impaired ascorbic acid metabolism along with increased oxidation of ascorbic acid to dehydro-ascorbic acid which may promote diabetic process.[26]

These studies confirmed that compared to apparently healthy persons, ascorbic acid levels are significantly depressed in plasma of diabetic patients. Therefore, diabetic patients have significant defects in anti-oxidant defense which may increase vulnerability to oxidative damage and progression of the disease. Diabetic patients showed a significant negative correlation between mean plasma MDA and ascorbic acid levels ($r=-0.47$) ($p<0.01$). Similar correlation was found in the studies of other investigators.[25, 28]

Therefore, diabetes may cause disorders in ascorbic acid metabolism; ascorbic acid deficiency may provoke disorders in diabetes. Therefore, increase in oxidative stress may be due to decrease in the antioxidant defense.

Conclusion

The present study revealed that diabetes mellitus is associated with enhanced tissue oxidative stress and alterations in anti-oxidant. Antioxidant therapy to combat the progression of free radical production may also be beneficial. Therefore, monitoring oxidative stress and antioxidant parameters in type 2 diabetes mellitus patients could be important in the progression of diabetes mellitus and in the prevention of diabetic complications.

Moreover, further studies on the measurement of other antioxidant parameters should be required to elucidate their roles in type 2 diabetes mellitus. It is suggested that these studies may help to develop effective strategies for therapeutic approaches to the treatment of diabetic complications.

ACKNOWLEDGEMENT

We would like to express our grateful thanks to Dr. Kyaw Zin Thant, Director-General, and Dr. Khin Phyu Phyu, Director (Clinical Research) from the Department of Medical Research (Upper Myanmar) for their thoughtful help and encouragement. We must also express my profound gratitude to Dr. Khin Than Aye, Associate Professor/Consultant, Medical Unit II, MGH for her constructive advice and generous help in collection of patients. Finally, We must not fail to mention our sincere thanks to Professor Dr. May Pyone Kyaw, Head of Biochemistry Department, University of Medicine (Mandalay) and Professor Dr. Khin Win Sein, Head of Biochemistry Department, University of Medicine (Magway) for their patient guidance and suggestions.

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