RESEARCH

Lipid Peroxidation and Antioxidant Status in Male and Female Patients with Type 1 and Type 2 Diabetes Mellitus

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ABSTRACT

Background: Free radicals are reactive oxygen species which cause lipid peroxidation precipitating many metabolic diseases including Diabetes Mellitus. However, these free radicals are quenched by substances known as antioxidants like vitamin C, vitamin E and several other compounds. Lipid peroxidation and antioxidant status were investigated in patients with Type 1 and Type 2 Diabetes mellitus- Pokhara, Nepal.

Methods: The extent of lipid peroxidation was assessed by thiobarbituric acid reactive substances and the antioxidant parameter estimations were total antioxidant activity, Vitamin C and Vitamin E assessed in Type 1 and 2 diabetes mellitus patients along with matched healthy counterparts.

Results: The lipid peroxidation was increased in male Type 1 and 2 diabetic patients whereas female group showed decreased level as compared to its healthy counterparts. Similarly, the total antioxidant activity was found to be decreased in the diabetic group. The lipid peroxidation parameter and antioxidant status were statistically significant at $p< 0.05$.

Conclusion: Oxidative stress and antioxidant status varied in male and female patients suffering from diabetes either Type 1 or Type 2. Apart from gender basis of evaluating oxidative stress, variables based on diet, habitat, socioeconomic status, education, etc. can also be considered.

Keywords: Antioxidant, Diabetes Mellitus, Lipid peroxidation, Oxidative stress

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INTRODUCTION

The growing global prevalence and health burden associated with the disease has recently led to the World Health Organization to classify high blood glucose level as the third leading cause of premature mortality globally. The annual global healthcare burden associated with diabetes is currently estimated to be at least 376 billion USD, accounting for 7, 10, 11 and 14 percents of total healthcare expenditure in Sub-Saharan Africa, Europe, South East Asia and North America, respectively.[1, 2] Furthermore, the projected growth in the global prevalence and economic burden of Type 2 diabetes will further disproportionately affect low and middle income countries in future. In India, the prevalence of diabetes is projected to increase by 60 percent to 80 million cases by 2030 and its economic burden is projected to increase by almost 50% to at least 4.8 billion USD over the same period.

Lipid peroxidation by reactive oxygen species (ROS) appears to precipitate many diseases which include Diabetes Mellitus (DM). Substances known as antioxidants reduce the power of free radicals. There are several compounds functioning as antioxidants including vitamin C, vitamin E, beta-carotene and vitamin A.[3, 4] The quantum of lipid peroxidation in plasma is generally determined by measurement of thiobarbituric acid reactive substances (TBARS). Vitamin C serves as antioxidants in the aqueous phase while vitamin E functions in the lipid phase.

Oxidative stress is defined as disturbance in the prooxidant-antioxidant balance in favor of the prooxidant, leading to potential damage producing oxidative stress. Free radical is any atom with at least one unpaired electron in the outermost shell, and is capable of independent existence. Raised oxidative stress has been implicated not only for chronic diseases like cardiovascular disease, cancer, cirrhosis, atherosclerosis, Alzheimer's disease and Parkinson's disease; it has also been found altered in diabetes mellitus.[5-8]

Oxidative stress is defined as an imbalance between the production of reactive oxygen species or free radicals and antioxidant defense, which may induce tissue injury. In diabetes, oxidative stress is caused by both an increased formation of plasma free radicals and a reduction in antioxidant defense. An unbalanced excess of free radicals due to lack of antioxidants may increase the risk of complications of diabetes.[4, 5]

Therefore, the aim of present study was to study the role of oxidative stress and antioxidants in the pathogenesis of type 1 and type 2 diabetes mellitus.

MATERIALS AND METHODS

In the present Case-Control investigation patients suffering from type 1 and type 2 DM and attending Manipal Teaching Hospital, Pokhara, Nepal was selected. The study was conducted from March 2004 to February 2016. The number of patients in type 1 diabetes mellitus (DM) was limited to 20 (10 males and 10 females) and 40 type 2 DM (20 male and 20 female) along with 60 healthy individuals as control.

Both patients and controls were selected along with matched controls (individuals who had no history of illness or disease). Type 1 Diabetic patients were individuals treated with insulin injections, followed by elevated ketone bodies on diagnosis, less body mass index whereas, type 2 Diabetic patients were associated with higher body mass, high cholesterol and blood pressure at diagnosis. All the subjects after obtaining their informed written consent were examined clinically and information pertaining to age, habits and health status were collected. Using standard protocols glucose estimation by glucose oxidase method-Randox diagnostic kit, total antioxidant activity (TAA), thiobarbituric acid reactive substances (TBARS), Vitamin C, Vitamin E levels were estimated in plasma using UV-visible Spectrophotometer.

Collection of blood sample

All glassware and plastic wares were soaked in 20% nitric acid for 24 hrs to make them mineral free. About 6ml of venous blood was collected by vein puncture by standard blood collection technique in acid washed vials containing ethylene diamine tetraacetic acid (EDTA). Plasma was separated by centrifugation for 10 minutes at 3000 revolution per minute and was analyzed with minimum possible delay.

Plasma TBARS (thiobarbituric acid reactive substances) level was carried out by Buege and Aust method[9], TAA (total antioxidant activity) level was measured by Benzie and Strain method[10], vitamin C level was estimated by Natelson Method[11] and vitamin E was estimated by the method of Baker and Frank.[12]

Statistical analyses

The results were expresses in mean ± standard deviation, the level of significance was determined by Student's Independent t-test at $p<0.05$. Statistical analysis was carried out using SPSS version 13.0.

RESULTS

As per inclusion criterion, we observed significant differences in lipid peroxidation and antioxidant status in male and female patients as given in Figure 1, which reveals the plasma glucose concentration of fasting (FBS) and post-prandial (PPBS) of Type 1 and Type 2 diabetic patients based on gender. Male patients revealed higher FBS and PPBS level as compared to healthy individuals. On the other hand, female diabetic patients exhibited decreased FBS and increased PPBS level as compared to healthy controls.

The various biochemical parameters in plasma were estimated in healthy controls, Type 1 and Type 2 diabetic patients. The lipid peroxidation was assessed by thiobarbituric acid reactive substances (TBARS) and antioxidant assays were total antioxidant activity (TAA), vitamin C and E. The basal metabolic rate (BMI) and surface area (SA) was decreased in Type 1 DM, but

Type 2 DM patients showed increased BMI and SA as compared to healthy males. Lipid peroxidation parameter TBARS was increased both in Type 1 and Type 2 DM when compared with healthy male controls. The total antioxidant activity (TAA) was lower in both type 1 and type 2 DM. The antioxidant vitamins C and E were also found to be in normal range in female diabetic patients.

Table 1: Plasma levels of TBARS, TAA, Vitamin C and Vitamin E in male control, Type 1 and Type 2 DM patients

Group (male)	BMI (kg/m^2)	SA (m^2)	TBARS (nmol/ml)	TAA (µmol/L)	Vit C (mg/dl)	Vit E (mg/dl)
Control	22.35±2.32	1.71±0.24	1.47±1.38	787.79±16.62	0.76±0.29	0.77±0.49
Type 1	21.16±1.59	1.69±0.27	2.23±1.38^a	548.80±10.37^c	0.91±0.33^e	0.82±0.72^g
Type 2	25.67±8.02	1.92±0.02	2.01±1.81^b	647.41±18.92^d	0.82±0.32^f	0.74±0.41^h

Note: SA= surface area, p<0.05 (level of significance) i.e. 95% confidence interval; a=0.04, b=0.002, c=0.015, d=0.039, f=0.023, g=0.034, h=0.02

Plasma TBARS was increased in both type 1 and 2 DM patients compared to controls. Between type 1 and Type 2 DM, the change in the value of TBARS was not statistically significant. The plasma TAA was significantly decreased in both type 1 and type 2 DM. There was an increase of TAA in type 2 over type 1 DM which was statistically significant. Vitamin C antioxidant is increased in both type 1 and type 2 DM. Vitamin E is not significantly altered in both type 1 and type 2 DM. The increase of vitamin C in type 2 was less than that in type 1 DM. The plasma levels of TBARS in female group is significantly decreased in type 1 without any significant change in type 2 DM. Plasma TAA is decreased in type 1 but not significantly altered in type 2 DM. Vitamin C in plasma is decreased in type 1 without any change in type 2 DM. Similarly vitamin E did not alter in type 1 but decreased in type 2 DM. On Independent t-test analysis, male and female patients (Table: 1 and 2) showed statistical significance with respect to their healthy individuals at p< 0.05 (level of significance).

Table 2: Plasma levels of TBARS, TAA, Vitamin C and Vitamin E in female control, Type 1 and Type 2 DM patients

Group (female)	BMI (kg/m^2)	SA (m^2)	TBARS (nmol/ml)	TAA (µmol/L)	Vit C (mg/dl)	Vit E (mg/dl)
Control	24.45±2.29	1.74±0.11	2.12±0.21	615.39±18.07	0.99±0.31	0.81±0.34
Type 1	22.38±3.36	1.72±0.36^b	0.95±0.54^d	585.50±16.24^f	0.83±0.38^h	0.82±0.06
Type 2	28.36±3.64^a	1.82±0.09^c	1.62±0.15^e	647.10±28.56^g	0.98±0.40	0.70±0.14^i

Note: SA=Surface area, p<0.05 (level of significance) i.e. 95% confidence interval, a<0.001, b=0.005, c=0.012, d=0.019, f=0.001, g=0.034, h=0.02, i=0.033

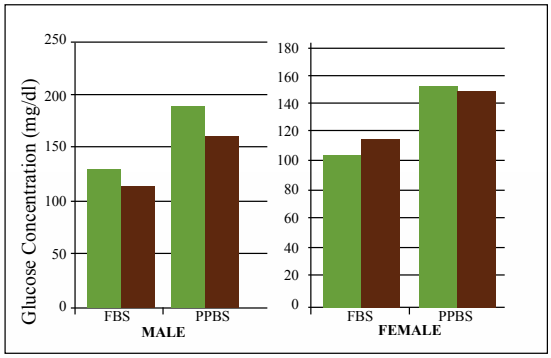

Fig 1: Glucose concentration of Type 1 and Type 2 of Diabetic patients based on gender
Note: *FBS- Fasting blood sugar, PPBS- Post-prandial blood sugar level*

DISCUSSION

The present study covers patients with diabetes mellitus both in type 1 and type 2 who attended Manipal Teaching Hospital, Pokhara, Nepal during the study period. The number of patients in type 1 is relatively less compared to type 2 DM, due to the fact that incidence of type 2 DM is globally more common than type 2 and Nepal is no exception. The results of this study have indicated that lipid peroxidation is increased in both the types of the diseases in male patients, but not in the female patients. The antioxidant status is generally related to the prooxidant status. This means that an increase of plasma TBARS should necessarily be associated with an increase in antioxidant levels. But, the present study has indicated that the antioxidants are marginally increased in male patients but not significantly altered in female group. Surprisingly, the female patients of type 2 DM showed decreased vitamin E level of plasma. The reason for this is not known.

The prooxidant and antioxidant status during any stress need to be balanced in patients with DM but such a balance is not encountered in the current investigation. A question arises whether the dietary status of both vitamins have to be taken care of to bring about a counter effect of lipid peroxidation which does exist in these patients. Experimental evidences suggest the involvement of free radicals in the onset of diabetes and more importantly in the development of diabetic complications.[7-9, 13-16] Scavengers of free radicals are effective in preventing experimental diabetes in animal models and in type 1 (IDDM) and type 2 (NIDDM) patients as well as reducing severity of diabetic complications. Persistent hyperglycemia in the diabetic patients leads to generation of oxidative stress due to auto-oxidation of glucose, non-enzymatic glycosylation and polyol pathway. [1-3] Auto-oxidation of glucose involves spontaneous reduction of molecular oxygen to superoxide and hydroxyl radicals, which are highly reactive and interact with all the biomolecules.[15, 16]

Lipid peroxidation was increased in the plasma of diabetic patients by 50% (TBARS). The activity of superoxide dismutase was enhanced by 9.4% in the erythrocytes of patients. Our results showed higher oxidative stress and generation of free radicals in the RBCs of diabetic patients. [17-20] Parthiban (1995), Oberley (1988) has reported oxidative stress is associated with development of diabetic complications. In diabetes mellitus, GSH formation is hampered due to diminished efficiency of the HMP shunt pathway of glucose oxidation, the second enzyme in the red blood cell rises to the passion and disposes off the hydrogen peroxide formed in the cell so that internal environment in the erythrocyte is taken care of to the maximum possible extent. But in spite of this mechanism, lipid peroxidation and free radical generation prevails in the RBCs of diabetics.[21-23]

The development and existence of an organism in the presence of O_2 is associated with the generation of reactive oxygen species (ROS), even under physiological conditions. Twenty four ROS are responsible for the oxidative damage of biological macromolecules such as DNA, carbohydrates and proteins. Some of the most relevant ROS are: peroxyl radicals ($ROO^{\cdot}$), nitric oxide radical ($NO^{\cdot}$), superoxide anion radical ($O_2^{\cdot-}$), singlet oxygen ($^1O_2$), peroxynitrite ($ONOO^-$) and hydrogen peroxide (H_2O_2). ROS are either radicals (molecules that contain at least one unpaired electron) or reactive non-radical compound capable of oxidizing biomolecules. ROS are also produced in the organism as a part of the primary immune defense. [22-26] Phagocytic cells such as neutrophils, monocytes, or macrophages defend against foreign organism by synthesizing large amount of superoxide anion radical and nitric oxide radical as a part of their killing mechanism. To counteract the prooxidant load a diversity of antioxidant defense systems are operative in biological systems including enzymatic and non-enzymatic antioxidants. Major enzymes directly involved in detoxification are superoxide dismutase as well as catalase and glutathione peroxidase. The human diet contains an array of different compounds that possess antioxidant activities or have been suggested to scavenge ROS based on their structural properties. The most prominent representatives of dietary antioxidants are ascorbate, tocopherols, carotenoids and flavonoids.[26, 27]

ROS are known to be involved in pathogenic processes of numerous diseases, such as CVD, cancer, cataract, rheumatoid arthritis, neurodegenerative disease. Oxidative damage to important biomolecules is a deleterious pathway, but also influences of ROS on gene regulation and immune system might impair bodily functions.[4-8]

CONCLUSION

Accounting all the information and data gathered in this study indicate that oxidative stress is not the etiologic factor in diabetes mellitus of local population. By implication, antioxidant defense system did not appear to be involved. Interestingly, the local population seems to be well endowed with antioxidant defense system. Whether the good antioxidant defense system or well antioxidant-balanced diet, habitat, socioeconomic status, education are responsible to keep the oxidative stress, in check forms a good platform for future study.

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