Original Article

Type 2 Diabetes Mellitus: The Role of Oxidative stress and Antioxidants

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Abstract:

Objective: Diabetes mellitus is a group of metabolic disease characterized by hyperglycemia. Oxidative stress contributes to the pathogenesis of diabetic microvascular and macrovascular complications. This study was undertaken to find the oxidative stress and antioxidant conditions in diabetic and healthy individuals. ***Method:*** A case-control study was carried out at Kantipur Dental College Teaching Hospital & Research center, Kathmandu, Nepal from January 2018 to January 2019 with 200 subjects. Amongst them 100 were diagnosed as diabetic individuals and rest 100 were healthy controls with age and gender matched. Blood samples were drawn after overnight fasting for the analysis of glucose, thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides, nitric oxide, total antioxidant activity, vitamin A, vitamin C, vitamin E, reduced glutathione, glutathione peroxidase, superoxidase and catalase. ***Result:*** The plasma glucose, lipid peroxidation parameters: TBARS, lipid hydroperoxide and nitric oxide were increased in diabetic patients. Antioxidants markers included: total antioxidant activity, reduced glutathione, vitamins A, C & E levels were significantly decreased in diabetic patients compared to healthy control counterpart. ***Conclusion:*** Increased evidence of free radicals/ oxidative stress with respect to decreased levels of antioxidants has implicated a strong role in progression of diabetes and its associated complications. Appropriate medications with antioxidants supplementation, physical exercise, and restricted diet can improve diabetes through the reduction of oxidative stress.

Keywords: Diabetes mellitus, free radicals, oxidative stress, antioxidant, vitamin.

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Introduction

Diabetes mellitus is a disease of epidemiological impact, characterized by hyperglycemia and glucose intolerance due to insulin deficiency, impaired effectiveness of insulin action or both. It is among the five leading causes of death in most developed nations, affected over 387 million populations globally and Nepal is no exception[1,2]. In normal physiological conditions, 95% of oxygen is consumed, undergoes tetravalent reduction by cytochrome oxidase system forming water and ATP. The remaining 5% oxygen is univalently reduced and four electrons are added one at a time, leads to the formation of a variety of highly reactive oxygen species (ROS)[3-5]. There are number of causes for increased oxidative stress in diabetes, of which hyperglycemia-driven mitochondrial overproduction of ROS is particularly noteworthy. Another major source of oxidative stress in diabetes is through glucose oxidation. Hyperglycemia may compromise antioxidant defenses such as glutathione and reduce vitamin E[6].

The prevalence of type 2 diabetes is increasing at alarming rates both in developing and developed world. Diabetes is considered as common

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chronic metabolic disorders characterized by hyperglycemia which results due to defects in insulin secretion, insulin action or both and accounts for at least 90% of all cases of diabetes[7,8]. It is also highly prevalent in the elderly and is associated with various co-morbidities, such as obesity, hypertension, hyperlipidemia and cardiovascular diseases leading to a condition called metabolic syndrome. On average, two persons develop diabetes and one person dies from diabetes related causes in the world every ten seconds. The International Diabetes Federation estimates the global prevalence of Type 2 diabetes at 6.6% (285 million cases) in 2010 and expects to reach to 7.8% (438 million cases) by 2030. This rapid increase in the global prevalence is attributed to population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity, sedentary lifestyle[9].

However, in developing countries like Nepal, rapid urbanization and changes in lifestyles that adversely affect carbohydrate and fat metabolism are causing a large increase in the number of type 2 diabetes in Nepalese urban population is second highest after India among various Asian populations[1,2]. The present study aimed to determine the role of antioxidants and free radicals in diabetes subjects attending Kantipur Dental College Teaching Hospital.

Methods

The study was carried out in the Department of Biochemistry, Kantipur Dental College Teaching Hospital & Research Center, Basundhara, Kathmandu, Nepal.12-hour fasting samples were collected from the outpatient department. A total of hundred (100) individuals suffering from diabetes more than one year were enrolled for the study whereas freshly diagnosed diabetes were excluded. Correspondingly, hundred (100) healthy individuals were selected as healthy controls. The age of diabetic subjects ranged from 35 to 60 years, hemoglobin levels were also estimated by cyanmethemoglobin method using Drabkin solution for the enzymatic antioxidant analysis. Venous blood was withdrawn from cases as well as control subjects. Lipid peroxidation as well as antioxidants (enzymatic and non-enzymatic) were estimated using standard protocols. Lipid peroxidation product was estimated using thiobarbituric acid reactive substances (TBARS) method, which measures pink colored malondialdehyde (MDA) reactive products (Buege and Aust method)[10] at 535nm. The total lipid hydroperoxide (LOOH) was determined by Jiang method[11]. LOOH was determined by their ability to oxidize ferrous ion under acidic condition in the presence of xylenol orange resulting in the formation of chromophore at 560nm. Nitrate and nitrite levels were estimated using reduced cadmium by the method of Cortas and Wakid[12]. Superoxide dismutase (SOD) activity was determined by Kakkar method[13] based on the 50% inhibition of the formation of nicotinamide adenine dinucleotide (NADH)-phenazine methosulfate-nitroblue tetrazolium formazan at 560nm. Catalase (CAT) activity was assessed by Sinha method[14] in which hydrogen peroxide was used as a substrate and the decrease in hydrogen peroxide concentration in phosphate buffer was read at 240nm. Glutathione peroxidase (GPx) was estimated by the method of Rotruck[15]. Reduced glutathione (GSH) was measured by Beutler method[16]. Vitamin A was estimated by Bessey OA[17] method at 327nm, Vitamin C was estimated by Natelson 2,4-dinitrophenyl hydrazine (DNPH) method[18], Vitamin E was measured by Baker method[19], total antioxidant activity (TAA) was estimated by Benzie and Strain method[20]. Values are presented as mean ± standard deviation. Statistical analysis of the data was carried using SPSS. Student's t-test was performed to find out the differences between diabetic case and control groups and p-value less than was considered as significant statistically.

Results & Discussion

Free radicals are substances that easily react with biomolecules and is accountable for the etiopathogenesis of several diseases including diabetes. Oxygen derivatives, which constitute a large portion of free radicals which gives rise to reversible or irreversible damages to nucleic acids, proteins, amino acids, lipids, carbohydrates and connective tissues. In this study we designed to investigate relation between free radicals and antioxidants in healthy and diabetic subjects. For this purpose, TBARS, LOOH, nitric oxide, as lipid peroxidation; enzymatic antioxidants- SOD, GPX, CAT and non-enzymatic antioxidants- Vitamin A, Vitamin C, Vitamin E, glutathione, TAA were evaluated.

The TBARS levels were increased in diabetic cases as compared to their healthy counterparts. Similarly, lipid hydroperoxide and nitrite and nitrates levels too were found to be increased in the

Table 1. Lipid peroxidation parameters of diabetic versus control subjects.

Lipid Peroxidation parameter	Unit	Case Mean ± SD	Control Mean ± SD	p-value
TBARS	nmol/ml	3.2±0.12*	1.1±0.24	0.032
Lipid hydroperoxide	nmol/ml	7.21±0.23*	3.28±0.15	0.029
Nitric oxide-NO_2	µmol/L	6.5±0.22*	3.22±0.83	0.041
Nitric oxide-NO_3	µmol/L	38.6±1.85*	28.42±1.12	0.021

Note: *= p<0.05 (level of significance)

Table 2. Enzymatic and non-enzymatic parameters of diabetic versus control subjects.

Unit	Antioxidants	Case Mean ± SD	Control Mean ± SD	p-value
µg/dl	Vitamin A	59.38±3.91*	88.62±10.62	0.011
mg/dl	Vitamin C	0.41± 0.11	1.1± 0.08	0.055
mg/dl	Vitamin E	0.75±0.03	0.92±0.21	0.062
µmol/L	TAA	600.12±2.24*	719.25±1.27	0.039
mg/dl	Glutathione	21.34±6.73*	59.15±8.16	0.035
U/gHb	Catalase	698.26±22.47*	460.17±12.38	0.010
U/gHb	SOD	49.61±2.12*	22.18±2.24	0.018
U/gHb	GPX	5.36±0.34*	1.42±0.28	0.008

Note: *= p<0.05 (level of significance)

cases as compared to normal healthy subjects. The increase in the lipid peroxidation parameters were significant as compared to their respective controls as shown in Table 1. The decrease in level of non-enzymatic antioxidants in diabetic subjects were also found to be statistically significant as shown in Table 2. The enzymatic antioxidants SOD, CAT and GPx of diabetes cases were statistically increased because of increased oxidative stress and as a result the non-enzymatic antioxidants were found to be diminished. Several studies have shown the chronic hyperglycemia induces an increase in oxidative stress in diabetic cases. Our results are in accordance with those of previous findings which shows that increased glucose level induces overproduction of oxygen free radicals and consequently increases the protein oxidation and lipid peroxidation[21-25]. Undeniably, the plasma concentration of MDA is a final product of the peroxidation of polyunsaturated fatty acids and diabetic subjects showed its increased level as compared with controls. These results agree with previous studies which expresses that glycemic control plays an important role in peroxidation of fatty acids and the well-controlled diabetic patients demonstrate a lower level of lipid peroxidation[1-5].

During oxidative stress, there is excessive generation of free radicals and its derivatives which are capable of causing extensive damage at cellular and tissue levels resulting in a variety of untoward patho-physiological conditions. Numerous reports show preventive, protective and curative role of natural antioxidants, especially vitamin C and Vitamin E against increasing oxidative stress.

It is well known since long time that carbohydrates, proteins and lipids are biologically active molecules involved in various metabolic and energy yielding processes of cellular systems. Metabolic disturbances of any of these molecules are known to contribute several chronic pathological states[21-25]. Free radical mediated lipid peroxidation is involved in many pathological processes and biological systems possess self-defensive mechanism against these peroxides mediated through enzymatic and non-enzymatic systems. Erythrocytes are intrinsically prone to oxidative stress because they are exposed to high oxygen tension and have a characteristic structural composition with polyunsaturated fatty acids in the membrane, besides the presence of hemoglobin bound iron. However, membrane and cytoplasmic compartments of RBC have an efficient antioxidant mechanism that maintains their integrity[26]. A detoxifying system consists of reduced glutathione, SOD, CAT, GPx and vitamin E prevent oxidative damage. In addition, there is also a system consisting of NADPH-dependent methemoglobin reductase, vitamin C, glutathione reductase whose main role is the repairing of damage that follows oxidative stress[27].

Tuleb SF, 2016 research presented increase in serum MDA, oxidized LDL and decreases in serum GSH, vitamin C, uric acid in diabetic population and increased non-enzymatic glycosylation and autooxidation of glucose is the possible mechanism in the excessive production of free radicals which induce lipid peroxidation

and increased MDA concentration[22]. Chronic hyperglycemia is the hallmark of diabetes mellitus, disproportionate amounts of glucose are delivered to the cells, results in enhanced glucose flux through glycolysis and tricarboxylic acid cycle leading to overdrive of the mitochondria electron transport chain, which generates greater amounts of superoxide radical more than mitochondrial SOD can dismutase. This tilts the normal delicate balance between mitochondrial ROS production and mitochondrial ROS degradation in favor of mitochondrial ROS generation, and oxidative stress ensues[26-28].

Wild et al, 2004 work concluded that the number of diabetic cases is increasing due to population growth, aging, rapid urbanization, increasing prevalence of obesity, physical inactivity and prevalence of diabetes in men are higher than women. Gyawali et al investigation on type 2 diabetes revealed high burden disease in Nepal and suggested a possible area of deliberately expanding preventive interventions as well as efforts to control the disease over weight, patients were found to be more prone to develop diabetes in comparison to non-obese patients. Reactive oxygen species are recognized to participate in pathogenic processes of numerous diseases such as cardiovascular, cancer, cataract, rheumatoid arthritis, neurodegenerative diseases. Oxidative damage to biomolecules causes deleterious effect and also influences ROS on gene regulations and immune system might impair bodily functions[22-24,29,30].

Conclusion

Oxidative stress is increased in diabetic patients as compared to healthy controls supporting hypothesis that links with the etio-pathophysiology of diabetes mellitus. Avoiding oxidative sources such as cigarette smoking, tobacco chewing/ consumption, alcohol, unhygienic/ fast foods/ unbalanced diet, stress must be considered and consumption of traditional balanced diet, spices rich in antioxidants on regular basis must be implemented. It is also necessary to generate public alertness about the possible impact of sedentary lifestyle and obesity on diabetes.

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