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Original Research Article

Association of systemic inflammation and oxidative stress in adolescent diabetes mellitus patients

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Abstract: Oxidative stress has been implicated in the pathophysiology of a number of diseases such as arthritis, hypertension and inflammatory diseases. Although previous evidences provided extensive literature about the biological role of antioxidant enzymes in diabetes mellitus (DM), there is a paucity of satisfactory explanation regarding the alteration in the level of antioxidant enzymes along with marker of systemic inflammation in adolescent DM patients. Therefore, the objective of present study was to estimate the level of C-reactive protein (CRP), Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GSHPx) and Ceruloplasmin in adolescent DM patients. 20 patients of either sex (10-19 years age group) suffering from DM and 20 normal healthy individuals served as control; were included in the study. Above mentioned parameters were estimated using standard methods and data from patients and controls were compared by using Student's t-test. Erythrocyte SOD, CAT and GSHPx activity were significantly low in DM subjects ($P<0.001$) whereas plasma ceruloplasmin level was found to be significantly high ($P<0.001$) as compared to healthy controls. These findings suggest that combined effect of inflammation and free radical generation is involved in the pathogenesis of DM, characterized by imbalance in antioxidant enzyme status and enhanced CRP levels, which served as an excellent marker of oxidative stress and systemic inflammation in adolescent diabetes mellitus patients.
Keywords: Antioxidant enzymes, Catalase, Ceruloplasmin, C-reactive protein, superoxide dismutase

INTRODUCTION:

Despite massive efforts, diabetes mellitus is still a major medical condition that affects the quality of human life of developed and developing countries as well [1, 2]. Previous evidences suggest that reactive oxygen species derived from molecular oxygen (Superoxide anion, hydrogen peroxide and hydroxyl radical) contribute to the diabetes mellitus and its associated complications [3, 4]. Moreover, hyperglycemia has been found to be the causal link between diabetes and increased oxidative stress [5]. A number of sources of free radical generation have been identified in biological tissues, which may be mutually interactive and once triggered, they may lead to loss of antioxidant defense system. The defense system to combat the potentially damaging effects of free radical species includes antioxidant enzymes and antioxidants. Superoxide dismutase (SOD, EC: 1.15.1.1), Catalase (CAT, EC: 1.11.1.6), Glutathione peroxidase (GSHPx, EC: 1.11.19) and Ceruloplasmin (EC: 1.16.3.1). SOD catalyses the conversion of superoxide radicals to hydrogen peroxide and molecular oxygen. Hydrogen peroxide is further detoxified by either heme containing enzyme CAT or selenium containing enzyme GSHPx. Ceruloplasmin, is a blue colored copper binding protein, function as antioxidant enzyme by virtue of its ferroxidase activity and scavenges superoxide anion radical [6,7].

Alterations in the levels of these antioxidant enzymes with subsequent increased ROS accumulation leads to the activation of stress-sensitive intracellular signaling pathways that, in turn, promote cellular damage and contribute to the diabetic complications development and progression[5]. C-reactive protein (CRP), a marker of systemic inflammation and synthesized in liver, has been received considering attention in hyperglycemia associated disorders such as diabetes mellitus. In addition, previous studies have demonstrated an association between diabetes

progression and inflammation as measured by plasma C-reactive protein [8].

Although several evidences provided extensive literature about the role of antioxidant enzymes and inflammation in DM, there is a paucity of satisfactory explanation regarding, alteration in the level of these antioxidant enzymes and systemic inflammation in adolescent DM. In addition, as best of our knowledge, previous studies on DM patients have not included systemic inflammation and oxidative stress in a single setting Therefore, the objective of present study was to estimate the level of these antioxidant enzymes and systemic inflammation in DM patients and statistically determine the variation in their level by comparing it with that of healthy subjects served as control.

MATERIAL AND METHODS:

In the present study 20 patients of either sex with diabetes mellitus belonged to age group 10-20 years and 20 age matched healthy individuals, served as control, were taken. A general information or pre-experimental questionnaire regarding demographic information, family history and limited physical examination including blood pressure measurement was completed from all the subjects after taking their informed consent and approval of protocol by ethics committee of college.

Inclusion criteria:

The inclusion criteria adopted were: age 10 to 19 years, newly diagnosed and untreated cases of Type 2 diabetes. Diabetes mellitus was diagnosed by following the American Diabetes Association criteria 2015 [9].

Exclusion criteria:

Patients suffering from cardiovascular disease, hepatic disease, tuberculosis, renal disease and taking drugs like steroid, amiodarone, lithium, antioxidant vitamin supplement or non-steroidal anti-inflammatory drugs, antihypertensive drugs and other medications that alter thyroid functions and lipid levels led to exclusion from the study.

Fasting blood samples were collected in EDTA vials from the anticubital vein of the study group subjects and processed immediately. Erythrocyte SOD activity was measured by Marklund and Marklund's method. The enzyme SOD inhibits the auto-oxidation of pyrogallol by catalyzing the breakdown of superoxide. The inhibition of pyrogallol oxidation by SOD is monitored at 420 nm and the amount of enzyme producing 50 % inhibition is defined as one unit of enzyme activity [10].

Plasma ceruloplasmin levels were estimated by Ravins's method (1961). Ceruloplasmin due to its oxidase activity, catalyses the oxidation of substrate p-Phenylene diamine chloride into purple coloured oxidation product, measured spectrophotometrically at 530 nm [11].

Erythrocyte glutathione peroxidase (GSHPx) activity was estimated by Beutler's method (1971), after preparation of hemolysate. GSHPx catalyse the oxidation of reduced glutathione (GSH) to oxidized glutathione (GSSG) by H_2O_2. The rate of formation of GSSG is measured by means of glutathione reductase reaction in which NADPH is oxidized and measured at 340 nm [12].

Erythrocyte catalase activity was estimated by Goth's method (1991) which involves the enzymatic breakdown of H_2O_2 under optimized condition followed by spectrophotometric assay of H_2O_2 (405 nm) based on formation of its stable complex with ammonium molybdate [13]. Plasma CRP levels were measured using commercially available ELISA kits (R&D Systems, USA), according to manufacturer's instructions.

STATISTICAL ANALYSIS:

The data collected from study group subjects were entered separately in Microsoft Excel sheet of windows 2007 and values were expressed as Mean ± SD. The significance of mean difference between study group subjects was compared by using Student's t test. The distribution of't'- probability was calculated depending on 'n' and significance of test was obtained. P value <0.05 and <0.001 were considered as significant and highly significant respectively.

RESULTS:

The level of antioxidant enzymes and systemic inflammation in adolescent DM patients and controls were depicted in Table 1. In the present study, erythrocyte SOD and GSHPx levels were significantly decreased in patients with DM (P <0.001; 42.0% low and P <0.001; 42.15% low respectively) as compared to controls. Plasma catalase activity was also significantly low in DM patients (P<0.001; 45.25% low) whereas plasma ceruloplasmin level was significantly elevated in DM subjects as compared to controls (P<0.05; 29.89% high). Plasma CRP levels were found to be significantly high (P<0.05; 32.92% high) in patient group as compared to healthy controls which reflect the role of inflammation and oxidative stress in disease process.

Table 1: Marker of systemic inflammation and antioxidant enzymes in study group subjects (Mean ± SD)

S.No.	Particulars	Control Group (n=20)	Patient group (n=20)	Level of Significance	% Increase	% Decrease
1.	CRP (mg/L)	3.25 ± 0.13	4.32 ± 0.16	P < 0.05	32.92 %	
2.	SOD (U/gm Hb)	1928.36 ±233.55	1118.42±313.48	*P < 0.001*	-	42.0
3.	Ceruloplasmin (mg %)	25.52 ± 4.7	33.15 ± 6.4	P < 0.05	29.89	-
4.	GSHPx (IU/ gm Hb)	32.5 ± 4.6	18.8 ± 3.5	*P < 0.001*	-	42.15
5.	Catalase (KU/L)	53.7 ± 16.5	29.4 ± 5.6	P < 0.001	-	45.25

Where, *p<0.1: Non-significant, **p<0.05: Significant , **p<0.001: Highly significant

DISCUSSION:

Adaptation of sedentary life style and changes in dietary pattern have been found to be associated with enhanced risk of disease development, even in early stages of life and establishment of diabetes in young adults is not a surprising incidence. Moreover, involvement of reactive oxygen species in association with sedentary life style have been implicated in the pathogenesis of many disease processes including diabetes mellitus [1, 2]. Superoxide anion, which is believed to be one of the initiators of free radical mediated pathological alterations leading to disease process, is efficiently removed by antioxidant enzyme SOD [14]. In the present study, we observed that the SOD activity in DM patients was significantly low (P<0.001) as compared to healthy controls, which direct towards its protective and superoxide radical scavenging action in DM patients. Our findings were in accordance with the findings of Kimura et al. According to them, reduced activity of SOD could be the result of inter and intramolecular cross-linking of proteins and thereby causing conformational changes in SOD which leads to accumulation of H_2O_2 followed by induction of lipid peroxidation leading disease progression [15].

Superoxide anion ($O_2^{\cdot-}$) scavenging action of SOD can be mimicked by other copper containing enzyme ceruloplasmin also has the capacity to scavenge $O_2^{\cdot-}$. In the present study, plasma ceruloplasmin level was found to be significantly increased (P<0.001) in DM patients as compared to healthy controls which indicate that high ceruloplasmin level is associated with its antioxidant activity to protect the body tissues against the deleterious effects of oxygen free radical and to compensate the loss of SOD activity, occur due to oxidative stress in DM patients [16]. Among the enzymatic systems of protecting the cell against free radical injury, GSHPx and CAT play a crucial role in the final detoxication of H_2O_2 to H_2O. In the present study, low GSHPx and CAT activity were observed (i.e. p<0.05 and p<0.001 respectively) in DM patients as compared to controls. Cojocaru *et al*. and Goyal *et al*. in their separate studies on DM patients also observed a direct relationship of free radical production with DM progression and concluded that reduced level of CAT and SOD may be due to their consumption during metabolism of oxygen free radicals. However, it is unclear whether the reduced activity of antioxidant enzymes is the cause or the consequence of the increased oxidative stress in DM [17, 18].

Free radicals and their intermediates are also serve as a mediator of inflammation in inflammatory disorders by promoting various culprit events such as inhibition of glycolytic enzymes, reduction of antioxidant reserves in body fluid, activation of proteolytic enzymes and induction of lipid peroxidation [19]. In the present study, plasma CRP levels were found to be significantly high (p<0.001) in DM patient which clarify the combined role of inflammation and oxidative stress in the etiopathogenesis of DM and its related complications [8, 20, 21].

CONCLUSION:

Thus, it can be inferred that alterations in antioxidant enzymes and CRP are excellent markers of oxidative stress and systemic inflammation in DM patients. It also authenticate the fact that combined effect of inflammation and oxidative stress plays a significant role in the etiopathogenesis of DM even in early stages of life and the patients are unable to counteract the augmented oxidative stress effectively. Thus, antioxidant supplementation in diet/drug regime regularly, avoidance of junk food and regular physical excercise along with regulation of blood glucose levels may reduce the DM progression and its complications.

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