Correlation between oxidative stress marker and antioxidant enzymes in patients suffering from type 2 Diabetes mellitus.

AUTHORS: Mohammad Zaid Kidwai, Maninder Bindra

AFFILIATIONS: Department of Biochemistry, L.N.C.T. University & J K Hospital, Bhopal (M.P) India

CORRESPONDING AUTHOR:

Dr Maninder Bindra

Professor

Department Of Biochemistry

LNCT University & J K Hospital, Bhopal (M.P) India

ABSTRACT

Introduction: Oxidative stress contributes to defective antioxidant defences, possibly leading to type 2 diabetes (T2D). This study aimed to elucidate the T2D risks and antioxidant defences by investigating the correlation between malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) in T2DM patients.

Methodology: In this case-control study, 63 newly diagnosed T2DM patients and 63 healthy individuals served as cases and controls, respectively. The socio-demographic information was recorded. In addition, plasma glucose, HbA1c, MDA, SOD, and catalase were calculated and statistically compared.

Results: The mean age of the cases was 47.14±8.16, and the controls were 45.12±6.09 years. The majority of the patients in both groups were married and intermediate-qualified. The mean duration of the smoking was significantly higher in Cases ($p=0.0050^*$). Mean serum urea and creatinine were substantially higher in the case group. The study revealed significant differences in plasma glucose and HbA1c levels between cases and controls ($p<0.05$). Moreover, MDA levels were substantially elevated in the case group ($p<0.0001^*$). In contrast, SOD ($p=0.0025^*$) and catalase ($p<0.0001^*$) levels were significantly lower in the case group.

Conclusion: T2DM is linked to heightened oxidative stress, evidenced by elevated MDA levels and reduced activity of antioxidant enzymes such as SOD and catalase. These antioxidant defence mechanism changes could serve as early markers for the onset of T2DM complications.

KEYWORDS: Diabetes Mellitus, Malondialdehyde Superoxide Dismutase, Reactive Oxygen Species

INTRODUCTION

The prevalence of diabetes mellitus has increased globally. 90% of all diabetes cases are Type 2 diabetes (T2D), particularly in middle-aged individuals. [1] The complications of diabetes mellitus vary from person to person and are affected by variables such as diet and general health. Diabetes mellitus affects approximately 190 million people of various ages, making it one of the most significant causes of disability and mortality worldwide. T2DM has reached epidemic proportions, especially in certain subgroups of the population. Due to its rising prevalence, it is expected to become one of the leading preventable causes of death. The prevalence of diabetes in India is projected to increase to 642 million by 2040, from an estimated 66.8 to 69.1 crores in 2014 and 2015, respectively. [2] Insulin resistance and abnormal secretion contribute to the development of type 2 diabetes. It is affected by obesity, age, ethnicity, and family history. The causes of type 2 diabetes are genetics and lifestyle. Hyperglycaemia is caused by insulin resistance. When beta cells are unable to compensate for insulin resistance, type 2 diabetes results. [3] Oxidative stress (OS) may be one of the most significant risk factors for early-onset T2D and the onset of diabetic complications. OS is associated with increased reactive oxygen species (ROS) production and decreased antioxidant system efficiency. According to reports, T2D is significantly associated with OS, and persistent long-term hyperglycaemia can result in excessive ROS formation in diabetic patients. [5,6] Enzymatic (superoxide dismutase (SOD) and catalase (CAT)) and nonenzymatic (vitamins A and E) antioxidants comprise the common ROS defence mechanism. [7]. Blood antioxidant defences can be evaluated to predict the risk of type 2 diabetes and diabetic complications. SOD is an initial antioxidant defence against ROS. It converts superoxide anion into hydrogen peroxide and oxygen, which CAT subsequently degrades into water and oxygen. [9] According to reports, SOD is associated with T2D by ameliorating hyperglycaemia-induced OS. [10] A decrease in SOD concentrations may

increase T2D patients' susceptibility to OS. [4] CAT is a significant antioxidant enzyme that converts hydrogen peroxide to oxygen and water. Given that CAT helps protect -cells from ROS-induced damage, CAT deficiency is associated with increased T2D risk, contributing to -cell dysfunction. Lipid peroxidation, measured by malondialdehyde (MDA) levels, is a frequently employed indicator of oxidative stress. [5] This study aimed to assess oxidative stress markers and antioxidant enzyme activity in newly diagnosed patients with type 2 diabetes mellitus.

MATERIAL AND METHODS

In this Case-Control study conducted at the Department of Biochemistry, L.N.C.T. University, Bhopal, over the course of one year, we obtained ethical clearance and informed consent before enrolling participants. The study included 63 newly diagnosed cases of type 2 diabetes mellitus (T2DM), defined as individuals with Fasting blood sugar levels ≥126mg/dl or 2-hour post-prandial blood sugar levels ≥200mg/dl. Additionally, 63 individuals were selected as controls, meeting criteria for Fasting blood sugar levels <110mg/dl and 2 hours post-prandial blood sugar levels <140mg/dl. Exclusion criteria involved patients with type 1 diabetes mellitus and individuals with other chronic conditions like cardiovascular disease, cancer, etc. Detailed clinical information, including age, sex, occupation, and relevant risk factors contributing to the illness, was gathered from the case and control groups. The levels of oxidative marker

: malondialdehyde (MDA) (Catalog Number KA3736), antioxidant enzymes: superoxide dismutase (Catalog Number KA0783), and catalase (ABNOVA Catalog Number KA0884), were assessed in accordance with the kit protocol.

Statistical Analysis:

Data were entered in Microsoft Excel and analyzed using Statistical Package for the Social Sciences version 26 (SPSS Inc., Chicago, IL, U.S.A.). Continuous variables were expressed as mean (standard deviation) or range, while dichotomous variables were presented as numbers/frequencies. The Chi-square test and Student t-test were used for analysis. Pearson r correlation determined correlations. Significance was set at $p < 0.05$ (95% confidence interval) or $p < 0.001$.

RESULTS

The majority of the patients were males aged between 61-65 years. [Figure-1] The male patients [(66.67%), (58.73%)] showed a predominance in both case and control groups [Figure-2]. Most of the patients were married and were non-vegetarian. [Figure-3] Most controls and cases were intermediate qualified [Figure-4]. We observed no significant difference in the demographics of the enrolled patients in both groups. The mean duration of the smoking was significantly higher in Cases [11.53±5.62] as compared to the control group [8.87±4.79] (p=0.0050*). In Cases group, majority of the patients' cigarette consumption per day were 4-5 times (46.03%). However, in control group most patients' cigarette consumption per day were 2-3 times (50.79%). Furthermore, a significant association was observed **(p=0.0002*)** in Frequency of cigarette /beedis consumption per day in cases patients. Among the cases, 65.08% reported alcohol consumption, compared to 46.03% in the control group. Most patients in both groups did not have any systemic diseases. The mean BMI was significantly higher in the case group [25.06±1.42] than in the control group [23.45±1.13]. The mean serum urea and creatinine were significantly higher in cases [30.14±6.23 and 0.49±0.05] as compared to controls [27.43±4.39 and 0.41±0.02]. Plasma glucose levels (fasting and postprandial) and HbA1C were significantly higher in the case group compared to the control group. Additionally, mean HDL-C was lower in the case group [p<0.0001*] [Table-1]. Regarding oxidative markers, the mean MDA was significantly higher in the case group [2.06±0.53] than in the control group [1.56±0.28]. On the other hand, the mean levels of antioxidant enzymes Superoxide dismutase and catalase were significantly higher in the case group than in the control group [Table-2]. Pearson correlation analysis revealed a significantly negative correlation between SOD [r=-0.1932; p=0.0302*] and Catalase [r=-0.3544; p<0.0001*] with HbA1C levels. Conversely, MDA showed a significantly positive correlation with HbA1C levels [r=0.6728; p<0.0001*]. [Table-3]

TABLE-1: Biochemical parameters of enrolled patients among the cases and control groups.

Biochemical parameters		**CASES [N=63]**		**CONTROL [N=63]**		**P-VALUE**
		MEAN	**SD**	**MEAN**	**SD**	
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-2: 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	MDA (nmol/ml)	2.06	0.53	1.56	0.28	t=6.621 **p<0.0001***
Antioxidant Enzymes	SOD (%)	78.53	8.86	83.65	9.74	t=3.086 **p=0.0025***
	CATALASE (nmol/min/mL)	7.13	1.75	17.86	3.44	t=22.07 **p<0.0001***

TABLE-3: Pearson correlation analysis of the HbA1C level with markers and enzymes.

CORRELATION ANALYSIS			
HBA1C Vs.	**MDA (nmol/ml)**	**SOD (%)**	**Catalase (nmol/min/mL)**
Pearson r	0.6728	-0.1932	-0.3544
95% confidence interval	0.5643 to 0.7584	-0.3560 to -0.01890	-0.4984 to -0.1913
P value	**<0.0001***	**0.0302***	**<0.0001***

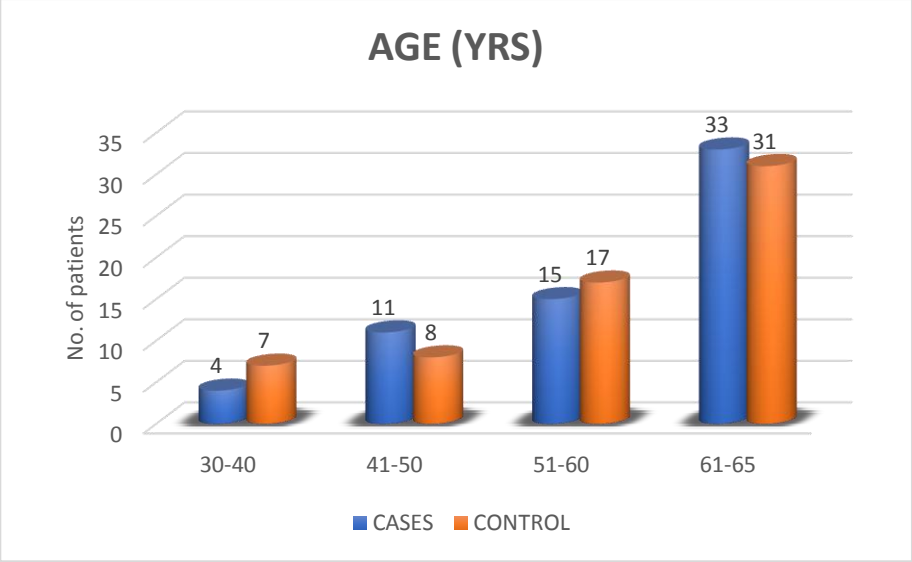

FIGURE-1.1: Age distribution of the enrolled patients among the case and control.

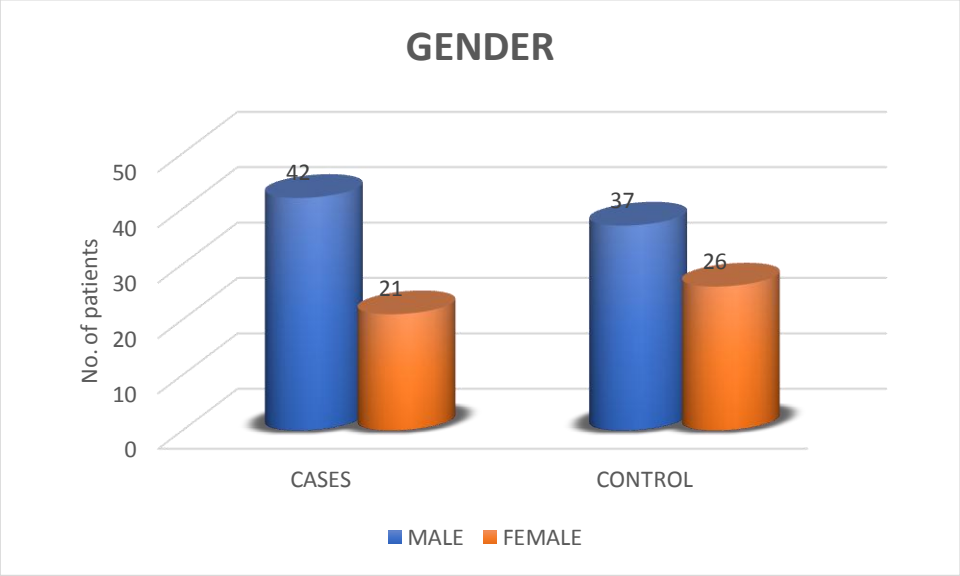

FIGURE-2: Gender-wise distribution of the enrolled patients among the case and control.

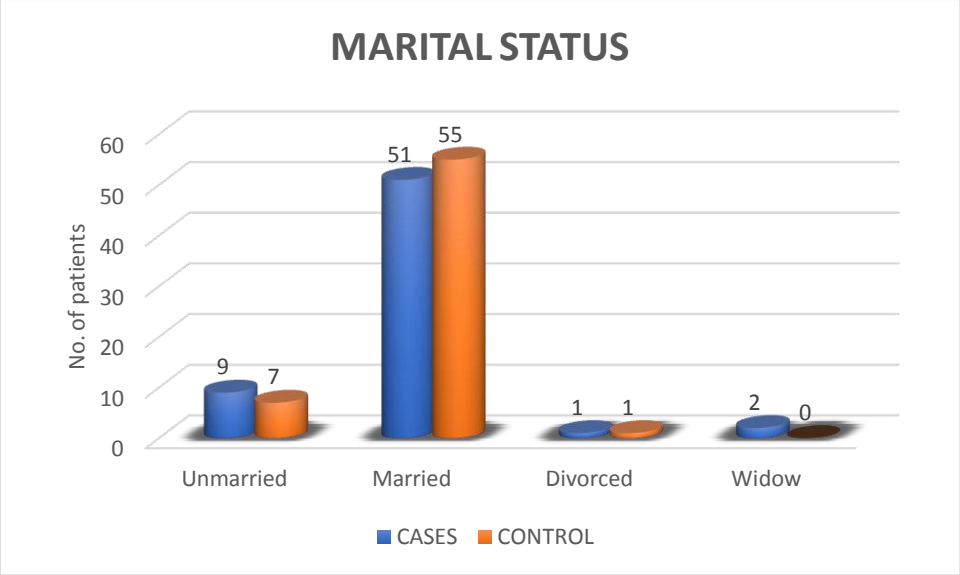

FIGURE-3: **Marital status of the enrolled patients among the case and controls.**

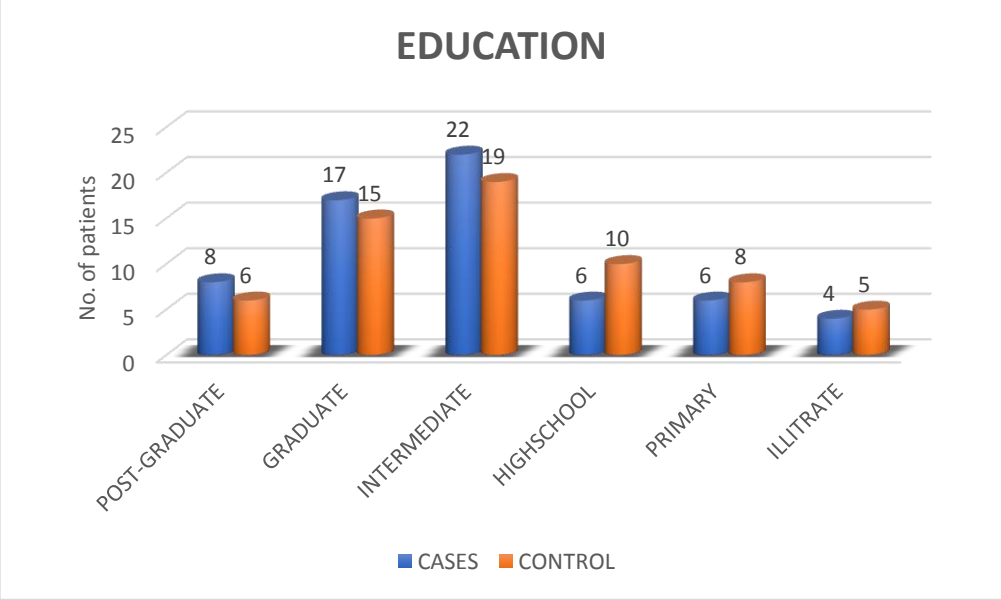

FIGURE-4: **Educational status of the enrolled patients among the case and controls.**

DISCUSSION

In the present investigation, the average age of the cases was 47.14±8.16 years, while the controls had an average age of 45.12±6.09 years. Most cases and controls fell within the 61-65 years age range, with 52.38% and 49.21%, respectively. This was followed by the 51-60 years age group, comprising 23.81% of cases and 26.98% of controls. The 41-50 age group accounted for 17.46% of cases and 12.70% of controls, while the 30-40 age group

represented 6.35% of cases and 11.11% of controls. Our study revealed a higher proportion of males, with 66.67% of the patients in the case group and 58.73% in the control group being male (p=0.3570). This male preponderance aligns with the findings of **Ghosh A et al.** [11], who also observed a higher mean age in the case group [49 ± 7.91 years] compared to the control group [47 ± 9.73 years]. However, the difference was not statistically significant. Similarly, **Beg A et al.** [12] reported mean ages of 43.9 ± 6.5 years for controls and 46.4 ± 8.5 years for cases in their study, with a male preponderance also observed. No significant differences were found in the mean age and male-to-female ratio across different study groups. Further, **El Eter et al.** [13] noted a significantly higher mean age in the study group [61.33 ± 11.14 years] compared to the control group [54.15 ± 15.39 years]. They also reported a male preponderance with 42 male patients and a higher number of female patients (n=28) in the study group. Furthermore, **Promyos N et al.** [14] found a median age of 44 years in the control group and 48 years in the case group. Most patients in both groups were aged between 41-50 years, and a significant difference in age distribution between the two groups was reported. Interestingly, they observed a female preponderance in the case (72.3%) and control groups (67.3%). In our study, the mean Malondialdehyde (MDA) were significantly higher in Cases groups [2.06±0.53 nmol/ml] as compared to the control group [1.56±0.28 nmol/ml]. Similarly, **Beg A et al.** [12] reported a significantly higher level of MDA in the case group [2.60 ± 0.35 nmol/ml] than in the control group [1.90 ± 0.47 nmol/ml]. Other studies have also reported that individuals with diabetes have an unfavourable lipid profile and altered plasma levels of oxidative stress markers than control subjects. [15,16] Under normal physiological conditions, there exists a balance between the generation of free radicals and the antioxidant defence mechanisms. However, in individuals with type 2 diabetes mellitus (T2DM), persistent hyperglycaemia leads to increased reactive oxygen species (ROS) production, overwhelming the available antioxidant mechanisms. Malondialdehyde (MDA) is often used as a marker of lipid peroxidation, which encompasses the combined activity of plasma antioxidants, including vitamins and enzymes. [17] **Chavan et al.** [18] reported elevated MDA levels in diabetic patients compared to controls. Similarly, **Gupta et al.** [19] found increased MDA levels in diabetics, with higher levels observed in subjects with uncontrolled hyperglycaemia and diabetic complications. Numerous other studies have also shown lower antioxidant levels and enhanced pro-oxidative status in diabetic conditions. [20,21] Furthermore, MDA levels were higher in people with diabetes compared to the control group. [12] We noted that the mean SOD and Catalase were significantly lower in cases [78.53±8.86% and 7.13±1.75 nmol/min/mL] than in the control

groups [83.65±9.74% and 17.86±3.44 nmol/min/mL]. Significant differences were observed in the levels of antioxidant enzymes among the groups. 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 H_2O_2 (hydrogen peroxide) production. This mechanism may necessitate higher activity of antioxidant enzymes to provide protection against the increased oxidative stress associated with adverse cardiovascular and metabolic conditions. [14] Decreased concentrations of superoxide dismutase (SOD) and catalase (CAT) in patients with type 2 diabetes (T2D) have been linked to oxidative stress-induced hyperglycaemia. [14] This finding is consistent with other studies that have also reported reduced levels of SOD and CAT in individuals with T2D. [22] For instance, **Hou et al.** [5] found a statistically significant decrease in SOD activity in participants with T2D compared to healthy individuals. Similarly, **Lipa et al.** [23] concluded that low serum levels of SOD were significantly associated with an increased risk of T2D and cataract development in individuals with T2D. Several previous studies have also reported a reduced activity of serum SOD in patients with T2D compared to those without T2D. [10,24] However, some studies have reported contrasting findings, showing elevated or unchanged levels of SOD and CAT in patients with T2D. **Dworzanski et al.** [25] presented elevated SOD and CAT levels in individuals with T2D, while others found no significant increase in SOD and CAT levels in T2D patients compared to non-diabetic individuals. [26,27] The elevated or unchanged levels of SOD and CAT could be attributed to the overexpression of these enzymes in an attempt to counteract oxidative attack and prevent peroxidation of polyunsaturated fatty acids in the cell membrane of patients with diabetes, thus compensating for free radicals. [5] These discrepancies in findings may be influenced by factors such as the duration of T2D, stage of diabetes, genotype background, variations in study populations, different sample sizes, and variations in SOD and CAT analysis methods. [10,25] After applying Pearson correlation analysis, we noted that SOD [r=-0.1932; p=0.0302*] and Catalase [r=-0.3544; p<0.0001*] showed a significantly negative correlation with HbA1C level. Whereas MDA [r=0.6728; p<0.0001*] showed a significantly positive correlation with HbA1C level. On the other hand, a positive correlation was found between HbA1c and MDA (a marker of lipid peroxidation), suggesting that higher HbA1c levels were associated with increased oxidative stress. [13] Furthermore, a significant negative correlation between MDA indicated that higher lipid peroxidation levels were associated with decreased antioxidant capacity. These results suggest that in type 2 diabetes, the increase in free radicals is

proportional to the degree of hyperglycemia, accompanied by a decrease in antioxidant capacity. The effects of oxidative markers levels on type 2 diabetes may be mediated by age, body mass index (BMI) and lipid profile. Reduced levels of these biomarkers may serve as screening tools for type 2 diabetes risk assessment. [11-13] In addition, measuring these biomarkers may aid in preventing and treating type 2 diabetes. To confirm these associations and elucidate the potential mechanisms underlying the activities of antioxidant enzymes in modulating the pathogenesis of type 2 diabetes, however, additional research with larger sample size is required.

CONCLUSION

This study revealed that Type 2 Diabetes Mellitus (T2DM) is linked to increased oxidative stress, evidenced by elevated MDA levels and reduced SOD and Catalase levels. These changes in antioxidant defence mechanisms may serve as early indicators of T2DM complications. The severity of hyperglycaemia, as reflected by higher HbA1c levels, is associated with escalated oxidative stress in T2DM. Regular monitoring of glycemic status may help mitigate oxidative stress and potentially delay diabetic complications. However, further research is necessary to validate the relationship between oxidative stress markers and antioxidant enzyme activity in newly diagnosed individuals with T2DM.

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:

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

REFERENCES

1. Nandi A, Yan LJ, Jana CK, Das N. Role of catalase in oxidative stress-and age-associated degenerative diseases. Oxidative medicine and cellular longevity. 2019 Nov 11;20191:9613090.
2. International Diabetes Federation. IDF diabetes atlas seventh edition: 2015. Brussels, Belgium: 2015 [updated 2015; cited 2016 Aug 16]. Available from: http://www.idf.org/diabetesatlas/update-2015.
3. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes care. 2004 May 1;27(5):1047-53.
4. Darenskaya MA, Kolesnikova LI, Kolesnikov SI. Oxidative stress: pathogenetic role in diabetes mellitus and its complications and therapeutic approaches to correction. Bulletin of experimental biology and medicine. 2021 May;171(2):179-89.
5. Hou Y, Lin M, Qiu X, He M, Zhang Y, Guo F. Effect of Type-2 Diabetes Mellitus in Retinopathy Patients on MDA, SOD Activity and its Correlation with HbA1c. Brazilian Archives of Biology and Technology. 2022 Jan 5;64:e21200075.
6. Ghasemi-Dehnoo M, Amini-Khoei H, Lorigooini Z, Rafieian-Kopaei M. Oxidative stress and antioxidants in diabetes mellitus. Asian Pacific Journal of Tropical Medicine. 2020 Oct 1;13(10):431-8.
7. Haddad NI, Nori E, Ali SH. The Effect of Type Two Diabetes Mellitus on Superoxide Dismutase (SOD) activity and its correlation with HbA1c in Iraqi Patients. Int. J. Eng. Res. 2016;2:7-15.
8. Pieme CA, Tatangmo JA, Simo G, Biapa Nya PC, Ama Moor VJ, Moukette Moukette B, Tankeu Nzufo F, Njinkio Nono BL, Sobngwi E. Relationship between hyperglycemia, antioxidant capacity and some enzymatic and non-enzymatic antioxidants in African patients with type 2 diabetes. BMC Research Notes. 2017 Dec;10(1):1-7.
9. Omoruyi F, Sparks J, Stennett D, Dilworth L. Superoxide dismutase as a measure of antioxidant status and its application to diabetes. InDiabetes 2020 Jan 1:409-417.
10. 56 Fei Z, Gao W, Xu X, Sheng H, Qu S, Cui R. Serum superoxide dismutase activity: a sensitive, convenient, and economical indicator associated with the prevalence of chronic type 2 diabetic complications, especially in men. Free Radical Research. 2021 Mar 4;55(3):275-81.

11. Ghosh A, Sherpa ML, Bhutia Y, Pal R, Dahal S. Serum nitric oxide status in patients with type 2 diabetes mellitus in Sikkim. International Journal of Applied and Basic Medical Research. 2011 Jan;1(1):31-35.
12. Beg A, Thakur RK, Saxena R, Rai G, Srivastava S, Gambhir JK. ComparativeEvaluation of Oxidative Stress in Type-2 Diabetes Mellitus in Relation to Controlled Vs Uncontrolled Diabetes. Annals of Pathology and Laboratory Medicine. 2019 Apr;6(4):1-6.
13. El Eter E, Al-Masri AA. Peroxiredoxin isoforms are associated with cardiovascular risk factors in type 2 diabetes mellitus. Brazilian Journal of Medical and Biological Research. 2015 Mar 3;48:465-9.
14. Promyos N, Phienluphon PP, Wechjakwen N, Lainampetch J, Prangthip P, Kwanbunjan K. Inverse Correlation of Superoxide Dismutase and Catalase with Type 2 Diabetes among Rural Thais. Nutrients. 2023 Apr 25;15(9):2071-85.
15. Konukoğlu D, Serin Ö, Turhan MS. Plasma total homocysteine concentrations in obese and non-obese female patients with type 2 diabetes mellitus; its relations with plasma oxidative stress and nitric oxide levels. Clinical hemorheology and microcirculation. 2005 Jan 1;33(1):41-6.
16. Cassone Faldetta M, Laurenti O, Desideri G, Bravi MC, De Luca O, Marinucci M, De Mattia G, Ferri C. L-arginine infusion decreases plasma total homocysteine concentrations through increased nitric oxide production and decreased oxidative status in Type II diabetic patients. Diabetologia. 2002 Aug;45:1120-7.
17. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical biochemistry. 1996 Jul 15;239(1):70-6.
18. Chavan VU, Melinkeri RR. Study of protein carbonyl group, nitric oxide and MDA (index of lipid peroxidation) as biomarkers of oxidative stress in type 2 diabetes mellitus. National Journal of Community Medicine. 2013 Jun 30;4(02):294-9.
19. Gupta S, Mehndiratta M, Kalra S, Kalra OP, Shukla R, Gambhir JK. Association of tumor necrosis factor (TNF) promoter polymorphisms with plasma TNF-α levels and susceptibility to diabetic nephropathy in North Indian population. Journal of Diabetes and its Complications. 2015 Apr 1;29(3):338-42.
20. Ahmed FN, Naqvi FN, Shafiq F. Lipid peroxidation and serum antioxidant enzymes in patients with type 2 diabetes mellitus. Annals of the New York Academy of Sciences. 2006 Nov;1084(1):481-9.

21. Kalaivanam KN, Dharmalingam M, Marcus SR. Lipid peroxidation in type 2 diabetes mellitus. Int J Diab Dev Ctries. 2006 Mar;26(1):30-2.
22. Mandal M, Varghese A, Gaviraju VK, Talwar SN, Malini SS. Impact of hyperglycaemia on molecular markers of oxidative stress and antioxidants in type 2 diabetes mellitus. Clinical Diabetology. 2019;8(4):215-22.
23. Lipa M, Madhusmita B, Ajay P. A study to assess serum levels of superoxide dismutase and catalase in senile cataract patients with and without diabetes mellitus at tertiary care hospital. International Journal of Research in Medical Sciences. 2016 Sep;4(9):3714-6.
24. Zhao JS, Jin HX, Gao JL, Pu C, Zhang P, Huang JJ, Cheng L, Feng G. Serum extracellular superoxide dismutase is associated with diabetic retinopathy stage in Chinese patients with type 2 diabetes mellitus. Disease markers. 2018 Apr 22;2018:1-8.
25. Dworzański J, Strycharz-Dudziak M, Kliszczewska E, Kiełczykowska M, Dworzańska A, Drop B, Polz-Dacewicz M. Glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity in patients with diabetes mellitus type 2 infected with Epstein-Barr virus. Plos one. 2020 Mar 25;15(3):e0230374.
26. Nwakulite A, Obeagu EI, Eze R, Vincent CC, Chukwurah EF, Okafor CJ, Ibekwe AM, Adike CN, Chukwuani U, Ifionu BI. Evaluation of Catalase and Manganese in Type 2 Diabetic Patients in University of Port Harcourt Teaching Hospital. Journal of Pharmaceutical Research International. 2021 Jun 3:40-5.
27. Jin C, Lin L, Han N, Zhao Z, Liu Z, Luo S, Xu X, Liu J, Wang H. Plasma retinol-binding protein 4 in the first and second trimester and risk of gestational diabetes mellitus in Chinese women: a nested case-control study. Nutrition & metabolism. 2020 Dec; 17:1-7.