Original Research Article

Comparative study of HbA1c and superoxide dismutase in type II diabetes mellitus

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Abstract

Introduction: Diabetes mellitus (DM) is a complex metabolic disorder characterised by chronic hyperglycemia with disturbances of carbohydrate, lipid and protein metabolism resulting from defects in insulin secretion, insulin action or both. Chronic hyperglycemia is linked to oxidative stress, which involves increased reactive oxygen species (ROS) production. **Materials and methods:** The is a prospective and observational study conducted at Tertiary care teaching hospital comprised of 60 patients between age groups 30-70 years of both sexes of DM Type 2 reporting for treatment. The patients were divided into Case group (Type 2 DM) and Control group (Healthy Subjects). The criteria for the diagnosis of Type 2DM were the same as the one which was given by the American Diabetes Association (ADA). **Result:** Among 60 patients, 38 were male (63.3%) and 22 were female (36.6 %) compared with control group 36 males (60%) and 24 females (40%) in control groups (p value 0.58). In this study, the maximum number of patients were in the age group of 51-60 years which were 31.6% (n =19) followed by age group 61–70 years having 28.3% (n = 17) in this group and 21.6% were 41-50 years. FBS in case group was 166.4±16.63 mg/dl and in control group 82.86±11.59 mg/dl. SOD in case group was 658.46+64.73 (U/gHb) and in control group 987.52+73.54 (U/gHb). HbA1c in case group was 7.98±1.31% and in control group 5.78±0.94%. **Conclusion:** The present study has proved the presence of oxidative stress in diabetic patients as assessed by decrease in SOD levels. Significant decrease in SOD in patients with poor glycemic control and negative correlation between HbA1c and SOD shows that the glycemic control has an influence on the oxidative stress in diabetic patients.

Keywords: Diabetes mellitus, Superoxide dismutase, HbA1c.

Introduction

Diabetes mellitus (DM) is a complex metabolic disorder characterised by chronic hyperglycemia with disturbances of carbohydrate, lipid and protein metabolism resulting from defects in insulin secretion, insulin action or both[1]. DM Type 2 is a heterogenous group of disorders characterised by variable degrees of insulin resistance and impaired insulin secretion contributing to hyperglycemia. The metabolic dysregulation associated with DM causes secondary pathophysiological changes in multiple organ systems that impose a tremendous burden on individual. It is the leading cause of end stage renal disease, non-traumatic lower extremity amputation and adult blindness[2].With an increasing incidence world-wide, DM is likely to continue to be a leading cause of morbidity and mortality. The identification of distinct pathogenic processes in DM Type 2 has important potential therapeutic implications[3]. Hyperglycemia, which is due to insulin resistance, impaired insulin secretion and increased glucose production resulting from environmental and genetic factors acting together is considered as the diagnostic parameter.

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The long-term control of DM is judged by levels of glycated hemoglobin (HbA1c)[4].

Chronic hyperglycemia is linked to oxidative stress, which involves increased reactive oxygen species (ROS) production. Antioxidant defense systems may be impaired and cellular/tissue damage may ultimately result. The antioxidant defense systems comprise enzymatic and non-enzymatic mechanisms, which ensure the balance between ROS production and scavenging[5]. The enzymatic antioxidant scavenging system includes enzymes, particularly superoxide dismutase (SOD) and glutathione peroxidase (GPx), while the non-enzymatic system involves circulating thiols (SH). Increased glycated hemoglobin levels (HbA1c) measured in the serum correlates with development and severity of micro and macrovascular complications and this might reflect the overall redox status of T2DM patients[6].

Strict glycemic control in type 2 diabetes mellitus (TDM2) is important to prevent DM complications. Hyperglycemia may be associated with increased SOD activity, as well as total thiols levels, to compensate for ROS generation. Moreover, it may influence circulating inflammatory cytokines (ICAM-1 and TNF-α) and serum ·NO levels[7]. To test this hypothesis, we sought to evaluate oxidative stress and inflammatory markers in patients with TDM2 stratified according to HbA1c levels compared to controls without DM.

Materials and methods

The is a prospective and observational study conducted at Tertiary care teaching hospital comprised of 60 patients between age groups 30-70 years of both sexes of DM Type 2 reporting for treatment. The patients were divided into Case group (Type 2 DM) and Control group (Healthy Subjects). The criteria for the diagnosis of Type 2DM were the same as the one which was given by the American Diabetes Association (ADA). 60 healthy subjects of similar age, sex and socioeconomic status served as controls. The controls were free from any major ailment which could alter the parameters under study.

Blood was drawn in the fasting state for Fasting Blood Sugar (FBS) in the fluoridated vial. For HbA1c estimation, the sample was collected in heparinized vial. The samples were collected in plain vials for the estimation of serum SOD. Sera were separated from samples and analysis was done. FBS was estimated by GOD-POD method. HbA1c was estimated by the method of Trivelli et al.SOD activity was determined by the method of Kakkar et al. [8] Homogenate was prepared by mixing serum and trichloroacetic acid (50%) in 1:1 ratio and centrifuged at 13,000 rpm for 10 min at 25 ℃. 15 µL supernatant was added to 120 µL sodium pyrophosphate buffer (52 mM, pH 8.3), 12 µL phenazine methosulphate, 36 µL nitroblue tetrazolium. Reaction was started by addition of 24 µL nicotinamide adenine dinucleotide. After incubation at 37 ℃ for 90 s, reaction was stopped by addition of 12 µL of glacial acetic acid. The reaction mixture was stirred vigorously with 400 µL of n-butanol. The mixture was incubated for 10 min and then centrifuged at 2000 rpm for 5 min at 25 ℃ and butanol layer was separated. The color intensity of chromogen in butanol layer was measured at 560 nm against n-butanol using a spectrophotometer.

Result

Among 60 patients, 38 were male (63.3%) and 22 were female (36.6 %) compared with control group 36 males (60%) and 24 females (40%) in control groups (p value 0.58) in Table 1.

Table 1: Gender distribution in the both group of study participants.

Gender	Case (N=60)		Control (N=60)		p value#
	No. of patients	Percentage	No. of patients	Percentage	
Male	38	63.3	36	60	0.58
Female	22	36.6	24	40	

Note: p value based on chi-square test

Table 2: Distribution of the number of subjects according to age group

Age group	Case (N=60)		Control (N=60)	
	No. of patients	Percentage	No. of patients	Percentage
31-40 years	**11**	**18.3**	**10**	
41-50 years	**13**	**21.6**	**12**	
51-60 years	**19**	**31.6**	**20**	
61-70 years	**17**	**28.3**	**18**	
Total	**60**	**100**	**60**	**100**

In this study, the maximum number of patients were in the age group of 51-60 years which were 31.6% (n =19) followed by age group 61–70 years having 28.3% (n = 17) in this group and 21.6% were 41-50 years in table 2.

Table 3: Biochemical parameters in cases and control group

Parameter	Case (N=60)	Control (N=60)	p-value
	Mean±SD	Mean±SD	
FBS (mg/dl)	166.4±16.63	82.86±11.59	< 0.001
SOD (U/gHb)	658.46+64.73	987.52+73.54	< 0.001
HbA1c (%)	7.98±1.31	5.78±0.94	< 0.001

In table 3, FBS in case group was 166.4±16.63 mg/dl and in control group 82.86±11.59 mg/dl. SOD in case group was 658.46+64.73 (U/gHb) and in control group 987.52+73.54 (U/gHb). HbA1c in case group was 7.98±1.31% and in control group 5.78±0.94%.

Table 4: Pearson Correlation Coefficient of SOD in Case group

Parameter	r	P
SOD (U/gHb)	-0.374	0.052

Discussion

Diabetes does not only alter the metabolism of carbohydrates, lipids and protein, but also the chemistry of these macromolecules. Poorly controlled diabetes accelerates the chemical modification of proteins and their functions which could lead to the development of diabetic complications. In this context, several hypotheses have been emitted in order to understand the origin of the complications observed in diabetic patients[9]. These hypotheses include mitochondria damage, mitochondrial defect in oxidative phosphorylation, increased formation of advanced glycation end products (AGES), increased activity of the polyol pathway, hypoxia, alteration of lipoprotein metabolism, increased protein kinase C activity, alteration of growth factors and cytokine, activities and increased oxidative and reductive stress[10]. Although oxidative stress appears as one of the metabolic events associated to diabetes and its complications the precise mechanisms by which oxidative stress may accelerate the development these complications are still not well understood [11]. Oxidative stress seems to be increased in a system where the rate of free radicals production is increased and/or the antioxidant mechanisms are impaired. Free radicals and oxidative stress are found to be responsible for the development of diabetes. Free radicals or reactive oxygen species (ROS) causes the oxidative stress which leads to development of diabetes, so an imbalance due to increased production of reactive oxygen and as well as reduction in antioxidant defenses which alter cellular redox status[12].

In our study, a total number of 120 subjects out of which 60 were controls without type 2 DM and the rest 60 were type 2 DM patients were included. Superoxide dismutase (SOD) is the antioxidant enzyme that catalyses the dismutation of superoxide anion (O2) into hydrogen peroxide and molecular oxygen[13]. SOD plays important protective roles against cellular and histological damages that are produced by ROS. It facilitates the conversion of superoxide radicals into hydrogen peroxide, and in the presence of other enzymes it converted into oxygen and water[14]. In the present study, SOD was significantly (p=0.0001*) lower among cases (658.46+64.73) as compared with controls (987.52+73.54).

Scientists had also observed similar finding in which an inverse relationship was observed of HbA1c with superoxide dismutase

(SOD) levels in type 2 diabetes groups[15]. In this study, HbA1c level was significantly (p=0.001) higher among cases as compared with controls. A significant decrease in SOD levels were noted in diabetic patients when compared to controls. These results are consistent with the findings of Sarita et al[16]. Glycated hemoglobin (HbA1c) is the marker of both severity and long-term control of the disease. It reflects the average level of blood glucose concentration over the preceding 6-8 weeks and is unaffected by diet, insulin therapy and other drugs. The values in our study are in accordance with several studies which have shown increase in HbA1c levels in diabetes[17]. Their study found significant association with Diabetes, hyperglycemia (as measured by HbA1c) is considered an important risk factor associated with diabetic retinopathy[18]. One of the most important risk factors for diabetes, regardless of the type of diabetes, is how well the diabetes is controlled. This is often measured by levels of a glycated hemoglobin or HbA1c, which is representative of blood sugar levels over a 3 to 4 month period[19].

Conclusion

The present study has proved the presence of oxidative stress in diabetic patients as assessed by decrease in SOD levels. Significant decrease in SOD in patients with poor glycemic control and negative correlation between HbA1c and SOD shows that the glycemic control has an influence on the oxidative stress in diabetic patients. Hyperglycemia, by causing alteration in the balance between pro-oxidants and antioxidants, serves as an important predictor of diabetic complications. Thus, good glycemic control can alleviate the long term complications of diabetes mellitus by decreasing oxidative stress.

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