ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 04 | April - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

PRELIMINARY STUDY OF GLYCOSYLATED HEMOGLOBIN, MALONDIALDEHYDE AND SUPEROXIDE DISMUTASE LEVELS IN IDDM PATIENTS UNDER GOING SURGERY.

Biochemistry

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ABSTRACT

OBJECTIVE - To study Glycosylated hemoglobin level, Superoxide dismutase level in fasting state and Malondialdehyde(MDA) level in fasting and post prandial (4 hours after meal) state in IDDM patients who are posted for surgery and comparing these values with the control group comprising of healthy subjects not suffering from IDDM and not posted for any surgery.

Methods - Patients with IDDM to undergo surgery admitted in Lokmanya Tilak Municipal General Hospital were enrolled for the study. Duly signed consent forms were taken from these patients. Control group comprised of age matched healthy subjects not suffering from NIDDM, IDDM or any other acute diseases and not posted for any surgery. The biochemical parameters estimated for IDDM patients to undergo surgery and healthy controls were Glycosylated hemoglobin, Superoxide dismutase and Malondialdehyde. The study protocol was approved by the Ethical Committee.

Results –The IDDM patients to undergo surgery had significantly high mean HbA1C in fasting state. In these patients the mean SOD level was less than that of the healthy controls in fasting state. In these patients the mean MDA level was significantly higher in fasting state and post-prandial (4 hours after meal) state as compared to healthy controls.

Conclusion –It will be beneficial to monitor Malondialdehyde and Superoxide dismutase levels besides HbA1C in IDDM patients posted for surgery to get clear insight of metabolic status.

KEYWORDS

Glycosylated hemoglobin (HbA1C), Insulin dependent diabetes mellitus (IDDM), Malondialdehyde (MDA), Superoxide dismutase (SOD).

INTRODUCTION

Diabetes mellitus is a clinical condition characterized by hyperglycemia caused due to insufficient insulin or insulin resistance. Diabetes mellitus is of two types namely insulin dependent diabetes mellitus and non-insulin dependent diabetes mellitus (NIDDM). IDDM also known as type-I or juvenile onset diabetes mellitus results from failure of β–cells to secrete insulin. Plasma insulin level is abnormally low. The absence of insulin level results in uncontrolled rate of lipolysis in adipose tissue[1]. The patients of IDDM require insulin therapy. Deficiency of insulin causes metabolic derangement. If patients with IDDM have to undergo surgical procedure, the trauma caused is tremendous. The insulin levels are to be adjusted and glycaemic control must be achieved before surgery.

HbA_{1C} is glycosylated form of HbA_1. Under normal physiological conditions, a small fraction of HbA_1 present in RBCs is non-enzymatically glycosylated to form HbA_{1C}. Since the extent of glycation of HbA_1 to HbA_{1C} depends on the plasma glucose level, the percentage of HbA_{1C} increases in diabetes mellitus. The determination percentage of HbA_{1C} reveals the mean blood glucose level over the previous three months because the half-life of erythrocytes is 120 days. Normally HbA_{1C} concentration is about 3 to 7% of the total hemoglobin.

Oxidative stress is defined as the state in which the balance between oxidants and antioxidants system in the body is lost in favour of oxidants[2]. Molecular oxygen is essential in the process of mitochondrial respiration for complete metabolism of glucose and other substrates during ATP production. Oxidative phosphorylation results in the generation of free radical superoxide, from 0.4 to 4% of consumed oxygen. The free radicals like superoxide, hydroxyl radical, hydrogen peroxide, nitric oxide are highly unstable due to presence of unpaired electrons, which may pair with other electrons, resulting in detrimental effect. SOD is an enzyme which metabolizes the hazardous superoxide ion to hydrogen peroxide and oxygen. The damaging effect of elevated toxic radical are due to an increase in the formation of superoxide radicals within cells which causes inactivation of superoxide dismutase enzyme in hyperglycemic conditions. SOD is the first line of defense to protect cells from injurious effect of superoxide radical. Lipid peroxidation is the free radical damage of lipids. MDA can be generated in vivo as a side product by enzymatic processes during biosynthesis of thromboxane A_2 which is a biologically active metabolite of arachidonic acid. Elevated levels of lipid peroxide in diabetes mellitus may be due to the alteration of function of erythrocytes membrane. This inhibits the activity of SOD enzyme leading to accumulation of superoxide radicals which causes the maximum lipid peroxidation and tissue damage in diabetes. Malondialdehyde (MDA) is used as biochemical marker for the assessment of lipid peroxidation. The estimation of serum MDA is used to assess oxidative stress and free radical damage to the body.

MATERIALS AND METHODS:

Group A consisted of 40 clinically diagnosed IDDM to undergo surgery admitted in wards of Lokmanya Tilak Municipal General Hospital. These were selected for this study after duly signing consent form. Group B consisted of 40 age matched control not suffering from diabetes mellitus and any other chronic infections and not posted for any surgery. Blood samples were collected after 12 hours fasting and post-prandial (4 hours after meal) from Group A and Group B subjects. The blood collected was transferred to appropriate containers as shown.

Volume of blood	Vacutainer	Parameters estimated	State
2ml	Ethylenediaminetetra acetic acid	1) Glycosylated hemoglobin 2) SOD	Fasting
3ml	Plain Vacutainer with red top	1) MDA	1) Fasting 2) Post prandial (4 hrs after meal)

1)The blood from EDTA vacutainer was centrifuged and plasma was discarded the sediment of red blood cells washed with ice cold saline thrice. An aliquot of the washed red blood cells was used to estimate Glycosylated hemoglobin and SOD in both Group A and Group B subjects in fasting state only.

2) Serum samples were obtained after centrifuging the blood in plain vacutainers. MDA estimation was carried out in the fasting state as well as post prandial (4 hours after meal) in both Group A and Group B subjects.

Glycosylated hemoglobin was estimated by method of Fluckleiger R, Winterhalter, (1976)[3]

Superoxide dismutase (SOD) was estimated by method of Winterbourn C C et al (1975)[4]

Malodiadehyde (MDA) was estimated by modified method Sasikala M et al (1999)[5]

Statistical significance was established using unpaired students 't' test[6].

RESULTS:

In this study there was significant increase in the mean HbA_{1C} level in Group A patients as compared to that of Group B controls in fasting state. The mean SOD level in Group A patients was non-significant as compared to that of Group B controls. In Group B controls there was significant increase in serum MDA level in post prandial (4 hours after meal) state as compared to the fasting state. Serum MDA level was significantly high in fasting and post prandial (4 hours after meal) states in Group A patients as compared to that of Group B controls. In Group A patients there was a significant decrease in the serum MDA level in post prandial (4 hours after meal) state as compared to the fasting state.

These results are highlighted in table 1 along with statistical significance.

Table 1. Comparison Of HbA_{1C} , SOD And MDA In The Study Groups

PARAMETERS	Group A n = 40 mean±SD	Group B n = 40 mean±SD	'p' value
HbA_{1C} fasting % of Hb	7.08±1.7	4.60 ±0.84	< 0.001
SOD fasting units/gm of Hb	2005±150	3780±116	< 0.05
MDA fasting umol/L	2.0±0.09	0.8±0.14	< 0.001
MDA post prandial (4 hours after meal) umol/L	1.20±0.22	1.01±0.06	< 0.001

HbA_{1C} - Glycosylated Hemoglobin
SOD - Superoxide dismutase
MDA - Malondiadehyde
Hb - Hemoglobin
Normal Range - HbA_{1C} - 3to 7% of Hb

The mean value of HbA_{1C} in Group A patients and Group B controls at fasting state was 7.08±1.7% of Hb and 4.6±0.84% of Hb respectively. The difference was statistically significant ($p<0.001$). The mean value of SOD in Group A patients and Group B controls at fasting state was 2005±150 units/gm of Hb and 3780±116 unit/gm of Hb respectively. The difference was statistically non-significant ($p < 0.05$). The mean value of MDA in Group A patients and Group B controls at fasting state was 2.0±0.09 umol/L and 0.8±1.4 umol/L respectively. The difference was statistically significant ($p <0.001$).

The mean value of MDA is Group A patients and Group B controls at post-prandial (4 hours after meal) state was 1.2±0.22 umol/L and 1.01±0.06 umol/L respectively. The difference was statistically significant ($p < 0.001$).

DISCUSSION:

In our study it was observed that in fasting state mean HbA_{1C} level, mean MDA level were significantly high in Group A patients when compared to the Group B controls. SOD level in fasting state was non-significant in Group A patients when compared to Group B controls. In post-prandial (4 hours after meal) state mean MDA level was significantly high in Group A when compared to Group B controls.

In Group B controls the mean MDA level in the post prandial (4 hours after meal) state is higher than the mean MDA level in the fasting state ($p <0.001$). The results show that there are lipid peroxidation products in the body of Group B controls but which are taken care by antioxidants defense mechanism such as SOD, catalase, glutathione, alpha tocopherol and ascorbic acid present in the body.

In Group A patients the mean MDA level in the post-prandial (4 hours after meal) state is reduced as compared to the mean MDA level in the fasting state ($p<0.001$). Group A patients were given insulin / hypoglycemic drugs to keep normal blood glucose levels which would favourably lower the oxidative stress. Lowered MDA levels served as a marker for reduced oxidative stress. In our study we found a positive correlation (r) between MDA level and HbA_{1C} in fasting state ($r=0.51$) in Group A patients. This is in accordance with Dr. Palem S. P.[7] Dr. Palem S. P found positive association between HbA_{1C} and MDA showing indication that HbA_{1C} also plays a key role in the pathogenesis of atherosclerosis and cardiovascular disease in diabetes mellitus patients.

Hyperglycemia is closely related to elevated lipid peroxidation. The antioxidant system weakens in diabetic patients compared to control group[8]. The research finding of Rabia Alghazeer et al revealed that oxidative status and antioxidant levels were affected in IDDM, decrease in SOD activity was observed ($p<0.05$) in IDDM patients. They suggested that the biomarkers such as MDA and SOD can be used to monitor the developing complication of diabetes in early diagnosed IDDM patients.[9] Ummugulusm et al demonstrated a decrease in antioxidant defenses in IDDM patients. Hence, IDDM patients experienced oxidative stress[10].

CONCLUSION:

It will be beneficial to monitor Malondialdehyde and Superoxide dismutase levels besides HbA_{1C} in IDDM patients posted for surgery to get clear insight of metabolic status.

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