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Assessment of nitric oxide and peroxiredoxin along with frap in newly diagnosed patients of type 2 diabetes mellitus

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Abstract

The pathophysiological mechanisms underlying Type 2 Diabetes Mellitus (T2DM) involve oxidative stress and impaired antioxidant defense systems. Hence, we aimed to assess Nitric Oxide (NO), Peroxiredoxin, and Ferric Reducing Ability of Plasma (FRAP) levels in newly diagnosed patients with T2DM. In this case-control study, we included 63 patients as cases (newly diagnosed T2DM patients) and 63 as controls (healthy individuals). Detailed clinic-demographic data were recorded for all participants. Furthermore, fasting and post-prandial plasma glucose, HbA1c, NO, FRAP and peroxiredoxin were calculated. Statistical analysis was conducted to compare the findings between the two groups. The preponderance of cases and controls were aged between 61 and 65. Male patients were the majority in both groups. The majority of reported cases involved alcohol consumption (p=0.0314*). The study revealed significant differences in kidney function, lipid profile, fasting and postprandial plasma glucose, and Hba1c levels between cases and controls (p<0.05). NO and FRAP levels in the case group were significantly lower (p<0.0001*), while peroxiredoxin levels were significantly higher (p<0.0001*). T2DM is associated with increased oxidative stress, indicated by elevated levels of Peroxiredoxin and decreased levels of FRAP and NO. These alterations in antioxidant defence mechanisms may serve as early indicators for the development of T2DM complications.

Keywords: Diabetes Mellitus, Ferric Reducing Ability, Total Antioxidant Capacity, Nitric oxide, Reactive Oxygen Species

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1. Introduction

Diabetes mellitus (DM) is a prevalent endocrine and metabolic disorder and a leading global cause of death. It is characterized by insufficient insulin production by the pancreas's beta cells, leading to metabolic dysregulation. [1] The complications of diabetes mellitus vary from person to person and are influenced by factors such as overall health and diet. Approximately 190 million people of various ages are affected by diabetes mellitus, making it one of the most significant causes of disability and mortality worldwide. [2] Type 2 diabetes mellitus (T2DM) has reached epidemic proportions, particularly in certain population subgroups. It is projected to become one of the leading preventable causes of death due to its increasing prevalence. In India alone, the number of diabetes cases is estimated to be around 66.8 and 69.1 crores in 2014 and 2015, and it is projected to rise to 642 million by 2040 [3]. Type 2 diabetes occurs due to insulin resistance and abnormal insulin secretion. It is influenced by factors such as obesity, age, ethnic origin, and family history.

While genetics plays a role, environmental and lifestyle factors also contribute to developing type 2 diabetes. Insulin resistance impairs the body's response to insulin, leading to hyperglycemia. When beta cells fail to produce sufficient insulin to compensate for insulin resistance, type 2 diabetes develops. [4] Oxidative stress, characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defence mechanisms, is implicated in the pathogenesis of diabetes and its complications. [5] High levels of ROS can result from glucose and lipid overload, which trigger oxidative stress. [6] Antioxidants, such as vitamins E, C, and A, play a vital role in counteracting oxidative stress. However, the efficacy of vitamin supplementation as an antioxidant therapy remains uncertain, and more research is needed. [6] Oxidative stress can have detrimental effects on cellular physiology, including damage to lipids, proteins, and DNA. It is implicated in the development and progression of diabetes complications, both

microvascular and macrovascular. [7] Mitochondrial superoxide overproduction has been identified as a primary cause of metabolic abnormalities in diabetes. [8-9] total antioxidant capacity (TAC) measurement is essential in evaluating oxidative stress. The ferric-reducing ability/antioxidant power (FRAP) assay is a direct test that provides a rapid, cost-effective, and robust measure of TAC. This study aimed to assess oxidative stress markers, FRAP, and antioxidant enzyme activity in newly diagnosed patients with type 2 diabetes mellitus.

2. Materials and Methods

We conducted this Case-Control study at the Department of Biochemistry, L.N.C.T. University, Bhopal, for one year. After obtaining the ethical clearance and informed consent, we included 63 newly diagnosed T2DM as cases (subjects with Fasting blood sugar ≥126mg/dl or 2-hour post-prandial blood sugar ≥200mg/dl are considered cases. Case was newly diagnosed as T2DM) and 63 as controls (Subjects with Fasting blood sugar <110mg/dl, or 2 hours post-prandial blood sugar <140mg/dl are considered controls). On the contrary, patients with type 1 diabetes mellitus and other chronic diseases, such as cardiovascular disease, cancer etc., were excluded from the study. A detailed clinical history, including age, sex, occupation, and other associated risk factors contributing to the illness, was elicited from the case and controls. Levels of markers such as oxidative markers: serum nitric oxide (NO) (Abnova Catalog Number KA1641) were detected per the kit protocol. Ferric reduction antioxidant potential (FRAP) (Catalog Number KA6199) and Antioxidant enzyme: peroxiredoxin (Catalog Number KA2121) was also evaluated as per the kit protocol.

2.1. Statistical Analysis

Data were entered in Microsoft Excel and analyzed using statistical software S.P.S.S. version 26 (S.P.S.S. Inc., Chicago, IL, U.S.A.). The continuous variables were evaluated by mean (standard deviation) or range value when required. The dichotomous variables were presented in number/frequency and were analyzed using the Chi-square test. To compare the means between the two groups, analysis by Student t-test was used. Correlation analysis was done using Pearson r correlation. At 95% confidence interval, a p-value of <0.05 or 0.001 was considered significant.

3. Results and discussion

The mean age of the patients in cases [47.14±8.16] was higher than in controls [45.12±6.09]. No substantial difference was noted in the socio-demographic parameters among groups. The mean duration of smoking was significantly higher in cases [11.53±5.62] than in controls [8.87±4.79] [Table-1]. In the case group, most of the patients' cigarette consumption per day was 4-5 times (46.03%); in the control group, most patients' cigarette consumption per day was 2-3 times (50.79%). In the cases group, 65.08% reported consuming alcohol, while in the control group, 46.03% reported the same [Figure-1]. In the case and control group, most patients did not suffer from any systemic disease [Figure-2]. The mean BMI was significantly higher in case groups [25.06±1.42] as compared to the control group [23.45±1.13] [Figure-3]. A substantial difference was noted in the kidney function test among both groups. The mean Plasma Glucose at fasting, at postprandial and HbA1C were significantly higher in Cases patients compared to Control groups [Table-1]. The mean total cholesterol and LDL-C were significantly higher in the cases than in the controls [p=0.0462*; p<0.0001*], while the HDL-C was lower in the case group than in the control group [p<0.0001*]. [Table-2] At the same time, mean nitric oxide was significantly higher in control patients [61.77±14.46] compared to cases patients [42.75±12.82]. The mean FRAP value was significantly higher in the control group [406.39±51.42] compared to the cases group [308.44±45.26]. The mean antioxidant enzyme, Peroxiredoxin, was significantly higher in the case groups than in the control group. [Table-3] After applying Pearson correlation analysis, FRAP [r=-0.6804; p<0.0001*]and NO [r=-0.3636; p<0.0001*] showed a significantly negative correlation with HbA1C level. Peroxiredoxin [r=0.3446; p<0.0001*] showed a significantly positive correlation with HbA1C level [Table-4 and Figure-4]. In our study, the mean NO was considerably higher in control patients [61.77±14.46 µmoles/L] as compared to cases patients [42.75±12.82 µmoles/L]. Another study reported the level of NO higher in controls [58.85 ± 12.81 µmoles/L] as compared to the cases [43.83 ± 11.31 µmoles/L] (p< 0.0001). In a study [10] conducted at West Glasgow Hospitals, researchers observed that individuals with type 2 diabetes exhibited decreased nitric oxide (NO) production, associated with factors such as age, body mass index (BMI), and lipid profile. Another study [11] has also reported that individuals with diabetes have an unfavourable lipid profile and altered plasma levels of oxidative stress markers, including lower nitric oxide levels than control subjects. Studies [12,13] have established that a reduction in NO bioavailability predicts dyslipidemia, as NO acts as an endogenous anti-atherosclerotic molecule. Dysfunction of the endothelial L-arginine-nitric oxide pathway, caused by various cardiovascular risk factors, including hypercholesterolemia, contributes to the deleterious effects on the vascular wall. Furthermore, researchers in China observed [14] that changes in NO levels and other markers of oxidative stress in patients with type 2 diabetes did not significantly correlate with changes in plasma lipid profile. Under normal physiological conditions, a balance exists between the generation of free radicals and the antioxidant defence mechanisms [15]. However, in individuals with type 2 diabetes mellitus (T2DM), persistent hyperglycaemia leads to increased reactive oxygen species (ROS) production, overwhelming the available antioxidant mechanisms. The Ferric Reducing Ability of Plasma (FRAP) is employed to assess the total antioxidant capacity (TAC) of plasma, which encompasses the combined activity of plasma antioxidants, including vitamins and enzymes. Numerous other studies have also demonstrated lower antioxidant levels and enhanced pro-oxidative status in diabetic conditions [7]. The mean FRAP value in our study was found to be lower in the case group [308.44±45.26 µmol/l] as compared to the control group [406.39±51.42 µmol/l], and a statistically significant difference was observed [p<0.0001*] in Mean FRAP level among both groups [16,17].

Table 1. Socio-demographic parameters of enrolled patients among the cases and control groups.

Socio-demographic parameters		CASES [n=63]		CONTROL [n=63]		P-VALUE
		MEAN/N	SD/%	MEAN/N	SD/%	
Age (years)	30-40	4	6.35%	7	11.11%	X=1.479 p=0.6870
	41-50	11	17.46%	8	12.70%	
	51-60	15	23.81%	17	26.98%	
	61-65	33	52.38%	31	49.21%	
	MEAN±SD	47.14±8.16		45.12±6.09		t=1.575 p=0.1179
BMI (K	MEAN±SD	25.06±1.42		23.45±1.13		t=7.042 **p<0.0001***
Gender	MALE	42	66.67%	37	58.73%	X=0.8484 p=0.3570
	FEMALE	21	33.33%	26	41.27%	
Marital Status	Unmarried	9	14.29%	7	11.11%	X=2.401 p=0.4935
	Married	51	80.95%	55	87.30%	
	Divorced	1	1.59%	1	1.59%	
	Widow	2	3.17%	0	0.00%	
Education	Post-Graduate	8	12.70%	6	9.52%	X=2.027 p=0.8454
	Graduate	17	26.98%	15	23.81%	
	Intermediate	22	34.92%	19	30.16%	
	Highschool	6	9.52%	10	15.87%	
	Primary	6	9.52%	8	12.70%	
	Illiterate	4	6.35%	5	7.94%	
Occupation	Business	22	34.92%	17	26.98%	X=3.153 p=0.5326
	Job	25	39.68%	23	36.51%	
	Housewife	13	20.63%	15	23.81%	
	Student	2	3.17%	5	7.94%	
	Labour	1	1.59%	3	4.76%	
Dietary Habit	Vegetarian	21	33.33%	20	31.75%	X=0.2408 p=0.6236
	Non-Vegetarian	42	66.67%	43	68.25%	

Table 2. Biochemical Analysis of the enrolled patients among the cases and control groups**.**

Biochemical Analysis		Cases [n=63]		Control [n=63]		P-value
		MEAN	SD	MEAN	SD	
Kidney Function Test	Serum Urea (mg/dL)	30.14	6.23	27.43	4.39	t=2.822 **p=0.0055***
	Serum Creatinine (mg/dL)	0.49	0.05	0.41	0.02	t=11.79 **p<0.0001***
Blood Sugar Level	Plasma Glucose Fasting (mg/dL)	141.46	4.67	91.39	10.16	t=35.54 **p<0.0001***
	Plasma Glucose Postprandial (mg/dL)	183.19	12.64	125.34	13.37	t=24.96 **p<0.0001***
	HbA1c (%)	6.46	0.52	5.08	0.49	t=15.33 **p<0.0001***
Lipid Profile	Triglyceride (mg/dL)	143.67	13.56	142.36	10.08	t=0.6154 p=0.5394
	Total Cholesterol (mg/dL)	181.06	30.42	171.49	22.31	t=2.014 **p=0.0462***
	HDL-C	35.61	5.74	41.56	5.34	t=6.024 **p<0.0001***
	LDL-C	118.36	17.08	102.68	16.59	t=5.227 **p<0.0001***
	VLDL-C	29.48	2.65	28.96	2.52	t=1.129 p=0.2612

Table 3. Level of markers and enzymes in the enrolled patients among the cases and control groups.

Markers and Enzymes		Cases [n=63]		Control [n=63]		P-value
		Mean	Sd	Mean	Sd	
Oxidative stress marker	NO (µmoles/L)	42.75	12.82	61.77	14.46	t=7.812 **p<0.0001***
Total Antioxidant Capacity	FRAP (µmol/l)	308.44	45.26	406.39	51.42	t=11.35 **p<0.0001***
Antioxidant Enzyme	Peroxiredoxin (ng/mL)	22.75	5.84	16.27	4.06	t=7.231 **p<0.0001***

Table 4. Pearson correlation analysis of the HbA1C level with various markers.

Correlation analysis			
HBA1C Vs.	**FRAP (µmol/l)**	**NO (µmoles/L)**	**Peroxiredoxin (ng/mL)**
Pearson r	-0.6804	-0.3636	0.3446
95% confidence interval	-0.7644 to -0.5738	-0.5063 to -0.2015	0.1806 to 0.4900
P value	**<0.0001***	**<0.0001***	**<0.0001***

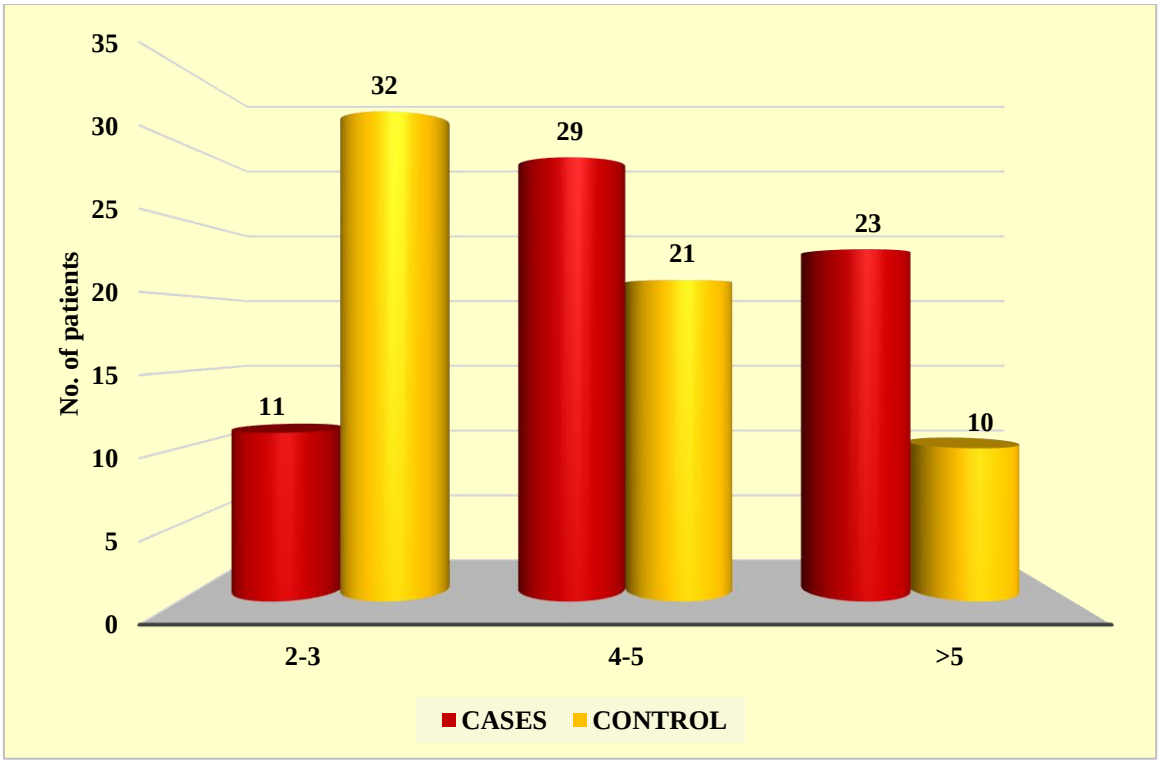

Figure 1. Frequency of the cigarettes/beedis consumption per day among the cases and control groups

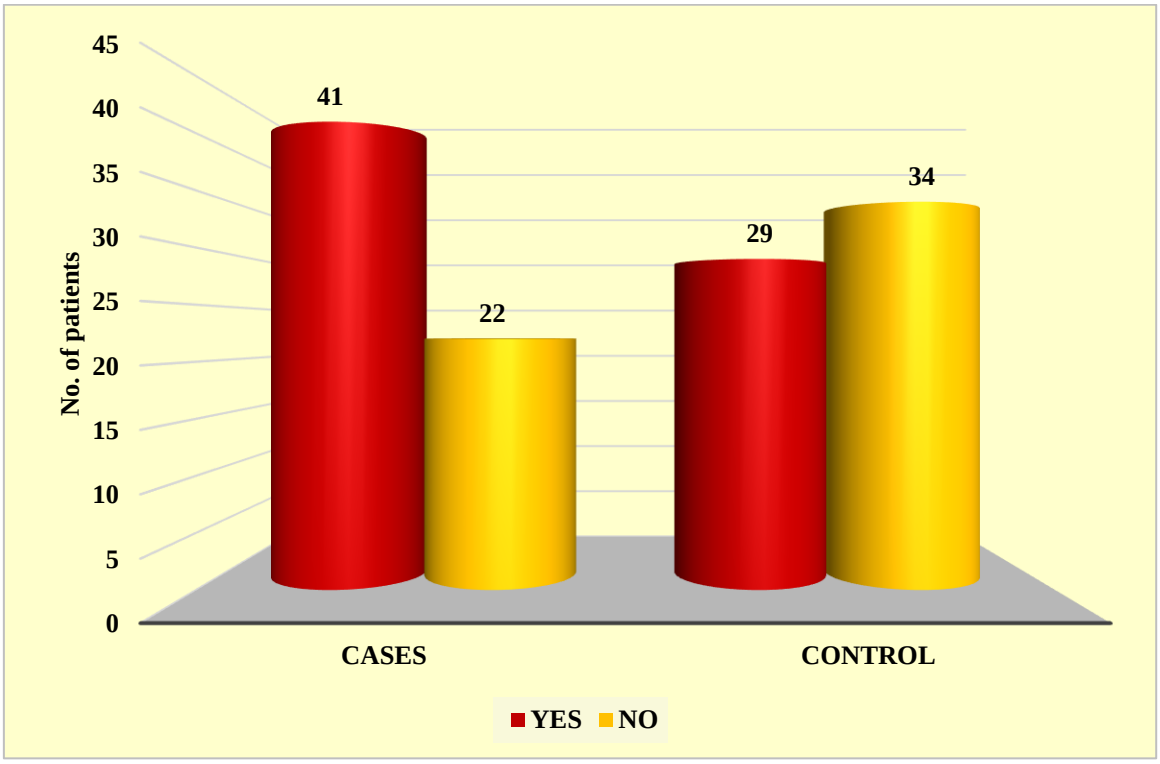

Figure 2. Alcohol consumption of the enrolled patients among the cases and control groups.

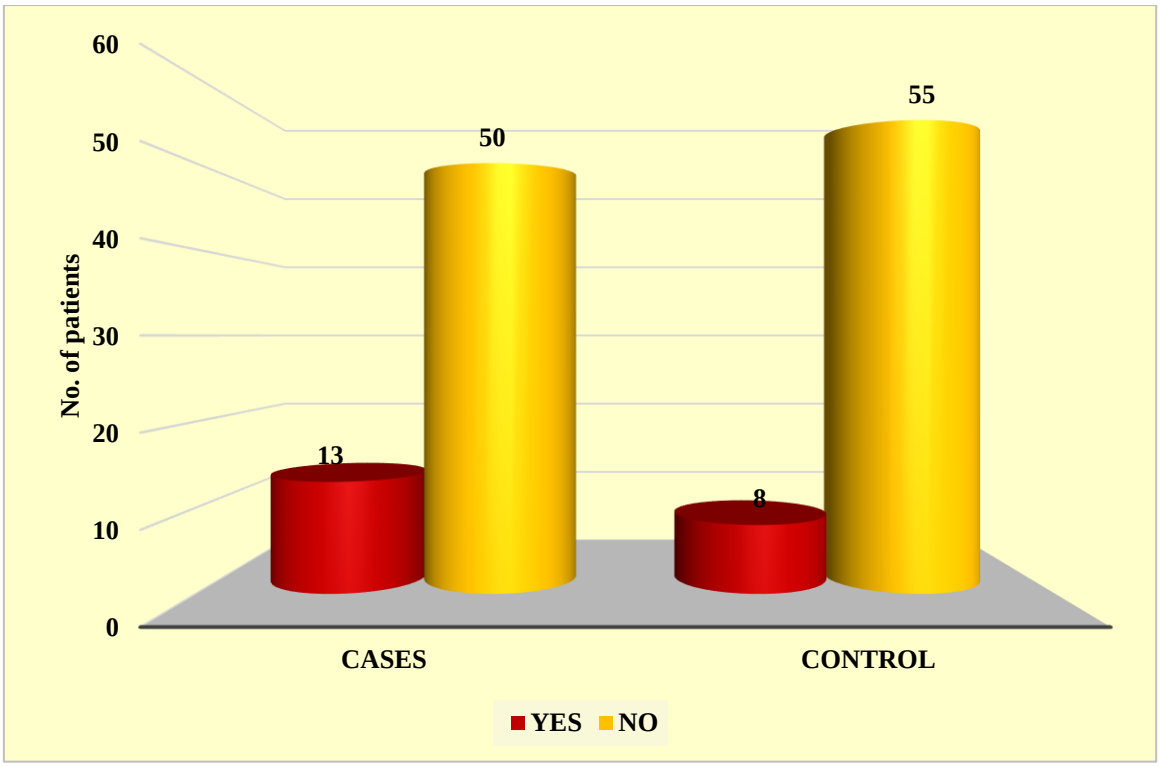

Figure 3. Suffering from any systemic disease of the enrolled patients among the case and control groups.

Beg A et al. [18] found the level of FRAP increased in the control group [407.6 ± 51.6 µmol/l] than in the case group [307.6 ± 45.62 µmol/l]. We noted that the mean antioxidant enzymes Peroxiredoxin were significantly higher in case groups [22.75±5.84 ng/mL] than in the control group [16.27±4.06 ng/mL]. A significant difference was observed in the antioxidant enzyme levels among the groups. In diabetic patients, plasma levels of peroxiredoxin isoforms (PRDX1, PRDX2, PRDX4, and PRDX6), were higher than in healthy subjects. [19] Similar findings were reported for PRDX1 activity in erythrocytes, which was greater in patients with type 2 diabetes mellitus (T2DM) than in non-diabetic individuals. [20] Another study also demonstrated higher total antioxidant capacity and increased concentrations of lipid peroxidation markers in T2DM patients compared to non-diabetic subjects. [21] Interestingly, our present study also showed higher levels of antioxidants, despite the well-known increase in oxidative stress in diabetes. These results suggest a possible adaptive response, which may be attributed to increased O2- (superoxide) production, leading to elevated H2O2 (hydrogen peroxide) production. This mechanism may necessitate higher activity of antioxidant enzymes to protect against the increased oxidative stress associated with adverse cardiovascular and metabolic conditions. [22] After applying Pearson correlation analysis, we noted that FRAP [r=-0.6804; p<0.0001*] and NO [r=-0.3636; p<0.0001*] showed a significantly negative correlation with HbA1C level. Peroxiredoxin [r=0.3446; p<0.0001*] showed a significantly positive correlation with HbA1C level. In the study conducted by Beg et al. [18], a significant negative correlation was observed between HbA1c and FRAP (total antioxidant capacity), indicating that higher HbA1c levels were associated with decreased antioxidant capacity. Furthermore, a significant negative correlation in FRAP indicated that higher lipid peroxidation levels were associated with decreased antioxidant capacity. These findings suggest that in T2DM, the increase in free radicals is directly proportional to the degree of hyperglycaemia, accompanied by a corresponding decrease in antioxidant capacity. El Eter et al. [19] observed that PRDX2 and PRDX6 levels were negatively correlated with diastolic blood pressure (DBP), fasting blood sugar (FBS), and HbA1c levels. Conversely, PRDX1 levels positively correlated with LDL cholesterol and C-reactive protein (CRP) levels, while PRDX4 levels negatively correlated with triglyceride (TG) levels. These results suggest PRDX isoforms may affect metabolic and inflammatory indicators in type 2 diabetes. Another study found a substantial negative correlation between blood nitric oxide (NO) levels, glucose, and HbA1c in diabetic hypertensive patients, suggesting a relationship between HbA1c and NO metabolism problems. This correlation suggests HbA1c and NO may interact in diabetes and hypertension [23]. Oxidative indicators and FRAP levels may affect type 2 diabetes through age, BMI, TC, HDL-C, LDL-C, and TG. Reduced markers may screen for type 2 diabetes risk. [10, 11, 30] These biomarkers may help prevent and treat type 2 diabetes. However, larger studies are needed to validate these relationships and determine how antioxidant enzymes modulate type 2 diabetes development.

4. Conclusions

Based on the findings of this study, it was observed that T2DM is associated with increased oxidative stress, indicated by elevated levels of Peroxiredoxin and decreased levels of FRAP and NO. These alterations in antioxidant defence mechanisms may serve as early indicators for the development of T2DM complications. These findings emphasize that oxidative stress escalates in T2DM in correlation with the degree of hyperglycaemia, as evidenced by higher HbA1c levels. Therefore, regular monitoring of glycemic status through glucose and HbA1c measurements, followed by a timely intervention such as lifestyle modifications, may mitigate the impact of oxidative stress and potentially delay the onset of diabetic complications. However, further studies are necessary to confirm the relationship between oxidative stress markers, FRAP levels, and antioxidant enzyme activity in newly diagnosed individuals with Type 2 Diabetes Mellitus.

Conflict of interest

All authors declare no conflict of interest.

Source of funding

None

Consent

The authors have collected and preserved written participant consent per international or university standards.

Ethical approval

The author(s) has collected and preserved written ethical permission per international or university standards.

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