Original Research Article

Assessment of Oxidative Stress and Antioxidant Levels in Individuals with type 2 Diabetes Mellitus and their Relationship with Glycosylated Hemoglobin

Muralidhar D[1], K Ravindranath[2*], Naganarasimhaiah S[3]

[1]Associate Professor, [2]Assistant Professor, [3]Professor, Department of General Medicine, SIMSRH, Tumkur, Karnataka, INDIA.
Email: dr.muralipavana@gmail.com

Abstract

Background: To assess serum Total Antioxidant Capacity (TAC) and serum Total Peroxidase (TP) Oxidative Stress Index (OSI), and to analyze their relationship with Glycosylated Hemoglobin (GHM). Study Design: This was an analytical case-control study. Setting and Duration: Conducted at the Department of Medicine, SIMSRH, from November 2013 to February 2014. **Methodology**: The study included 110 participants divided into two groups: 55 individuals with Type 2 Diabetes Mellitus (T2DM) and 55 healthy controls, based on serum Fasting Blood Sugar (FBS), Post Prandial Blood Sugar (PPBS), clinical history, and medication. Serum levels of TAC and TP were assessed, and OSI was calculated and correlated with GHM. Additionally, biochemical markers such as serum urea, creatinine, protein, albumin, and bilirubin were evaluated in all participants. **Results:** The findings indicated no significant differences between the two groups regarding age, body mass index, and blood pressure (all $p > 0.05$). However, serum levels of FBS, PPBS, GHB, and mean blood glucose (MBG) were significantly elevated in T2DM subjects ($p < 0.05$). Spearman's correlation analysis in T2DM participants revealed a statistically significant positive correlation between FBS and OSI, PPBS and TP, as well as GHB and OSI, TP. Conversely, a negative correlation was observed between GHB and TAC, and between TP and OSI with total protein and total bilirubin, while no significant correlation was found with urea and creatinine. **Conclusion:** Individuals with T2DM exhibited elevated levels of TP and OSI, alongside reduced TAC, correlating with diminished individual antioxidants such as total protein, albumin, uric acid, and total bilirubin.

Keywords: Type 2 Diabetes Mellitus, Total Antioxidant Capacity, Serum Total Peroxidase, Oxidative Stress Index, Glycated Hemoglobin.

***Address for Correspondence:**
Dr K Ravindranath, Assistant Professor, Department of General Medicine, SIMSRH, Tumkur, Karnataka, INDIA.
Email: drravikatta@gmail.com

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INTRODUCTION

Diabetes mellitus (DM) refers to a metabolic disorder with various causes, marked by chronic hyperglycemia and disruptions in carbohydrate, fat, and protein metabolism. These issues arise from deficiencies in insulin secretion, insulin action, or both. Type 2 diabetes mellitus (T2DM) is the most prevalent type of diabetes, characterized by a reduced effectiveness of insulin on peripheral tissues (insulin resistance, IR) and a relative inability of the pancreas to produce adequate insulin, which can range from early relative deficiency to late absolute deficiency, with either aspect potentially being the primary issue. Oxidative stress, resulting from the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), has been identified as a fundamental factor contributing to the onset of insulin resistance, β-cell dysfunction, impaired glucose tolerance, and T2DM. Cells can manage moderate oxidative stress by enhancing gene expression to boost their antioxidant defenses and restore the balance

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between oxidants and antioxidants. However, if this compensatory mechanism fails due to enzyme damage, substrate shortages, or excessive oxidative stress, an imbalance occurs, leading to oxidative stress. This persistent imbalance can cause damage to DNA, proteins, and lipids, ultimately resulting in cell death. Antioxidants in the body can be categorized based on their mechanisms of action into three primary groups: preventive antioxidants, which inhibit the formation of new ROS (such as ceruloplasmin, metallothionein, albumin, myoglobin, ferritin, and transferrin); scavenging antioxidants, which eliminate existing ROS to prevent radical chain reactions (including reduced glutathione, α-tocopherols, ascorbic acid, β-carotene, uric acid, and bilirubin); and enzyme antioxidants, which catalyze the oxidation of other molecules (such as superoxide dismutase (SOD), glutathione reductase, glutathione peroxidase, and catalase). Additionally, antioxidants can be classified as endogenous, including SOD, catalase, glutathione, uric acid, and NADPH.

Glycated hemoglobin is a variant of hemoglobin that is primarily assessed to determine the average plasma glucose levels over extended periods. It is produced through a non-enzymatic glycation process when hemoglobin interacts with plasma glucose. Reactive oxygen species (ROS) arise from dysfunctions in the mitochondrial electron transport chain or from the activation of hexosamines in conditions of hyperglycemia. These ROS interfere with the signaling pathways between the insulin receptor and the glucose transport system, contributing to insulin resistance. They also play a role in the inactivation of essential anti-atherosclerotic enzymes, such as endothelial nitric oxide synthase and prostacyclin synthase, and are involved in the formation of advanced glycated end products (AGEs), which can lead to clinical complications and metabolic disorders. Additionally, ROS can be generated when AGEs bind to the receptor for AGE (RAGE) on endothelial cells, stimulating ROS production via NADPH oxidase, particularly in the form of superoxide anions, or through glucose overload in the mitochondria. Once generated, ROS activate nuclear factor (NFkB), leading to the transcriptional activation of genes associated with inflammation, immunity, and atherosclerosis. The American Diabetes Association has established diagnostic criteria for diabetes mellitus, which include classic symptoms alongside casual plasma glucose levels of ≥200 mg/dL (11.1 mmol/L), fasting plasma glucose levels of ≥126 mg/dL (7.0 mmol/L), and two-hour postprandial blood sugar levels of ≥200 mg/dL. The oxidative stress index (OSI) is assessed by measuring oxidants, individual antioxidants, or both. Our research posits that in patients with type 2 diabetes mellitus (T2DM), oxidant levels will rise while antioxidant levels will decline, resulting in a shift in the oxidative/antioxidative balance towards the oxidative side. Consequently, increased oxidative stress may be linked to both affected individuals and healthy subjects, as measured by total antioxidant capacity (TAC), total protein levels, and OSI, and correlated with glycated hemoglobin values.

MATERIALS AND METHODS

The study involved native residents from Tumkur and its neighboring regions. It was conducted with 110 individuals attending the outpatient department of SIMSRH. Participants were categorized into two groups based on their health status: those with Type 2 Diabetes Mellitus (T2DM) and healthy controls, determined by serum fasting and post-prandial glucose levels, clinical history, and medication use. The study comprised 55 subjects with T2DM (32 males and 23 females) and 55 control subjects (37 males and 18 females). The median age of the entire study cohort was 51.0 years, with an inter-quartile range of 45.0 to 59.25 years. Specifically, the median age for control subjects was 45 years (inter-quartile range: 37 to 51), while for T2DM subjects, it was 52 years (inter-quartile range: 44 to 61). Participants' ages ranged from 15 to 59 years. Clinical assessments were conducted through personal interviews. Recruitment was managed by the principal investigators, who selected individuals with T2DM that did not use supplemental vitamins and had no history of chronic heart disease, rheumatoid arthritis, malignancy, hypertension, or liver and kidney diseases. Participants were also required to complete a questionnaire.

Laboratory Procedure

A total of 7 ml of venous blood was collected. Of this, 1 ml was transferred into fluoride oxalate tubes for glucose estimation, and 2 ml was allocated to potassium salt of ethylene diaminetetraacetic acid (EDTA) tubes for glycated hemoglobin (HbA1C) analysis. The remaining 5 ml was placed in plain tubes and centrifuged at 1500 g for 10 minutes. The resulting serum was divided into 2 ml aliquots and stored at -20°C until needed for the analysis of blood glucose, total protein, albumin, bilirubin, urea, and creatinine, with diagnostic kits sourced from Human Diagnostics, Germany. The determination of glycated hemoglobin was performed using the ion exchange chromatography method provided by Tulip Diagnostics (P) Ltd, India. All tests adhered to the manufacturers' protocols, and a semi-automated analyzer was utilized for biochemical assessments. The total antioxidant status of the plasma was evaluated using the Ferric Reducing Antioxidant

Power (FRAP) automated colorimetric method developed by Erel. Additionally, total plasma peroxide concentrations were measured using the FOX2 method, with slight modifications, and the oxidative stress index was calculated by multiplying the ratio of total protein to total antioxidant capacity by 100.

Statistical Analysis

Patients' data were collected in prescribed forms containing history, clinical findings, available laboratory data, and socio-economic background. The data was analyzed by the statistical software. Data were presented median (interquartile range). Correlations analysis was done using Spearman's coefficient. Comparison of parameters between T2DM and healthy controls was performed with Mann-Whitney test. Two tailed probability values were calculated throughout, and $p<0.05$ was considered statistically significant.

RESULTS

A total of 55 subjects with type 2 diabetes mellitus (T2DM) were matched with 55 control subjects based on age, body mass index (BMI), and blood pressure. The demographic characteristics of the study population are presented in Table 1. No significant differences were observed between the T2DM subjects and the control group regarding age, BMI, and blood pressure (all $p > 0.05$).

Table 1: Demographic profile of controls andT2DMpatients

Parameters	T2DM (N=55)	Controls (n=55)	P
Age (Years)	52(61-44)	45(51-37)	0.368
BMI (kg/m2)	24.14(27.02-22.06)	24(27.2-22.4)	0.86
SBP (mmHg)	135(145-125)	120(124-116)	0.013
DBP (mmHg)	84(86-82)	78(82-76)	0.009

The values presented include the median (interquartile range), with a significant mean difference ($p<0.05$) and a highly significant mean difference ($p<0.0001$). The abbreviations used are as follows: BMI refers to Body Mass Index; SBP denotes Systolic Blood Pressure; and DBP stands for Diastolic Blood Pressure. Table 2 displays the clinical and biochemical parameters of the study population. The levels of Fasting Blood Sugar (FBS), Postprandial Blood Sugar (PPBS), glycated hemoglobin (HbA1c), and Mean Blood Glucose (MBG) were significantly elevated in individuals with Type 2 Diabetes Mellitus (T2DM) compared to the control group ($p>0.05$).

Table 2: Biochemical parameter in controls and T2DM patients

Parameters	Controls (N=55)	T2DM (n=55)	P
FBS	90(100-84)	127(137-109)	<0.001
PPBS	123(129-117)	173(219-132)	<0.001
GHB	7.7(7.9-7.5)	9.1(9.7-8.5)	<0.001
MBG	104(110-99)	144(160-127)	<0.001
Total Protein	6.8(7.1-6.4)	6.8(7.1-6.5)	0.513
Albumin	3.7(4.0-3.5)	3.7(4.0-3.5)	0.42
Total Bilirubin	0.8(1.0-0.7)	0.8(1.0-0.7)	0.791
Urea	30(34-25)	25(31-21)	0.011
Creatinine	0.9(1.0-0.8)	0.9(1.0-0.8)	0.149

Values are presented as median (interquartile range), with a significant mean difference ($p<0.05$) and a highly significant mean difference ($p<0.0001$). FBS refers to Fasting Blood Sugar, PPBS to Post Prandial Blood Sugar, and GHB to glycated hemoglobin. The oxidative and antioxidative parameters of the study population are detailed in Table 2. Total Protein (TP), Total Antioxidant Capacity (TAC), and Oxidative Stress Index (OSI) were found to be significantly elevated in subjects with Type 2 Diabetes Mellitus (T2DM) compared to control subjects ($p<0.05$).

Table 3: Oxidative and antioxidative parameters in controls and T2DM patients

Parameters	Controls (N=55)	T2DM (n=55)	P
Total Peroxide	1.92(2.26-1.69)	3.57(4.66-2.42)	<0.001
TAC	1165.35(1771.65-1547.24)	980.31(1234.25-785.43)	<0.001
OSI	0.0011(0.0014-0.0009)	0.0039(0.0050-0.0021)	<0.001

Values are presented as median (interquartile range), with a significant mean difference ($p<0.05$) and a highly significant mean difference ($p<0.0001$). TP refers to Total Peroxidase, TAC to Total Antioxidant Capacity, and OSI to the oxidative stress index. The Spearman's correlation between oxidative stress indices and biochemical variables is detailed in Tables 4 and 5. In the control group, Spearman's correlation analysis revealed a positive but statistically non-significant correlation between fasting blood sugar (FBS) and both TP and OSI, while showing a negative and non-significant correlation with TAC. Additionally, postprandial blood sugar (PPBS) exhibited a positive but non-significant correlation with TAC, alongside negative and non-significant correlations with TP and OSI. Furthermore, glycosylated hemoglobin (GHB) demonstrated a positive but non-significant correlation with TP, TAC, and OSI. In contrast, the analysis for subjects with Type 2 Diabetes Mellitus (T2DM) indicated a positive and statistically significant correlation between FBS and OSI, as well as a positive correlation with TP, while showing a negative correlation with TAC that was not statistically significant. PPBS also displayed a positive and statistically significant correlation with TP, a positive correlation with OSI, and a negative correlation with TAC that was not statistically significant. Lastly, GHB showed a positive correlation with both OSI and TP, but a negative correlation with TAC, with all correlations being statistically significant.

Spearman's correlation coefficients for the biochemical variables are presented in Tables 6 and 7. In the control group, the analysis revealed a negative and statistically non-significant correlation between total protein (TP) and oxidative stress index (OSI) with total protein, albumin, total bilirubin, and creatinine. Conversely, a positive but statistically non-significant correlation was observed with urea. Additionally, the correlation between TP and total antioxidant capacity (TAC) was negative and not statistically significant, while the correlation between OSI and TAC was negative and statistically significant. In contrast, the analysis for subjects with type 2 diabetes mellitus (T2DM) indicated a negative and statistically significant correlation between TP and OSI with TAC. The correlations of TP with total protein, total bilirubin, urea, and creatinine, as well as the correlations of OSI with albumin, total bilirubin, and urea, were negative but not statistically significant. Furthermore, the correlation of total peroxide with albumin and the correlations of OSI with total protein and creatinine were positive yet statistically non-significant.

Table 4: Spearman's correlation analysis in the control subjects with given parameter

Parameter	TP	TAC	OSI
FBS	r= 0.181	r= -0.112	r= 0.213
	p= 0.186	p= 0.417	p= 0.118
PPBS	r= -0.064	r= 0.113	r= -0.057
	p= 0.643	p= 0.411	p= 0.680
GHB	r= 0.017	r= 0.009	r= 0.048
	p= 0.904	p= 0.951	p= 0.727

FBS: Fasting Blood Sugar, PPBS: Post Prandial Blood Sugar, GHB: glycated Hb

Table 5: Spearman's correlation analysis in the T2DM with given parameter

Parameter	TP	TAC	OSI
FBS	r= 0.264	r= -0.353	r= 0.382
	p=0.052	p=0.008	p=0.004
PPBS	r= 0.377	r= -0.201	r= 0.362
	p=0.004	p= 0.141	p=0.006
GHB	r= 0.505	r= -0.528	r= 0.539
	p<0.001	p<0.001	p<0.001

FBS: Fasting Blood Sugar, PPBS: Post Prandial Blood Sugar, GHB: glycated Hb

Table 6: Spearman's correlation analysis in the Control subjects with given parameter

Parameter	TAC	Total Protein	Albumin	Total Bilirubin	Urea	Creatinine
TP	r= -0.115	r= -0.047	r= -0.065	r= -0.148	r= 0.250	r= -0.013
	p=0.402	p= 0.734	p=0.638	p=0.281	p= 0.066	p=0.927
OSI	r= -0.388	r= -0.040	r= -0.079	r= -0.220	r= 0.270	r= -0.022
	p= 0.003	p= 0.771	p= 0.566	p= 0.106	p= 0.046	p=0.874

TP -Total Peroxidase, OSI- Oxidative Stress Index

Table7: Spearman's correlation analysis in the T2DM with given parameter

Parameter	TAC	Total Protein	Albumin	Total Bilirubin	Urea	Creatinine
TP	r= -0.353	r= -0.031	r= 0.007	r= -0.135	r= -0.114	r= -0.088
	p =0.008	p= 0.820	p=0.959	p=0.325	p= 0.409	p=0.525
OSI	r= -0.749	r= 0.009	r= -0.042	r= -0.027	r= -0.108	r= 0.052
	p<0.001	p= 0.945	p= 0.759	p= 0.842	p= 0.434	p=0.708

TP -Total Peroxidase, OSI- Oxidative Stress Index

DISCUSSION

Chemical reduction through oxidation-reduction (redox) reactions has been fundamental to the emergence of life. Research by Hulbert et al. demonstrated that aerobic cellular respiration inevitably produces pro-oxidative byproducts, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), which can inflict oxidative damage on DNA, proteins, and lipids if not adequately neutralized by the body's antioxidant systems. Goodarzi et al. found that lipid peroxidation and oxidative stress are heightened in diabetes, establishing a positive correlation between hyperglycemia levels and oxidative stress. Their study also highlighted a significant relationship between malondialdehyde (MDA) levels and mean HbA1c in both diabetic and healthy individuals. In our investigation, subjects with Type 2 Diabetes Mellitus (T2DM) exhibited a notable increase in HbA1c levels (6.89 [7.40-6.39]), total peroxide (3.57 [4.66-2.42]), and oxidative stress index (OSI) (0.0039 [0.0050-0.0021]) compared to control values of 5.72 [5.89-5.55], 1.92 [2.26-1.69], and 0.0039 [0.0050-0.0021] for HbA1c, total peroxide, and OSI, respectively. Additionally, T2DM subjects showed a significantly reduced total antioxidant capacity (TAC) of 980.31 [1234.25-785.43] compared to controls at 1165.35 [1771.65-1547.24]. Jain et al. (1989) proposed that hyperglycemia triggers lipid peroxidation in red blood cells, leading to increased oxidative stress due to excessive glycosylation of hemoglobin, a finding corroborated by our results, which indicated significantly higher oxidative stress in T2DM patients (mean total peroxide of 3.57 µmol in T2DM versus 1.92 µmol in controls). Our study focuses on the well-researched chemical intermediates, lipid peroxides, and late-stage lipid oxidation products, along with the antioxidant defense mechanisms in T2DM. The observed lower TAC in T2DM patients aligns with previous studies, reinforcing the notion that antioxidant inhibition plays a role in oxidative stress associated with diabetes.

CONCLUSION

The research conducted was a prospective cohort cross-sectional study involving the community population of Tumkur and its surrounding areas. Given the absence of prior studies on oxidative stress and total antioxidant status in this region, we believe that our findings may reflect the broader population of Nepal. The study included 110 participants who voluntarily took part, consisting of 55 individuals with Type 2 Diabetes Mellitus (T2DM) and 55 healthy controls. Our findings indicated a clear positive correlation between total peroxide levels and total antioxidant status in both T2DM and healthy subjects. Specifically, T2DM participants exhibited significantly elevated levels of total peroxide and the oxidative stress index (OSI), alongside notably lower total antioxidant status compared to healthy controls. Additionally, when examining other individual antioxidants such as total protein, albumin, and total bilirubin, all except uric acid were found to be significantly reduced in T2DM subjects relative to healthy controls. Therefore, the increase in total peroxide and OSI is indicative of oxidative stress.

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