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The Effect and Clinical Relevance of Superoxide Dismutase and RBC Glutathione Levels in Type-2 Diabetic Male and Female in Kolkata, West Bengal, India

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Abstract: Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. This study reveals the role of antioxidants enzymes SOD and RBC Glutathione as major source of antioxidant status in type-2 diabetes. A total 360 subjects were involved in the study of which 90 were male diabetics (cases), 90 were female diabetics (cases) and 90 male non diabetics(control), 90 females non diabetics (control). The mean values SOD and RBC Glutathione were significantly lower (p<0.05) among cases for both sexes compared to control. The mean values for FBS, PPBS, HbA1c%, were significantly higher (p<0.05) among cases for both sexes compared to controls. In DM there is increased level of oxidative stress in humans.
Keywords: Diabetes mellitus, Superoxide dismutase, RBC Glutathione, Antioxidants, Hyperglycemia

I. INTRODUCTION

Diabetes mellitus may be recognized as a group of metabolic disease which might be characterized by increased level hyperglycemia which is a result of the defects in insulin secretion and it's consecutive action or both.(1) Chronic hyperglycemia is associated with the damages in long run, of various organ in the body which includes eyes, kidneys, nerves, heart and blood vessels etc. It has also been seen that there is altered metabolism of carbohydrates lipids, proteins with increased risk of vascular disease. Regardless of the exact mechanism the reactive oxygen species (ROS) has been implicated to gear these diseases in the humans. Reactive oxygen species or free radicals has a very important role either in-vivo or in-vitro of lipids resulting in tissue damage and death of affected cells.

The primary target of ROS attack are PUFA(poly unsaturated fatty acid) in a membrane lipids causing lipid peroxidation which leads to disorganization of cells structure and function.(2)The harmful effect of ROS are counter attacked by class of protective agents termed as antioxidants which prevents the oxidative damage by reaction of free radicals before any other molecule can become a target.

The major types of antioxidants enzymes are superoxide dismutase (SOD), catalase, RBC Glutathione which are found to play a very important role in the protection of cells and tissues.(3,4). The evidence suggests that the oxidative stress plays a role in the diabetes mellitus pathogenesis and complication (5).

Hyperglycemia always increase oxidative stress which contribute to the main process of impairment that is the insulin action and insulin secretion .

Thus to protect the cell and the organ of the body against ROS humans have a very complex antioxidative protection(6,7,8). Free radicals has been found to effect the insulin signaling cascade (9). In certain case oxidative stress caused by hyperglycemia in diabetes patients impair insulin signaling which leads to insulin resistance and certain other mechanism which have not been completely established. (10,11).

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Several studies have also shown that the diabetes mellitus is accompanied by increased formation of free radicals and decreased antioxidant activity which leads to oxidative damage of cell component.(12). Under diabetic condition the reactive oxygen species are produced through the glycation reaction which occurs in various tissues playing the major role in the development of diabetic complication (13). Although the induction of glycation reaction in diabetes has been found in neural cells and lens crystal which are actually known as target diabetic complication. In this background a study has been formulated to estimate the antioxidant status in chronic type 2 Diabetes mellitus (T2DM) patients.

II. MATERIALS AND METHODS

The study was carried out at Department of Biochemistry, Calcutta National Medical college and Hospital, Kolkata, West Bengal. Total three hundred and sixty (360) subjects age between 18 to 45 years constituted the study population. One hundred and eighty (180) apparently healthy and non diabetic were used as control and one hundred and eighty (180) diabetes used to test subjects. A total one hundred and eighty (180) males and one hundred and eighty (180) females were involved in the study. These subjects of both the sexes were categorized under three age groups such as 18 to 26 years, 27 to 36 years and 37 to 45 years. In females samples are collected in between 1-4 days of menstrual cycle. The research protocol was approved by ethics committee of the Institution. Sample collected aseptically from cases and control was subjected to serum analysis. Proper vein puncture technique was employed in the collection of the 5m blood sample from the subjects. Blood samples was centrifuged at 3000rpm for 10 minutes Fasting blood glucose and post pondrial blood glucose were measured by GOD-POD method(14) and HbA1c% was measured by Ion exchange resin method by coral clinical system kit. The superoxide dismutase (SOD) was performed by Kakkar's et. al method (15) and Glutathione peroxidase was performed by DTNB method(16).

Data generated were analyzed using statistical package for social science (SPSS) version 20.00 and Microsoft excel 2007.

Comparison mean and standard deviation values were made for the various parameters for test and control subjects using student-t test. Results were considered statistically significant 95% confidence interval ($p<0.05$).

III. RESULTS

Age group (years)	18 to 26		27 to 36		37 to 45	
Male	Case (Mean and SD)	Control (Mean and SD)	Case (Mean and SD)	Control (Mean and SD)	Case (Mean and SD)	Control (Mean and SD)
SOD(milliunits/ g of hb)	3.51± 0.65	5.15±0.87	3.44±0.57	4.18±0.42	2.99±0.75	4.10±0.81
Glutathione(miu M/g of hb)	11.65±1.39	16.68±3.22	8.35±0.39	15.86±2.06	7.58±0.59	15.59±3.80
FBS (mg/dl)	175±5.45	78±5.63	201±7.40	88±5.34	167±8.58	98±7.01
PPBS(mg/dl)	254±16.99	102±13.51	236±8.23	110±8.44	210±9.35	115±6.09
HbA1C%	9.10±0.71	4.15±0.57	7.4±0.66	4.15±0.65	7.53±1.10	4.25±0.66
P value	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05

Table:-1 Age based comparison between case & controls for serum SOD, RBC Glutathione, FBS, PPBS and HbA1C% parameters among males.

Age group (years)	18 to 26		27 to 36		37 to 45	
Female	Case (Mean and SD)	Control (Mean and SD)	Case (Mean and SD)	Control (Mean and SD)	Case (Mean and SD)	Control (Mean and SD)
SOD(milliunits/g of hb)	2.50±0.57	5.32±1.17	3.21±0.88	4.58±0.50	2.83±0.34	4.09±0.45
Glutathione(miuM/ g of hb)	10.21±0.98	15.94±2.61	7.24±1.40	14.67±1.37	8.25±0.27	15.4±0.65
FBS (mg/dl)	179.96±16.70	82.33±8.35	159.33±10.01	92.89±3.92	158.43±9.29	105.10±3.18
PPBS(mg/dl)	232.76±36.77	105.16±9.30	222.80±25.60	111.58±5.68	210.96±13.57	118±5.73
HbA1C%	9.64±1.00	4.67±0.67	8.13±0.77	4.54±0.49	7.53±0.26	5.17±0.32
P value	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05

Table:-2 Table:-Age based comparison between case & controls for serum SOD, RBC Glutathione, FBS, PPBS and HbA1C% parameters among females

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IV. DISCUSSION

The result of this study showed that the mean and Sd values of FBS, PPBS, HbA1c%, SOD and RBC Glutathione where calculated for all Type 2 Diabetic cases and controls. The mean and Sd values of FBS, PPBS, HbA1c%, were significantly higher among cases **(** $p \leq 0.05$**)** for both sexes compared to controls as shown in Table 1 , 2

The mean and Sd values of SOD and RBC Glutathione for type -2 diabetics were significantly lower ($p \leq 0.05$**)** when compared with that of the control subjects as shown in Table 1 and 2.These findings show that type-2 diabetes is associated with decrease in antioxidant enzyme resulting in increase oxidative stress. These findings do agree with Djordjević et. al. findings which discovered a reduced antioxidative defense in patients with type-2 diabetes mellitus. Similarly in Table -1 and Table -2 ,the level of antioxidant SOD and glutathione were found to be significantly lower in diabetic patients than that of control subjects. These findings also show that type-2 diabetes is associated with decrease in antioxidant enzyme resulting in increase oxidative stress. These findings also do agree with Djordjević et. al. findings which discovered a reduced antioxidative defense in patients with type-2 diabetes mellitus (17).

Our finding also show that the level of antioxidant enzymes SOD and RBC Glutathione decreases with ages of diabetic subjects. This finding is similar with that of with Chandankhede et.al and Bohr et. al.(18,19) With increase in mitochondrial ROS production endogeneous antioxidant activity get diminished in aging individuals. Excessive production of ROS and reduced antioxidant defence with age significantly contribute to aging. This findings agrees with the literature of Poljsak and Milisav, 2013) (20)

V. CONCLUSION

From this research paper it has been found that type-2 diabetes is associated with decreased antioxidative status as the levels of antioxidant enzymes like SOD and RBC Glutathione were found to be significantly reduced in diabetic patients. This is because hyperglycemia is the cause of type-2 diabetes which increases the generation of ROS and that might depress the antioxidant defense system exposing the cells to damage from oxidative stress which leads to the development of diabetic complication. Moreover with the increase in ROS production of the antioxidant enzymes can suppressed the antioxidant system as well as its adaptive responses.

It can be concluded that T2DM is related to decreased antioxidative status as result in decrease in level of antioxidant enzymes SOD and RBC Glutathione where reduce significantly in diabetic subjects. The antioxidative system can be depressed if the subject is suffered from the illness for a prolonged period of time. Progression of disease is inversely proportional to antioxidative parameters, that is, antioxidative parameters are depleted with the progress in the condition of the disease.

It can be suggested that the antioxidant therapy is to be incorporated in the management of type-2 diabetes to supplement the antioxidative system as this prevent the progression of disease and other complication in diabetes. These imbalances lead to the tissue damage and inflammation in disease thus it can be said the antioxidant therapy along with conventional drugs should be used to prevent the disease progression.

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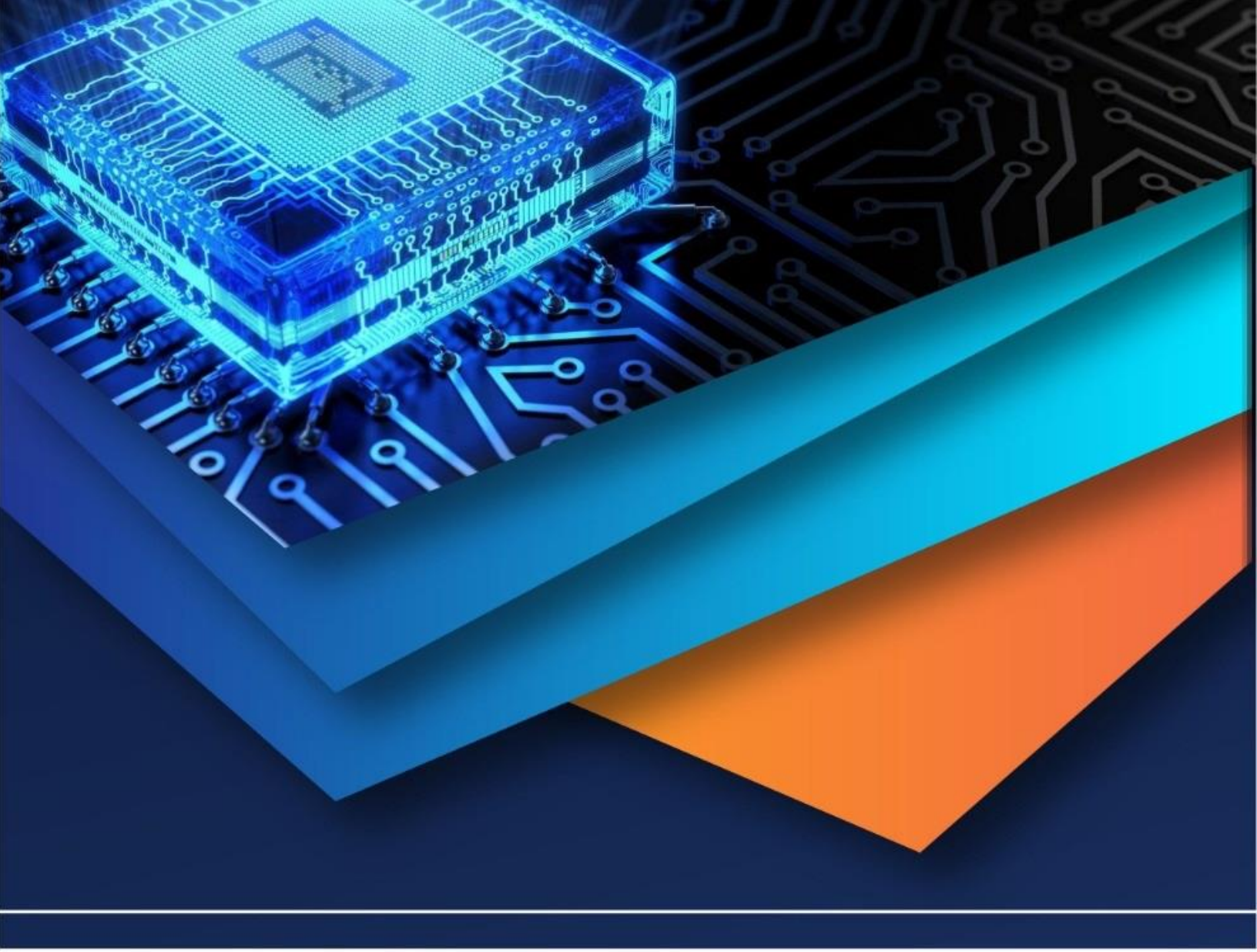

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