Evaluation of Induced Oxidative Stress and Status of Antioxidant Defense System in Acute Myocardial Infarction Patients with Hyperglycemia

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Abstract:-

Background:

Diabetes mellitus is a common metabolic disorder, represented by hyperglycemia and chronic hyperglycemia is associated with long-term complications. There is much evidence that oxidative stress is involved in the origination of several diabetic complications. Oxidative stress occurs when the production of oxidant exceeds the rate of oxidant scavenging. Thus the all individual antioxidant markers may deliver more appropriate information compared to that obtained by the measurement of total antioxidant capacity.

Aims and Objectives: The aim of this study evaluates the status of oxidative stress in type 2 diabetic patients and non-diabetic healthy individuals and the correlation between the antioxidant level and hyperglycemia.

Material and Methods: A total 140 individual were chosen, 70 patients with Type-2DM from the last 1-2 years and 70 normal healthy individuals as a control group of both sexes. All subjects were undergo to the following investigated parameters; BSL (F, PP), Vitamin C, Vitamin E, Catalase, MDA, SOD, Glutathione peroxidase, and Glutathione reductase enzymes.

Results: The mean values of BSL (F, PP) and HbA1c were significantly increased ($P<0.001$) in patients as compared to control. Whereas the mean value of Vitamin C, E, Catalase, SOD, Glutathione peroxidase, and Glutathione reductase enzymes was significantly decreased in Type-2 diabetic patients as compared to control. The value of malondialdehyde (MDA) was significantly increased in diabetic patients as compared to control.

Conclusion: The antioxidant and oxidative stress markers can be used for follow-up in patients suffering from diabetes mellitus type 2 and predict other complications.

Keywords: Diabetes Mellitus, Antioxidants, oxidative stress, Free Radicals

Introduction:-

Diabetes mellitus is a metabolic disarrangement, characterized by increased blood glucose levels and basically in which the beta cells of the pancreas produce insufficient insulin [1]. Diabetes mellitus problems differ from one individual to another but are being determined by the health and patient diet [2]. Diabetes mellitus is the dominant causation of death in most developing countries. Type-2 diabetes is the most frequent sort of diabetes and has increased parallel to cultural and societal changes. In high-income countries, up to 91% of adults with the disease have type 2 diabetes [3]. Chronic hyperglycemia is associated with long-term

damage, dysfunction, and failure of the normal functioning of various organs, especially the eyes (diabetic retinopathy), kidneys (diabetic nephropathy), nerves (diabetic neuropathy), heart (mainly myocardial infarction), and blood vessels [4].

Globally, about 190 million populations are affected by diabetes mellitus and are one of the most prominent causes of impairment and death in the world [5]. The metabolic theory of diabetes mellitus suggests that complications such as endothelial damage and cellular damage belong to long-term hyperglycemia, while genetic theory suggests that diabetes complications are genetically established. In1983-1993 a study is carried for diabetes control and complications which determine that maintained blood glucose can be very effective [6].

Free radicals are extremely reactive and unstable chemical species that are short-lived entities that consist of one or more unpaired electrons. They can also be considered as a necessary evil for signaling involved in the normal process of differentiation and migration. The free radicals cause damage to cells by transferring the unpaired electron resulting in the oxidation of cell components and molecules [7].

Free radicals in the biological system are products of the normal metabolism of cells. . They have free electrons to react with various organic substrates such as proteins, lipids, and deoxyribonucleic acid (DNA). Free radicals are well known for playing a dual role as both disastrous and beneficial species since they can be either harmful or beneficial to living systems [8].

An imbalance of free radicals and antioxidants can cause oxidative stress.Whenthe production of free radicals overwhelms the detoxification capacity of the cellular antioxidant system causing biological damage [9]. The deleterious process is the result of a higher concentration of free radicals that can damage cell structure which is caused by oxidative stress [10]. Oxidative stress causes loss of function and structure by attacking the healthy cells of the body. Now to counterbalance the deleterious effects of these free radicals, the

body has different mechanisms to produce antioxidants, which will neutralize the elevated amount of free radicals and keep the cells protected against their harmful effects, and contributing toward the prevention of diseases [11].

Free radicals produced under physiological conditions are maintained at steady-state levels by endogenous or exogenous antioxidants which act as free radical scavengers. The endogenous antioxidants comprise of the enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and non-enzymatic antioxidants including glutathione (GSH), vitamins C, E, and α lipoic acid [12, 13]. On the other hand, the exogenous antioxidants include micronutrients and other exogenously administered compounds such as vitamin A, C, E, trace metals (Se, Mg, Zn), carotenoid [14].

Enormously free radicals are formed in diabetic conditions by the oxidation of glucose and non-enzymatic glycation of protein. Abnormally high levels of free radicals and the simultaneous attenuation of antioxidant defense mechanisms can lead to the destruction of cellular organelles and enzymes increased lipid peroxidation and the development of insulin resistance. [15] These consequences of oxidative stress can encourage the progression of complications of diabetes mellitus. [16]

Malondialdehyde (MDA) is the product of lipid peroxidation and is presumed to be a critical indicator for oxidative stress. Catalase is a haem-containing ubiquitin enzyme, in eukaryotes, it is found in peroxisomes. The enzyme probably involves degrading hydrogen peroxide produced by peroxisomal oxidases to water and oxygen. Superoxide dismutase (SOD) performs a significant role in the conversion of the superoxide anion to hydrogen peroxide and oxygen. Glutathione peroxidase (GPx) is a Selenium-containing enzyme that can act on lipid peroxides as well as H2O2 using glutathione. Vitamin E is important for preventing the peroxidation of lipids by donating a single electron. Vitamin C is been documented as a type

of antioxidant vitamin that plays an important role in protecting free radical-induced damage and a decrease in basal vitamin C levels has Type-2 DM [17].

The intention of the present study was to determine the importance of oxidative stress in type 2 diabetic patients and non-diabetic healthy individuals and the correlation between the antioxidant level and hyperglycemia.

Materials and Methods:-

The proposed comparative cross-sectional observational study was conducted at Integral Institute of Medical Sciences & Research, Integral University, Lucknow. The present study includes a total of 140 subjects were classified into two groups:

-The Control group consisted of 70 clinically healthy persons free from any liver, kidney, or heart diseases.

-The diabetes mellitus Type-2 group consisted of 70 patients who were attended OPD last year.

Both groups were subjects of both sexes and ages (35-55 years) Control subjects were selected from relatives accompanied by the patients and staff members. The patients were diagnosed on a clinical basis and with laboratory data. They were stabilized with hypoglycemic drugs. None were current users of antioxidants. The patients suffering from hepatic disease, cardiovascular disease, cancer, chronic or acute inflammatory illness, alcoholics, and smokers were excluded from the study. The present research study was approved by the institutional ethical committee of the college. Individual informed consent was taken before enrolment of type 2 diabetes patients and healthy subjects for the study. The history of type 2 diabetes and other socio-demographic baseline data were collected by using a pre-structured questionnaire in both study groups.

The various parameters which were studied include the age of the patient (in years), sex, smoking history, blood pressure (mm Hg), BMI (kg/m2), history of ischemic heart disease (IHD). Blood samples from both groups were collected for determination the level of Blood Sugar levels (FBS, PPBS), glycated hemoglobin(%), Oxidative Stress Markers by MDA, and Catalase and Antioxidant status by SOD & Glutathione reductase, vitamin C and E.

Blood Collection:

Blood samples (08 ml) were collected in the morning after overnight fasting under all aseptic conditions in a sterile dry vial. The collected blood sample was divided into three separate vials to assess the different biochemical assays. 2 ml of blood was collected into potassium oxalate and sodium fluoride-containing vial and then centrifuged at 3000 rpm for 10 min. The plasma was used for the analysis of fasting glucose levels.

6 ml of the blood was collected into EDTA vials and centrifuged at 3000 rpm for 15 min at 4°C and the plasma was used for the analysis of malondialdehyde (MDA), vitamin E & C, and RBCs were hemolysed by adding ice-cold ultrapure water to yield a 50% hemolysate. An aliquot of hemolysate was used in the estimation of superoxide dismutase activity (SOD) and catalase. A heparinized whole blood sample was used for the estimation of HbAlc and glutathione reductase.

Biochemical assays

Blood glucose (F and PP) was estimated using Trinder's method (GOD-POD, endpoint) [18] and Glycosylated Hemoglobin was measured by Ion - exchange chromatography [19]. Analysis of Oxidative Stress by serum malondialdehyde (MDA) was estimated done by the thiobarbituric acid method [20] as an index of lipid peroxidation and catalase [21] was evaluated in RBCs. The activity of Superoxide dismutase SOD levels in plasma was measured using a Biovision kit and GPx was measured using the RANSEL kit (Randox

Laboratories Ltd., Crumlin, United Kingdom). This method is based on the study of Paglia and Valentine [22]. Glutathione Reductase (GR) activity was estimated by applying the method of Goldberg and Spooner [23]. A spectrophotometer was used to estimate the Vitamin-E level at Optical Density (OD) of 536nm by Desai's method [24] in plasma. Jacob's method [25] was employed for an estimate of the Vitamin C level at Optical Density (OD) of 520nm.

Statistical Analysis

Statistical evaluation was performed using the Statistical Package for the Social Sciences 16.0 (SPSS) software. Data obtained from the study groups were compared using the Student t-test. The results were expressed as mean ± SD and the $p < 0.001$ value was expressed as statistically significant.

Results:-

Table 1. Defining characteristics of individuals participating in the study

Characteristics	Case (n=70)	Control(n=70)	p-value
Male	37	39	N S
Female	33	31	N S
Age(Mean ± SD)	47.6±10.81	44.5 ±10.22	N S
BMI(Mean ± SD)	24.78 ± 4.45	24.49 ± 4.55	N S
Systolic Blood Pressure (Mean ± SD)	139.10 ±9.20	137.65±8.39	N S
Diastolic Blood Pressure (Mean ± SD)	81.55 ± 4.47	78.20±5.95	N S

Values are given as mean ± SD from 70 subjects in each group.

Table 2. Comparison of Glycemic parameters between in control and Type-2 Diabetic patients

Parameters	T-2 DM(n=70) Mean±SD	Control(n=70) Mean±SD	t-value	p-value
FBG (mg/dl)	185±47	82.91±9.52	28.45	<0.0001
PPBS (mg/dl)	215±50	113.19±11.46	29.53	<0.0001
G HbA1c	8.2±1.5	5.08±0.39	16.04	<0.0001

Values are given as mean ± SD from 70 subjects in each group.

The statistical significance between the patient group-II and control group-I

Table 3. Comparison of Oxidant and antioxidant parameters between in control and Type-2 Diabetic patients

Parameters	T-2 DM (n=70) Mean±SD	Control(n=70) Mean±SD	t-value	p-value
MDA(µmol/L)	5.63±0.53	2.69±0.43	35.9734	<0.0001
GR(µmol/L)	2.88±0.46	4.29±0.48	23.49	<0.0001
Catalase(U/gmHb)	2.18±0.10	2.42±0.17	9.98	<0.0001
SOD(U/ml)	1.79±0.31	3.03 ± 0.28	24.72	<0.0001
Glutathione peroxidase(U/gmHb)	27.85±2.18	30.43±2.54	6.43	<0.0001
Vitamin C(mg/dl)	0.97±0.17	1.17±0.15	7.42	<0.0001
Vitamin E(mg/dl)	0.89±0.20	1.22±0.23	9.19	<0.0001

Values are given as mean ± SD from 70 subjects in each group.

The statistical significance between the patient group-II and control group-I

Table 4. Spearman's correlation between oxidant and anti-oxidant levels with blood glucose levels and HbA1c in Type -2 Diabetic patients.(n=70)

Parameters	Fasting Blood Glucose		HbA1c	
	r-value	p-value	r-value	p-value
MDA	0.797	<0.001	0.385	<0.001
GR	-0.111	0.359	-0.239	0.046
Catalase	-0.494	0.007	-0.286	0.016
SOD	-0.228	0.056	-0537	0.008
Glutathione peroxidase	-0.332	0.004	-0.239	0.046
Vitamin C	-0.158	0.189	-0.245	0.040
Vitamin E	-0.125	0.299	0.173	0.151

**Correlation is significant at the P<0.05 level (2-tailed)

Discussion:-

Diabetes mellitus is considered to be a state of excess generation of free radicals which are contributed oxidative stress and result impair the endogenous antioxidant defense system. The endogenous antioxidant defense system includes both enzymatic and non-enzymatic approaches. Antioxidant includes superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase enzymes and vitamin A, C, E [13, 14].

In the present study, all the diabetic patients were poorly controlled, as BSL (F & PP) and GHbA1c level was found to be more prominently increased as compared to control subjects. These changes are highly statistically significant as compared to normal healthy subjects.

In the present study, Oxidative stress is observed more in diabetes mellitus patients as compared to normal. Lipid peroxidation is a remarkable marker for oxidative stress. Lipid

peroxidation product as MDA was increased significantly in diabetic patients compared to healthy subjects. Our results are similar to earlier workers who found increased MDA levels in serum of Type-2 DM patients [26]. There is a positive association between F & PP blood glucose, GHbA1c, and MDA which is statistically significant. The rise in the MDA suggested increased oxidative stress, caused by free radical mediated lipid peroxidation in the cell membrane during diabetes [27]. Elevated levels of lipid peroxide in diabetes mellitus may be due to the alteration of function of the erythrocytes membrane [28]. Therefore MDA is a critical indicator for oxidative stress lipid peroxidation.

Superoxide dismutase neutralizes superoxide ions by going through successive oxidative and reductive cycles of transition metal ions at their active site. SOD is acknowledged as a first-line defense against ROS. This enzyme is present in nearly all cells, and converts $^{\bullet}O_2^-$ into H_2O_2[29]. In this study, SOD activity was significantly decreasing in type-2 diabetics patients. This inhibits the activity of the superoxide dismutase enzyme contributing to the aggregation of superoxide radicals which generate the maximum lipid peroxidation and causes tissue damage in diabetes.

In the present study, GR was significantly higher among diabetic cases than controls. Any alteration in their levels makes the cells prone to oxidative stress and hence cell injury.The present data exhibited that hyperglycemia produced marked oxidant impact as evidenced by the significant rise in lipid peroxidation products along with a significant fall in antioxidant enzyme glutathione reductase. Similar findings were also reported by Mosaad A. Abou-Seif et.al [30]

GPx enzyme was accepted as biologically essential in the reduction of hydrogen peroxide. In the present investigation, the antioxidant enzyme activities of GPx, and Catalase were found to decrease in diabetic subjects as compared to healthy persons. Catalase is an enzyme exerting a dual function; it catalyzes the decomposition of hydrogen peroxide to produce

water and oxygen, which is a catalytic function, and oxidation of H donors, which is a peroxidative function [31]. In this study, the catalase enzyme was decreased in diabetes mellitus and this may be due to oxidative stress involved in the origin of diabetes, low efficiency of the scavenging antioxidant system has been shown to be related to the pathogenesis of the disease, which suggests that genes encoding antioxidant enzymes may involve in the diabetes development.

A deficiency of the antioxidant activity of superoxide dismutase and glutathione peroxidase has been related to a higher concentration of peroxide. There may be an imbalance between the production and scavenging of free radicals produced due to the lack of an antioxidant system. Spearman's correlation test shows negative correlation between SOD, GPx, GR, Catalase, and BSL (F & PP), GHbA1c.The antioxidant enzyme, GPx, GR, Catalase, and SOD activities were deceased which was found to be statistically significant in our diabetic patient's group. Rise in ROS level or maybe fall in catalase SOD, GPx, etc. to be significant to this oxidative stress. These results were in agreement with previous studies [26,32], which demonstrated a strong association between poor glycemic control and the reduction of the protective antioxidant defense mechanisms in diabetes mellitus.

Lipid peroxidation is prevented through vitamin E. Other recent research studies suggested that nitric oxide production in epithelial cells is increased through vitamin C [32]. Vitamin C &Eact as an antioxidant and detoxify the free radicals. In this study ascorbic acid and vitamin E were decreased and this may also be possible that metabolic disturbs diabetic patients, including missing antioxidants, resulting in major oxidation. High oxidative insult and missing antioxidants may be concurrent in the diabetic patient [33]. The association between the Vitamins which were assessed in this study showed a significant negative correlation of BSL (F & PP) and GHbA1c with vitamins C and E, which suggested the depleted antioxidant status causes an increased state of oxidative stress.

Oxidative stress plays a pivotal role in the development of diabetes complications, both at the micro-vascular and macro-vascular levels. Results originate from two decades of diabetes complications investigation point towards overproduction of mitochondrial superoxide as the main cause of metabolic abnormalities of diabetes. Thus, all of the above-reviewed pathways are involved in the microvasculature and macro-vasculature hyperglycemic damage [34].

It has been hypothesized that uncontrolled hyperglycemia is the major factor in various secondary complications. When intracellular glucose levels are high polyol pathway for glucose metabolism is activated [35] and which is immediately related to hyperglycemia and as a result, it progresses complications [35,36]. Increased oxidative stress also induces diabetic complications like retinopathy, nephropathy, neuropathy, etc. [37]

Conclusion:-

In our study increased MDA levels indicate the increased oxidative stress. Enzymatic antioxidant like SOD activity is significantly decreased in our study. Non-enzymatic antioxidants vitamin C and E levels are also decreased which indicates the reduced antioxidant status in Type-2 DM patients.

In conclusion, there is considerable evidence that induction of oxidative stress plays a key role in the onset of diabetic complications. The evidence in present studies has demonstrated that the increased oxidative stress marker MDA and the decreased level of antioxidants can predict the micro-vascular complications in diabetes mellitus. Oxidative stress seems to be more worrying in metabolic disorders specially Type-2diabetes.Strict glycemic control is needed for reducing oxidative stress in diabetes mellitus patients and to prevent its complications.

Acknowledgements- The study was carried out the permission granted from the Integral Institute of Medical Sciences & Research, Integral University, Lucknow.

Ethical Approval: - The study was approved by the Ethical Committee of Integral Institute of Medical Sciences & Research Integral University, Lucknow-India.

Source of Funding: - Not applicable

Conflict of interest: - The authors declare that they have no known competing financial Interests or personal relationships that could have appeared to influence the work reported in this paper.

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