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Effect of hyperglycemia on superoxide dismutase defense system and erythrocyte indices in diabetic patients

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Abstract: The Oxidative stress in diabetes is the result of persistent hyperglycemia that may cause several perturbations in biochemical constituents as well as variations in Erythrocyte indices that may ultimately result in the pathogenesis of various complications. The antioxidant defense system that exists naturally in human body is altered during the hyperglycemic states. In this study the effect of hyperglycemia on superoxide dismutase level along with variations in erythrocyte indices were determined in type -2 diabetic patients and compared with normal health control subjects. The blood samples were collected from 38 control subjects and 51 diabetic patients (type 2) and their blood glucose level was estimated using the Randox kit method, the HbA1c was analyzed by automatic analyzer Backman Coulter. The estimation of the antioxidant enzyme Superoxide dismutase (SOD) activity was also done using the Randox kit on chemistry analyzer. The hematological parameters were assayed using the ABX micros 60 hematoanalyzer. The results were evaluated statistically by using Graph pad prism (5.0). A significant elevation was found in the Fasting blood glucose level, HbA1c, RBCs Count, hemoglobin concentration, hematocrit value and MCH level of diabetic patients in comparison with the control ($P<0.001$). Whereas significant elevation in SOD level was also found in the diabetic patient when compared with control subjects ($P< 0.001$). The study revealed that poor glycemic control (high FBG and HbA1c level) is positively correlated with high levels of Erythrocyte indices i.e. RBCs, HGB, HCT, MCV, MCHC along with elevated level of SOD and can be used as potential indicators in finding the risk of developing micro and macro vascular complications in diabetic patients. The analysis of these parameters may be useful if included in routine lab tests to prevent patients who are on increased risk of developing complications.

Keywords: Diabetes Mellitus, HbA1c, MCV, Mean Corpuscular hemoglobin concentration, superoxide dismutase**.**
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INTRODUCTION

Diabetes mellitus, a metabolic syndrome manifested by varying degree of hyperglycemia, is also accompanied with the biochemical alterations in carbohydrate, protein and lipid metabolism, associated with various micro and macro vascular complications, such as renal failure, nerve damage and atherosclerotic plaques[1]. The increase in the incidence of diabetes is largely due to the changing lifestyles, including excessive intake of calories, less physical activity and sedentary lifestyle, which ultimately may lead to obesity, insulin resistance, resulting in impaired glucose tolerance and type 2 diabetes[2]. In the recent decades, it has become evident that hyperglycemia has been the leading cause of the excessive generation of highly reactive free radicals that may ultimately lead to the oxidative stress[3]. The imbalanced production of free radicals may result through the enhanced oxidative phosphorylation, glucose autoxidation, non-enzymatic protein glycation and increased flux of glucose through sorbitol pathway. Consequences, such as formation of advanced glycation end (AGE) products, lipid peroxidation, and altered expression of certain genes may result, and these may cause perturbations in various biological molecules, leading to the tissue damage. Also, the entry of the altered levels of biochemical and tissue products, as a result of hyperglycemia and the associated oxidative damage, into the blood and their interactions with the blood constituents, may cause the structural and certain functional abnormalities of the blood cells[4].

For the prevention of oxidative damage to the tissues, there exist a natural defense mechanism, that possess several components including antioxidant enzymes (Superoxide Dismutase, Glutathione system and Catalase) and other small molecules such as nutrient derived antioxidants (Ascorbic acid, carotenoids etc.) that have the scavenging ability[5].The insufficient removal of these free radicals, due to the alterations in the defense system, may also result in vascular dysfunction that may contribute to the pathogenesis of diabetic complications. The damaging effects of elevated toxic radicals are due to an increase in the formation of superoxide radicals within cells which causes inactivation of superoxide dismutase enzyme in hyperglycemic condition. This affects the tissue damage and secondary complications in diabetes mellitus[6]. A deficiency of the antioxidant activity of superoxide dismutase and glutathione peroxidase has been related to higher concentration of peroxide. There may be imbalance between production and scavenging of free radicals produced due to the lack of antioxidant system[7]. The status of antioxidant defense mechanisms in diabetes mellitus is very contradictory; as both increases and decreases of antioxidant status have been reported in diabetes mellitus[8]. Hyperglycemia associated oxidative damage may cause the morphological and functional abnormalities of blood cells. Hemorheological parameters in diabetes mellitus are often disturbed. These parameters include hematocrit,

plasma proteins, erythrocyte aggregation, and erythrocyte deformability. The abnormalities associated with each of these parameters have been shown to markedly increase both plasma and whole blood viscosity (WBV).Whether and not blood viscosity determines blood flow resistance and microcirculation, and moreover, whether or not increases in viscosity can lead to the development of microvascular complications. In addition, when the diameters of the capillaries of the eye and kidney are reduced in patients with diabetes because of the thickening of the basement membrane of capillaries[9]. The altered capillary structure increases flow resistance, leading to the impaired microcirculation. This resultant disturbance may be a risk factor for the progression of retinal failure in diabetic retinopathy and renal failure in diabetic nephropathy.

Hyperglycemia is a crucial feature in diabetes. Abnormal glycation, which can adversely affect hemoglobin and membrane proteins in erythrocytes, has been shown to correlate with reduced membrane fluidity[10]. Separately, high values of glycosylated hemoglobin have been found to correlate with decreased deformability of erythrocytes[11]. Several studies revealed that poor glycemic control with imbalance between production and scavenging of free radical due to lack of antioxidant defense system may affect the hematological indices that leads to micro and macro vascular complications in diabetic patients. The use of oral hypoglycemic drugs along
antioxidant dietary supplements can prevent the patients for further complications.

MATERIAL AND METHODS

The study was conducted on 51 diabetic patients (Type-2), both males (n =23) and females (n=28) between the age group 35-65 years who were registered at the diabetic clinic of Baqui Institute of Diabetology and Endocrinology Karachi, Pakistan. 38 age and sex matched control subjects (18 males and 20 females) were also selected from the general population at random for comparison. Ethical approval was obtained from the institutional ethical review board (IERB) before the commencement of the study. Informed consent of the patients was taken with the help of questionnaire. All patients who were diagnosed diabetes mellitus of type -2 using the ADA criteria of fasting blood glucose (FBG) of >126 mg/dl were included in the study. The patients with any recent critical illness were excluded from the study. The blood samples were collected in tubes with EDTA as anticoagulant and analyzed within 2 hours of venepuncture for erythrocyte indices and HbA1c. Plasma was also separated and analyzed for other fasting blood glucose level. The cell lysates were prepared from the remaining erythrocytes, for the evaluation of SOD level and stored in freezer. The stored plasma and lysates were allowed to attain room temperature before analysis. Fasting blood glucose was estimated by following glucose oxidase method on UV- visible spectrophotometer Jenway 6305.The HbA1c was analyzed by automatic analyzer Backman Coulter, Italy. Erythrocyte indices such as Red Blood Cells count (RBCs), Hemoglobin (HGB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were analyzed by using hematological analyzer ABX micros 60 fully automated analyzer HORIBA (France). The SOD activity was estimated in erythrocytes samples (cell lysates) using the commercially available Randox Kit (RANSOD, Cat. No. SD 125). Data were statistically analyzed using Graph Pad Prism (5.0) Software. Independent samples were examined with student's t test. P-values at 95% confidence intervals (CI) were also calculated. P-value of < 0.05 was taken as significant for all comparisons.

RESULTS

A total 51 diabetic patients (type 2) and 38 healthy control subjects fulfilling the selection criteria were allocated to male and female groups. Mean age of control is 45±3 years and 52±7 years for patients. Blood samples were collected and analyzed for FBG, HbA1c, erythrocyte indices and SOD level. The results of biochemical parameters and Erythrocyte indices were compared in patients and control subjects. Significant increase in FBG was found in diabetic patients 164±6.13 as compare to control group 94.36±1.82 .The level of HbA1c was also greater in diabetic patients 6.8±0.169 as compare to control group 4.79±0.052. The level of FBG and HbA1c was found increased in diabetic male and female patients when compared to their respective controls (Figures 1 and 2). The difference between diabetics and control was also significant for Super oxide dismutase (SOD) activity 250.4±12.89 and 204.13±5.77 respectively with p value of ($p<0.0001$) (Figure 3). Among Erythrocyte indices the difference of RBCs, HGB, HCT, MCV and MCHC in diabetic patients was highly significant ($p<0.0001$) as compared to healthy control subjects (Table 2).

DISCUSSION

In the present study diabetic patients and control subjects with respect to their Fasting blood glucose

level ,Erythrocyte indices, and the antioxidant enzyme, Superoxide Dismutase were investigated. The results of the diabetic patients were compared with the control subjects (Table-1). As seen in the results, the blood glucose level was significantly increased in the diabetic patients as compared to the control subjects. This indicates the disturbances in the regulation of blood glucose level, either due to the insulin deficiency or as a result of insulin resistance.

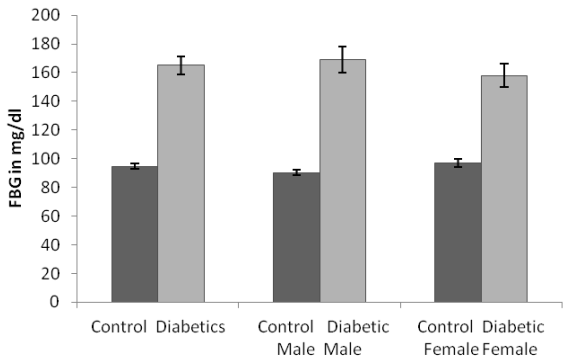

Figure 1: Fasting blood glucose (FBG) level in Diabetic patients (male and Female) in comparison with control (male and Female) subjects. Each bar represents mean±SEM. of n=51 for diabetic patients (23 male and 28 female) and n= 38 for normal control (18 male and 20 female) subjects. Where, *P<0.05 is considered to be statistically significant.

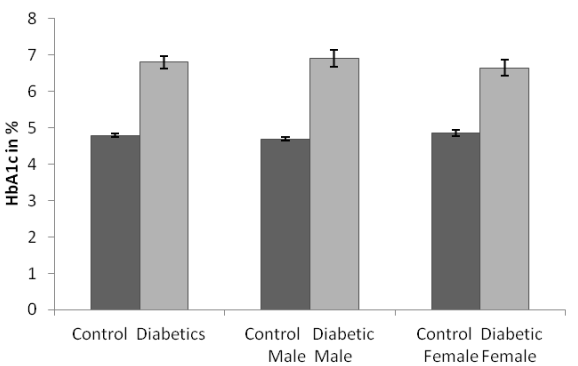

Figure 2: Concentration of glycosylated hemoglobin (HbA1c) in diabetic patients (male and Female) in comparison with control (male and Female) subjects. Each bar represents mean±SEM. of n=51 for diabetic patients (23 male and 28 female) and n=38 for normal control (18 male and 20 female) subjects. Where, *P<0.05 is considered to be statistically significant.

In the present study, the activity of antioxidant enzyme, Superoxide Dismutase was found to be elevated in diabetic patients who were statistically significant in comparison with the control subjects. The results also indicate that the SOD activity in diabetic female was higher than the diabetic male. One possible explanation may be that, the diabetic patients, irrespective of the gender, were exposed to an increased oxidative stress via lipid peroxidation. This suggests a direct correlation of enhanced lipid peroxidation with the increased SOD activity[12], and is also indicative of the later stages of diabetes mellitus. This might not be 100% true as some of the reports show an inverse relation between plasma lipid peroxidation and SOD activity[13].

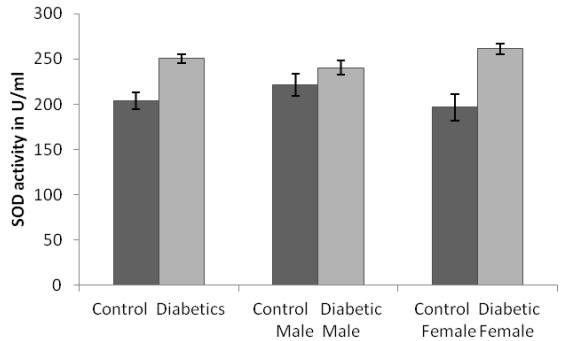

Figure 3: Superoxide dismutase (SOD) level in diabetic patients (male and Female) in comparison with control (male and Female) subjects. Each bar represents mean±SEM. of n=51 for diabetic patients (23 male and 28 female) and n=38 for normal control (18 male and 20 female) subjects. Where, * P<0.05 is considered to be statistically significant.

Table 1: Comparison between control and diabetic subjects with respect to the, FBG, HbA1c, and erythrocyte indices and SOD activity.

Parameters	Control (n=38)	Diabetic Patients (n=51)	P-Value
FBG (mg/dl)	94.36 ± 1.82	164.4 ± 6.13	0.0001*
HbA1c (%)	4.79 ± 0.05	6.8 ± 0.16	0.0002*
RBCs (10^6/mm^3)	4.33 ± 0.13	5.72 ± 0.18	0.0001*
HGB (g/dl)	12.87 ± 0.34	15.82 ± 0.60	0.0001*
HCT (%)	32.17 ± 1.09	39.22 ± 1.09	0.0001*
MCV (μm^3)	74.65 ± 2.081	81.08 ± 1.29	0.0072*
MCH (pg)	30.17 ± 1.02	28.39 ± 0.70	0.14
MCHC (g/dl)	40.35 ± 0.797	34.96 ± 0.52	0.0001*
SOD (U/ml)	204.13 ± 5.77	250.4 ± 12.89	0.0043*

Values are represented as mean±SEM

The effect of diabetes on the activity of SOD is erratic, with no discernable pattern based on gender or species of animal, or duration of diabetes, or tissue studied. The reports about the SOD activity in diabetes mellitus are controversial[14]. Some of the studies show an increase in the SOD activity, while other reports show no change in SOD activity[15].

There are also reports of decreased SOD activity in diabetic patients[16]. our result also indicate the marked increase in SOD activity in diabetic patients in comparison with control subjects which might be

due to compensatory action of enzyme SOD against oxidative stress in diabetics**.** The Erythrocyte indices were also evaluated in the diabetic and control subjects and a comparison was made to find out the relation of hematological variation with the hyperglycemia in the diabetic patients. Total erythrocyte count, hemoglobin level and the Hematocrit, mean cell volume was found to be significantly elevated in the diabetic patients in comparison with the control subjects. This might be due to the fact that erythrocytes are continuously subjected to various morphological changes, due to the compositional changes in plasma, associated with some variation in types 1 and 2 diabetes[17].

Table 2: Variations of FBG, HbA1c, erythrocyte indices (RBCs, HGB, HCT, MVC, MCH and MCHC) and SOD status in control and diabetic subjects on the bases of sex.

Variables	Control (n=38)		Diabetic Patients (n=51)	
	Male (n=18)	Female (n=20)	Male (n=23)	Female (n=28)
FBG (mg/dl)	90.34 ± 2.92	96.97 ± 2.24	*169 ± 8.91	*158 ± 8.21
HbA1c (%)	4.69 ± 0.08	4.86 ± 0.06	*6.91 ± 0.24	*6.65 ± 0.22
RBCs (10^6/mm^3)	4.46 ± 0.25	4.278 ± 0.16	*5.05 ± 0.18	4.65 ± 0.215
HGB (g/dl)	14.6 ± 0.38	12.11 ± 0.30	*15.25 ± 0.71	*14.93 ± 0.51
HCT (%)	35.70 ± 1.92	30.63 ± 1.17	*42.35 ± 1.34	35.83 ± 1.1
MCV (μm^3)	80.00 ± 1.36	72.31 ± 2.75	*84.0 ± 0.86	77.83 ± 2.21
MCH (pg)	33.07 ± 1.15	28.90 ± 1.26	29.59 ± 0.30	27.1 ± 1.35
MCHC (g/dl)	41.29 ± 1.27	39.95 ± 1.00	35.23 ± 0.30	34.67 ± 1.05
SOD (U/ml)	221.43 ± 11.93	196.56 ± 5.73	*240.38 ± 17.79	*261.25 ± 19

Values are represented as mean±SEM

The increase in the glucose concentration is one of the changes that effect the erythrocyte morphology i.e., the severity in the change of erythrocyte shape depends upon the plasma glucose level. This in turn, causes changes in deformability and aggregation of erythrocytes, finally affecting their flow properties[18]. There is often a reduction or change in Blood viscosity, the resistance of blood flow. Blood viscosity predisposes a person's system to reacting poorly to insulin. It also disrupts the transfer of glucose, insulin, and oxygen to metabolically active tissues in the body. We also found marked increase in glycemic index along with elevation in erythrocyte indices and SOD activity in male patients than female patients (Table-2),indicating that the male diabetic patients are at higher risk of developing micro and macrovascular complications than females.

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