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STUDY OF SERUM ADENOSINE DEAMINASE AS AN EFFECTOR OF OXIDATIVE STRESS IN DIABETES MELLITUS TYPE 2

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ABSTRACT

Diabetes Mellitus (DM) is the most common disorder characterized by metabolic abnormalities and long term complications. Hyperglycemia is considered a primary cause of diabetic vascular complications and is associated with oxidative stress. The purpose of this study was to investigate the role of hyperglycemia in generating oxidative stress if any, in DM Type 2. The study was carried out in 55 patients of DM Type 2 and also in 50 healthy controls. The patients were divided into two groups Group I (n=35) comprising patients of DM Type 2 with out complications and Group II (n=21) comprising patients of DM Type 2 with complications. Various parameters like Adenosine Deaminase (ADA) activity which is found to be a marker of abnormal lymphocyte response and a producer of reactive oxygen species (ROS), Malondialdehyde (MDA) which is a parameter to study increased oxidative stress leading to lipid peroxidation and Superoxide dismutase (SOD) activity which is an indicator of antioxidant status were taken into consideration to establish the existence of oxidative stress in DM Type 2. Glycosylated hemoglobin (HbA_{1c}) was also assessed to determine the status of diabetic control. The parameters like HbA_{1c}, serum ADA and serum MDA were significantly raised in DM Type 2 cases (both Group I and Group II) when compared to controls. Likewise the mean serum SOD was significantly lower in DM Type 2 patients (both Group I and Group II) as compared to Control group. When the levels of HbA1c, serum ADA and serum MDA in Group II patients of DM Type 2 when compared with those of Group I patients of DM Type 2, they were significantly increased. Likewise the serum SOD in Group II patients of DM Type 2 registered a significant decline as compared to Group I patients of DM Type 2. These alterations in DM Type 2 especially in Group II are ascribed to increased glycation, T-lymphocyte response, oxidative stress and limited defense against the radicals ,which act as harbinger of various metabolic complications.

Keywords: HbA_{1c}; ADA; MDA; SOD; Oxidative stress; Lipid Peroxidation.

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. 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.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.

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[1]. The long term control of DM is judged by levels of glycated hemoglobin (HbA_{1c}).

The purpose of this study was to investigate the role of hyperglycemia in generating oxidative stress, if any, in Type 2 DM. To this effect, various parametes like Adenosine Deaminase (ADA) activity which is found to be a marker of abnormal lymphocyte response and a producer of reactive oxygen species (ROS), Malondialdehyde (MDA) which is a parameter to study increased oxidative stress leading to lipid peroxidation and Superoxide dismutase (SOD) activity which is an indicator of antioxidant status were taken into consideration, to establish the existence of oxidative stress in DM Type 2.

MATERIALS AND METHODS

The study comprised of 55 patients between age groups 45-65 years of both sexes of DM Type 2 reporting to MIMS, Nellimarla, Vizianagaram for treatment.The patients were divided into Group I (DM Type 2 without complications) and Group II (DM Type 2 with complications). The criteria for the diagnosis of DM were the same as the one which was given by the National Diabetes Data Group 1999. 50 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.

Informed consent was taken from the patients and subjects who participated in the present study.

Institutional ethical committee approval has also been obtained.

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 ADA, MDA and SOD.Sera were separated from samples and analysis was done.

FBS was estimated by GOD-POD method (Trinder 1969)[2].HbA_{1c} was estimated by the method of Trivelli et al 1979[3].ADA was estimated by the method of Giusti 1984[4] .MDA was measured by colorimetric assay with 2-thiobarbituric acid by the method of Satoh 1978[5]. SOD was estimated by the method of Kakkar et al 1984[6].

RESULTS

Table 1: Showing plasma FBS and HbA1c in Control and Group I

	Control (n=50)	Group I (n=34)
FBS (mg/dl)	82.86±11.59	166.4±16.63**
Hb A_{1c} (%)	5.08±0.43	8.10±0.5**

** ($p< 0.001$)

The mean plasma FBS and HbA1c were found to be signicantly higher in Group I as compared to Control ($p<0.001$ for both).

Table 2: Showing plasma FBS and HbA1c in Control and Group II

	Control (n=50)	Group II (n=21)
FBS (mg/dl)	82.86±11.59	202.5±20.3**
Hb A_{1c} (%)	5.08±0.43	9.03±0.43**

** ($p< 0.001$)

The mean plasma FBS and Hb A_{1c} (%) were found to be signicantly higher in Group II as compared to Control.($p<0.001$ for both)

Table 3: Showing plasma FBS and HbA1c in Group I and Group II

	Group I (n=34)	Group II (n=21)
FBS (mg/dl)	166.4±16.63	202.5±20.3**
Hb A_{1c} (%)	8.10±0.5	9.03±0.43**

** ($p< 0.001$)

The mean plasma FBS and Hb A_{1c} were found to be signicantly higher in Group II as compared to Group I.($p<0.001$ for both).

Table 4: Showing serum ADA, MDA and SOD in Control and Group I

	Control (n=50)	Group I (n=34)
ADA (U/L)	16.32±1.71	26.91±4.20**
MDA(n mol/ ml)	3.20±0.63	5.75±.32**
SOD (U/ml)	9.07±1.58	5.27±0.93**

** ($p< 0.001$)

The mean serum ADA and MDA were found to be signicantly higher in Group I as compared to Control group. ($p < 0.001$). Likewise the mean serum SOD was significantly lower in Group I as compared to Control group. ($p < 0.001$).

Table 5: Showing serum ADA, MDA and SOD in Control and Group II

	Control (n=50)	Group II (n=21)
ADA (U/L)	16.32±1.71	37.80±6.07**
MDA (n mol/ml)	3.20±0.63	6.50±0.71**
SOD (U/ml)	9.07±1.58	4.44±1.04**

** ($p< 0.001$)

The mean serum ADA and MDA were found to be signicantly higher in Group II as compared to Control group. ($p<0.001$).Likewise the mean serum SOD was significantly lower in Group II as compared to Control group. ($p < 0.001$).

Table 6: Showing serum ADA, MDA and SOD in Group I and Group II

	Group I (n=21)	Group II(n=34)
ADA (U/L)	26.91±4.20	37.80±6.07**
MDA (n mol/ ml)	5.75±.0.32	6.50±0.71**
SOD (U/ml)	5.27±0.93	4.44±1.04*

** ($p< 0.001$) * ($p< 0.05$)

The mean serum ADA and MDA were found to be signicantly higher in Group II as compared to Group I ($p<0.001$). The mean serum SOD were found to be signicantly lower in Group II as compared to Group I. ($p< 0.05$)

DISCUSSION

The diagnosis of DM in the present study was based on criteria issued by consensus panel of experts from National Diabetes Data Group (NDDG) 1979[7] and World Health Organisation (WHO) 1980[8], According to these criteria, DM was diagnosed on the basis of the fasting plasma glucose ≥ 126 mg/dl and symptoms of diabetes. The classic symptoms of DM include polyuria, polydypsia, polyphagia and unexplained weight loss. The principal complications associated with DM are retinopathy, neuropathy, nephropathy, angiopathy, susceptibility to infection, dyslipidemia, ketoacidosis and hyperosmolar hyperglycemic non ketotic coma.

It is observed that in the present study the mean fasting plasma glucose level was significantly raised in both Group I without complications(166.4mg/dl±16.63) and Group II with complications (202.5 mg/dl±20.3) as compared to Control Group(82.86 mg/dl ±11.59). This rise is statistically significant ($p<0.001$; Table 1&2). The mean values of FBS in both the groups confirmed to the criteria laid down by WHO for diagnosis of DM. The comparison of plasma FBS between Group I and Group II (Table 3) established a higher level in the later and the increase was statistically significant ($p< 0.001$). Hyperglycemia is due to insulin resistance, impaired insulin secretion and increased glucose production resulting from environmental and genetic factors in DM Type2. The long term control of DM is judged by levels of glycated hemoglobin (HbA_{1c}). Glucose reacts spontaneously and non- enzymatically with free amino groups on hemoglobin (Hb) to form

covalent glycated hemoglobin. The extent of glycation depends on the average blood glucose to which hemoglobin is exposed and on the half life of hemoglobin. Several glycated derivatives of hemoglobin exist, derived from the reaction of Hb with glucose, glucose-6phosphate etc. These are collectively known as HbA_1. The principal constituent of HbA_1 is the one formed with glucose itself, termed as HbA_{1c}, which normally comprises about 5% of circulating hemoglobin (Becket et al 2006)[9]. It is observed that in the present study the mean Hb A_{1c} level was significantly raised in both Group I (8.10%±0.5) and Group II (9.03%±0.43) as compared to Control Group (5.08%±0.43) This rise is statistically significant ($p<0.001$;Table 1&2). The mean values of HbA1c in both the groups exceeded 5%. HbA_{1c} once formed stays within the red cell for its life time. Since half life of the red cell is 60 days, HbA_{1c} value reflects the average level of blood glucose over the previous 1-2 months. The comparision of HbA_{1c} between Group I and Group II (Table 3) established a higher level in the later and the increase is statistically significant ($p< 0.001$) indicating poor diabetic control responsible for the incidence of micro and macro vascular complications.

ADA is an enzyme of purine metabolism. It acts on adenosine and other adenosine nucleoside analogues. It plays a crucial role in lymphocyte proliferation and differentiation (Hovi 1976)[10], and shows highest activity in T-lymphocytes (Sullivan 1977)[11]. It is an enzyme distributed in the human tissues and was considered as a good marker of cell mediated immunity (Baghanha 1990)[12].

It is observed that in the present study the mean serum ADA is significantly increased in both Group I (26.91 U/L ±4.20) and Group II (37.80 U/L ±6.07) as compared to Control Group (16.32 U/L±1.71). This increase is statistically significant ($p<0.001$;Table 5) . Hoshino et al 1994[13], Kurthul et al 2004[14], Prakash et al 2006 [15] and Mokthari et al 2010 [16] reported in their study an increased level of serum ADA associated with hyperglycemia in DM Type2.High serum ADA activity in DM Type2 might be due to abnormal T-lymphocyte responses or proliferation and its release into circulation which depends on altered insulin levels (Shivaprakash et al 2006)[15]. This increased level of ADA in diabetic patients could result in increased hypoxanthine which oxidizes into xanthine and uric acid by xanthine oxidase and concomitant generation of free radicals (Siddiqi et al 2011)[17]. The comparision of serum ADA between Group I and Group II (Table 6) established a higher level in the later and the increase is statistically significant ($p< 0.001$) indicating an increased T-lymphocyte proliferation and free radical production in DM Type 2 with complications.

It is observed that in the present study the mean serum MDA was significantly increased in Group I (5.75 n mol/ml±.0.32) and Group II (6.50 n mol/ ml ± 0.71)) when compared to Control Group (3.20 n mol/ ml ±0.63). This increase is statistically significant ($p < 0.001$; Table 4, 5). The various mechanisms included in the increase of serum MDA in DM Type 2 include:

a) Chronic Hyperglycemic status in Diabetes mellitus Type 2 favours auto oxidation of glucose which is subjected to enediol rearrangements that result in the formation of an enediol radical anion. (Githanjali et al2010)[18].
b) Chronic Hyperglycemic status in Diabetes mellitus Type 2 also favours non enzymatic glycation reaction thereby leading to advanced glycosylation end products (AGE). Glycation of proteins can lead to direct release of O_2^-(superoxide radical) and hydrogen peroxide through specialized

receptors for AGE (Abuja and Albertini 2001)[19].

c) Increased level of serum ADA in diabetic patients could result in increased hypoxanthine which oxidizes to xanthine and uric acid and accompanying generation of free radicals. (Siddiqi et al 2011)[17].

The free radicals generated by above reactions are exteremely reactive.When a free radical reacts with a normal compound; other free radicals are generated through propagation phase. Peroxidation of poly unsaturated fattyacids in plasma membrane by free radicals leads to loss of membrane functions.Lipid peroxidation and consequent degradation products such as malondialdehyde are seen in biological fluids (Vasudevan 2011)[20]. The comparison of MDA between Group I and Group II (Table 6) established a higher level in the later and the increase is statistically significant ($p< 0.001$) indicating more lipid damages leading to the destruction of fine architecture and integrity of the membranes responsible for its complications.

SOD is an enzyme that repairs cells and reduces the damage done to them by free radicals by its antioxidant properties.It is observed that in the present study the mean serum SOD is significantly decreased in Group I (5.27 U/ml ±0.93) and Group II (4.44 U/ml ±1.04) when compared to Control Group (9.07 U/ml ±1.58). This decrease is statistically significant ($p < 0.001$; Table 4, 5).It has been reported that diabetic patients have significant defects of antioxidant protection (Aria et al 1989, Lin 1996 and Zbronska et al 1995) as manifested by a decrease in serum SOD.

This decrease can be explained as follows:

a) The decrease of superoxide dismutase activity might be due to the increase of glycosylated SOD that leads to the inactivation this enzyme (Aria et al 1989)[21]

b) The SOD activity is further decreased due to loss of its cofactors Zn^{2+} and Cu^{2+} in DM Type 2 (Lin 1996, Zbronska et al 1995) [22,23].

The comparison of SOD between Group I and Group II (Table 6) established a lower level in the later and the decrease is statistically significant ($p< 0.05$) indicating lowered antioxidant status in DM Type 2 with complications.

CONCLUSION

In the present study, it has been established that the various complications in DM Type 2 may be linked to the alteration in two of the biochemical parameters: HbA_{1c}.and free radical generation.

Statistically significant increase in HbA_{1c} in complicated DM Type 2 in comparison to uncomplicated DM Type 2 indicates an improper diabetic control which is responsible for the higher incidence of micro and macro vascular complications.

High serum ADA activity, formation of enediol radical ion and advanced glycosylation end products contribute to the generation of excess free radicals, as evidenced by a statistically significant rise in serum MDA and are responsible for inflicting gross damage to cell membranes and initiating as well as aggravating various complications of DM Type 2.The significant decrease of SOD in DM Type 2 as explained also contributes to the damage caused by the free radicals because of the curtailed defensive mechanism against them.

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