EVALUATION OF OXIDATIVE STRESS BIOMARKERS IN TYPE 2 DIABETES PATIENTS

Preeti Sharma[1], Shreya Nigoshkar[2*]

[1,2*]Department of Biochemistry, Index Medical College Hospital and Research Centre, Malwanchal University, Indore (MP), India (dr.preetibiochem@gmail.com, Shreyanigoskar@gmail.com)

Abstract:

Hyperglycemia is a chronic medical condition that occurs when the body is unable to properly regulate blood sugar levels. This condition is commonly associated with diabetes, both type 1 and type 2, when the body either does not produce enough insulin or is unable to use insulin effectively. Insulin is a hormone that helps regulate blood sugar by allowing cells to absorb glucose for energy refers to high levels of glucose in the blood. Untreated or poorly managed diabetes can lead to serious complications, including heart disease, stroke, kidney disease, nerve damage, and eye problems.. Type 2 diabetes mellitus (T2DM) is commonly regarded as a severe public health problem due to its potentially lethal implications and increased mortality risk. Reactive oxygen species (ROS) generation and antioxidant defence are in balance in Oxidative stress, which allows the body to detoxify its negative effects and prevent cell damage. Hyperglycemia generates reactive oxygen species (ROS), which in turn cause many types of cell damage. Secondary problems in diabetes mellitus ultimately occur from cell damage. The evaluation of oxidative stress markers in type 2 diabetic patients was the primary goal of the current investigation, 150 diagnosed Type 2 DM patients with no systemic complication and co-morbidities were chosen as the cases, while 150 healthy volunteers with similar age and sex were chosen as the controls. Catalase (CAT), Superoxide dismutase (SOD), Glutathione Peroxidase (GPx), Reduce Glutathione reductase (GSH), Glutathione reductase (GR) and Protein carbonyl was estimated by colorimetric methods while spectrophotometric analysis was used to determine lipid peroxidation. Significant increase ($P < 0.05$) were seen as Catalase (CAT), Superoxide dismutase (SOD), Glutathione Peroxidase (GPx), Reduce Glutathione reductase (GSH) ,Glutathione reductase (GR) Lipid peroxidation(LPO) and Protein carbonyl contrasted with the control group in DM patients. The findings of the present investigation revealed that diabetic patients experience higher levels of oxidative stress. It is possible to employ oxidative stress markers as early indicators of diabetic problems. Patients with DM may benefit from new therapeutic approaches that attempt to reduce oxidative stress.

Keywords: oxidative stress, Type 2 diabetes (T2DM), hyperglycemia

***Corresponding Author:** Dr. Shreya Nigoshkar
*Professor & Head, Department of Biochemistry, Index Medical College Hospital and Research Centre, Malwanchal University, Indore (MP), India Email:Shreyanigoskar@gmail.com

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Introduction

Hyperglycemia caused by deficits in insulin secretion, action, or both characterizes a group of metabolic diseases collectively known as diabetes mellitus. Chronic hyperglycemia can cause long-term harm, malfunction, and loss of normal functioning to the different body organs.[1,2] According to a 2017 estimate from the International Diabetes Federation, 8.8% of adults globally (424.9 million individuals) had diabetes.[3] Among these, type 2 diabetes accounts for the bulk of cases (87%–91%). Type 2 diabetes mellitus (T2DM) is commonly regarded as a severe public health problem due to its potentially lethal implications and increased mortality risk.[4] The autoimmune diseases, genetics, and environmental variables are all believed to play a substantial role in the development of T2DM, despite the fact that the precise causes of T2DM are not well understood.[5] Leading contributors to blindness, renal failure, and nerve damage are diabetes-specific microvascular diseases.[6] Due to factors including population expansion, age, urbanization, and a rise in obesity brought on by physical inactivity, the prevalence of diabetes is rising everywhere in the world. In Asian nations, young to middle-aged people are more likely to have diabetes than in Western nations, where disease mostly affects the elderly. All of these issues have detrimental long-term repercussions

on the economy and health of a country, particularly developing nations. Based on projections made by the International Diabetes Federation (IDF), the country of India would have 87.0 million individuals with diabetes by 2030, up from a total of 50.8 million in 2010.[7] Reactive oxygen species (ROS) generation and antioxidant defence are in balance in Oxidative stress, which allows the body to detoxify its negative effects and prevent cell damage. Hyperglycemia generates reactive oxygen species (ROS), which in turn cause many types of cell damage. Secondary problems in diabetes mellitus ultimately occur from cell damage.[8-9] Although the present research indicates that ROS created as a result of many cell's physiological and homeostatic processes, ROS formation was previously believed to be a kind of harmful cellular stress. There are different examples of ROS that can develop in excess and destroy various biological components, including proteins, lipids, and nucleic acids.[10] By neutralizing ROS or preventing their generation, antioxidants which are produced by the body combat their effects. Stopping OS from causing the start of numerous diseases may entail maintaining a balance between ROS and antioxidant levels.[11-12]

Material & Methods

At the Index Medical College Hospital and Research Centre, Malwanchal University, Indore (MP), India, this study was designed and carried out. Prior to beginning the investigation; approval from the institutional ethics committee was obtained. All subjects provided their informed consent. A convenient sampling technique was used to determine the sample size, 150 diagnosed Type 2 DM patients with no systemic complication and co-morbidities were chosen as the cases, while 150 healthy volunteers with similar age and sex were chosen as the controls.

Inclusion criteria

Case: Age between 35 and 65 years old amle and female, T2DM (American Diabetes Association's diagnostic criteria were used to make the diagnosis) and HbA1c >9%.

Controls: Male and female volunteers between the ages of 35 and 65 who were in good health were chosen as the control subjects.

Exclusion criteria

Case: Person suffering from endocrine disorder, smokers, and those taking drugs that reduce inflammation, Systemic hypertension patients who took antioxidants, avoided using insulin or oral hypoglycaemic drugs, and underwent treatment, People who are unwilling to participate in the trial were also excluded from the analysis.

Sample Collection and Laboratory analysis: A venipuncture was used to collect blood EDTA-K2 vacutainer. Blood was drawn, separated by centrifugation, and then stored at -80°C in order to provide plasma for the biochemical testing. Commercially available kits were used to determine fasting and postprandial blood glucose levels, Liver function, Kidney function markers, and for HbA1C.

Statistical analysis

Using the SPSS-17 program, the results of the biochemical analyses were reported as mean value standard deviation (S.D.). Statistical significance was set at P values less than 0.05.

Diabetic Markers

Glycosylated Haemoglobin (HbA1C) was calculated using an affinity chromatographic method, while the GOD-POD (Glucose Oxidase-Peroxidase) method was used to measure blood sugar levels while fasting and after meals (PPBS).

Oxidative stress markers

Catalase (CAT),[13] Superoxide dismutase (SOD),[14] Glutathione Peroxidase (GPx),[15] Reduce Glutathione reductase (GSH),[16] Glutathione reductase (GR)[17] and Protein carbonyl was estimated by colorimetric methods[18] while spectrophotometric analysis was used to determine lipid peroxidation.[19]

Kidney Function markers

The Berthelot method was used to quantify serum urea, while the Modified Jaffe's method was used to measure creatinine.

Liver Function Markers

Enzymatic colorimetric methods were used to estimate SGOT, SGPT, and ALP, while bromocresol green was used to estimate albumin. The colorimetric approach (biuret method) was used to estimate total protein and the colorimetric approach was used to estimate direct bilirubin.

Results:

Diabetic markers

Fasting blood sugar, HbA1C, and Postprandial blood sugar in Diabetes Mellitus exhibit substantial increases ($P < 0.05$) in comparison to the control group, as displayed in Table1.

Kidney function markers

The information in [Table 2] indicates DM patients' blood urea and creatinine readings did not significantly differ from those of the control group in any discernible way (P > 0.05).

Liver Function markers

Alkaline phosphatase (ALP), direct bilirubin, and indirect bilirubin were all noticeably greater in the DM group compared to the control group (P > 0.05), although total protein, SGOT, SGPT, and serum albumin as seen in [Table 3], there were no discernible differences.

Oxidative stress markers

Significant gains ($P < 0.05$) are seen in [Table 4] as Catalase (CAT), Superoxide dismutase (SOD), Glutathione Peroxidase (GPx), Reduce Glutathione reductase (GSH), Glutathione reductase (GR) Lipid peroxidation (LPO) and Protein carbonyl contrasted with the control group in DM patients.

Table 1 - Diabetes indicators in diabetic and control groups.

Diabetic Markers	Control (N=150)	T2DM (N=150)	P value
HbA1c (%)	4.99 ± 0.28	8.21 ± 1.56	0.001
FBS (mg/dl)	78.76 ± 11.51	202.33 ± 55.10	0.001
PPBS (mg/dl)	98.87 ± 10.59	277.10 ± 76.45	0.001

Table 2 -Renal function markers in the diabetes and control groups.

Renal Markers	Control (N=150)	T2DM (N=150)	P value
Serum Urea (mg/dl)	31.72 ± 7.99	31.97 ± 8.01	0.39
Creatinine (mg/dl)	0.93 ± 0.32	0.95 ± 0.41	0.37

Table 3 - The diabetes and control groups' indicators of liver function.

Liver Markers	Control (N=150)	T2DM (N=150)	P value
SGOT (IU/L)	32.94 ± 11.06	32.58 ± 10.90	0.39
SGPT (IU/L)	32.28 ± 11.12	32.82 ± 10.62	0.33
ALP (IU/L)	71.21 ± 26.91	79.66 ± 26.85	0.003
Direct Bilirubin (mg/dl)	0.13 ± 0.07	0.12 ± 0.07	0.057
Indirect Bilirubin (mg/dl)	1.09 ± 0.05	0.59 ± 0.23	0.001
Total Protein (gm/dl)	7.39 ± 0.44	4.45 ± 0.47	0.103
Albumin (gm/dl)	4.30 ± 0.53	4.25 ± 0.52	0.191

Table 4 -Oxidative stress markers in both the diabetes and control groups.

Oxidative stress markers	Control (N=150)	T2DM (N=150)	P value
LPO (nmol MDA/ml)	2.06 ± 0.87	5.56 ± 1.58	< 0.0001*
SOD (unit/mg protein)	6.42 ± 2.27	3.02 ± 1.29	< 0.0001*
Catalase (unit/mg protein)	12.38 ± 1.61	9.94 ± 2.34	< 0.0001*
GR (unit/min/mg protein)	1.17 ± 0.22	0.94 ± 0.21	< 0.0001*
GPx (nmol NADPH oxidized/min/mg protein)	49.37 ± 9.91	26.48 ± 4.42	< 0.0001*
GSH umolGSH/mg protein	3.71 ± 1.01	1.63 ± 0.63	< 0.0001*
Protein Corbonyl (µmol/L)	0.071 ± 0.02	0.17 ± 0.06	< 0.0001*

**p<0.05 considered statistically significant, Lipid peroxidise (LPO), Superoxide dismutase (SOD), Catalase, (CAT), Glutathione reductase (GR), Glutathione Peroxidase (GPx), Reduce Glutathione reductase (GSH)*

Discussion

T2DM is a metabolic condition that affects more than 400 million individuals globally and is extremely dangerous to both the economy and human health.[20] Diabetes mellitus and its subsequent consequences are thought to be connected by oxidative stress. Measuring antioxidant capacity might be a valid way to gauge oxidative stress and could be helpful in estimating the likelihood of developing diabetes problems.[21,22] It is believed that oxidative damage is the primary cause of insulin resistance and Diabetes Mellitus.[23] SOD, an enzyme that scavenges superoxide radicals, is regarded as the first line of defence against oxygen radicals' harmful effects on cells. The ability of SOD to quickly dismutate free oxygen radicals and shield the cells from damage by oxidative stress which is made possible by its distribution throughout the body. In our study we found that Type 2 diabetic patients had considerably lower serum SOD levels as compared to control group Moreover, our results supported those of the studies conducted by Sundaram *et al.*[24] In the course of our analysis, we discovered that the GSH levels of Type 2 diabetic patients were much lower than those of controls. Singh *et al.* also obtained a similar result. [25] Our findings were consistent with the work conducted by Arwa *et al.* in that the catalase (CAT) level was also considerably lower

in diabetic patients in the current study when compared to control groups.[27] An enzyme called glutathione peroxidise, which contains selenium, catalyzes the reduction of hydrogen peroxide by using glutathione as a substrate, protecting cells from oxidative stress. We have also found that the Glutathione reducatse (GR) level was also significantly decreased in diabetic patient's as compared to control group. We found that whereas the diabetes patient group's lipid peroxidation and protein carbonyl activities were considerably greater than those of the control group in the current investigation compared to the controls, the glutathione peroxidase activity in the diabetic patient group was considerably lower. This finding supports the idea that increased protein carbonyl activity, decreased GPx levels, and increased lipid peroxidation may contribute to tissue injury.[28, 29, 30]

Conclusion

The findings of the present investigation revealed that diabetic patients experience higher levels of oxidative stress. It is possible to employ oxidative stress markers as early indicators of diabetic problems. Patients with DM may benefit from new therapeutic approaches that attempt to reduce oxidative stress.

Conflict of Interest: There is no conflict of interest declared by authors.

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