



ASSESSMENT OF OXIDATIVE STRESS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS IN CORRELATION TO HBA1C

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ABSTRACT

Introduction: Diabetes mellitus is a complex disorder characterized by either insufficient insulin production or ineffective insulin action. Chronic hyperglycemia can lead to various diabetic complications, including neuropathy, nephropathy, and retinopathy. Oxidative stress is a primary factor contributing to these complications.

Aim: This study aims to assess the oxidative stress levels in patients with type 2 diabetes mellitus (type 2 DM) in comparison to healthy individuals.

Materials & Methods: The research involved 140 participants, comprising 70 individuals diagnosed with type 2 DM (Diabetic group) and 70 healthy controls (Control group). Following a thorough medical history assessment, 5 ml of venous blood was drawn from each participant in both groups and sent to a central laboratory for the evaluation of blood glucose parameters (HbA1c%, fasting blood sugar mg/dl, and postprandial blood sugar mg/dl) as well as oxidative stress markers (total antioxidant capacity in mmol/dl and malondialdehyde in mmol/dl). The collected data were analyzed using SPSS software version 23.0.

Results: The diabetic group exhibited a significant increase in HbA1c, fasting blood sugar, and postprandial blood sugar levels when compared to the healthy individuals. Furthermore, oxidative stress levels were found to be elevated in diabetic patients, indicated by an increase in mean malondialdehyde and a decrease in total antioxidant capacity ($P < 0.05$). A strong positive correlation was observed between HbA1c and malondialdehyde.

Conclusion: The diabetic group demonstrated elevated blood glucose levels alongside increased oxidative stress. The study also established a significant correlation between glycemic levels and oxidative stress, as evidenced by the rise in malondialdehyde levels with increasing blood glucose levels.

Keywords: TAC (total antioxidant capacity), MDA (malondialdehyde), HbA1c (glycosylated hemoglobin), Oxidative Stress, Diabetes Mellitus.

INTRODUCTION

Diabetes mellitus is a metabolic disorder marked by the disruption of glucose, lipid, and protein metabolism, resulting from either defects in insulin secretion (beta cell dysfunction) or insulin action (insulin resistance), or a combination of both.¹ There are numerous risk factors associated with diabetes, such as obesity, a lack of physical activity, stress, smoking, alcohol use, polycystic ovary syndrome, mental health issues, and sleep disturbances.² Individuals diagnosed with diabetes may encounter long-term complications, including diabetic nephropathy, neuropathy, retinopathy, and cardiovascular diseases, which not only have a significant impact on their health but also impose a considerable economic burden on society.^{3,4} The International Diabetes Foundation (IDF) projected that in 2011, there were 366 million adults living with diabetes globally, with estimates suggesting this number could rise to 552 million by 2030 and 642 million by 2040.⁵ In India, the prevalence of diabetes mellitus stands at 8.7%, affecting around 69.2 million individuals, with projections indicating it could reach 109 million by 2035.^{6,7} The worldwide increase in diabetes prevalence can be linked to shifts in human lifestyle and behavior over the last century.² As per the American Diabetes Association, 2018,⁸ the diagnostic criteria for diabetes mellitus include fasting blood glucose levels of ≥ 126 mg/dl, post-load glucose levels of ≥ 200 mg/dl, HbA1c levels of $\geq 6.5\%$, and random plasma glucose concentrations of ≥ 200 mg/dl. If elevated values are detected in individuals suspected of having diabetes, the same test should be repeated as soon as possible on a subsequent day to confirm the diagnosis (WHO. 2011).

Oxidative stress is characterized as a disruption in the balance between the generation and elimination of free radicals, including reactive oxygen species (ROS) and reactive nitrogen species (RNS), which leads to their accumulation within the body.⁹ Certain conditions, such as rheumatoid arthritis, diabetes, and even cancer, may be triggered by oxidative stress.¹⁰ Sources of oxidative stress in diabetes mellitus encompass non-enzymatic, enzymatic, and mitochondrial pathways. Although ROS and RNS function as signaling molecules under normal circumstances, their excessive levels in oxidative environments can result in cellular harm. This damage manifests through detrimental interactions with proteins, lipids, and DNA, ultimately facilitating the emergence of diseases associated with oxidative stress, including diabetes, obesity, Alzheimer's disease (AD), and Parkinson's disease (PD). Free radicals inflict damage on proteins, lipids, and DNA; therefore, oxidative stress plays a significant role in the initiation and advancement of diabetic complications.¹¹ It is crucial to keep the free radical burden at minimal levels, which is achieved through various antioxidant systems present in the body,¹² including both in vivo antioxidant enzymes and in vitro dietary sources of antioxidants. Enzymatic antioxidants comprise superoxide dismutase (SOD),¹³ Glutathione Peroxidase (GPx), and Catalase. Dietary antioxidants, such as tocopherol and vitamin C, can inhibit the chain-breaking reaction of lipid peroxidation across all cell membranes.

In the current research, two significant parameters were utilized to assess oxidative stress in patients diagnosed with type 2 diabetes mellitus: Total Antioxidant Capacity (TAC) and Malondialdehyde (MDA). The former is crucial for evaluating the risks linked to free radical activity in individuals suffering from type 2 diabetes mellitus,¹⁴ while the latter is a stable byproduct resulting from lipid peroxidation. Consequently, this study aims to analyze the levels of blood glucose and oxidative stress in diabetic patients compared to healthy individuals, as well as to determine whether there exists any correlation between blood glucose levels and oxidative stress.

MATERIALS AND METHODS

A comparative analysis was conducted in the Department of Biochemistry in collaboration with the Department of General Medicine at PES University Institute of Medical Sciences and Research, Bangalore, Karnataka, from June 2024 to May 2025. Prior to the initiation of the project, approval was secured from the institutional ethics committee. Participants were chosen based on established inclusion and exclusion criteria.

Inclusion criteria:

- ✓ Patients diagnosed with type 2 diabetes mellitus of both sexes
- ✓ A history of diabetes mellitus lasting 5 years or longer
- ✓ Patients aged between 30 to 60 years
- ✓ Diabetes mellitus patients who consented to provide written informed consent
- ✓ Healthy individuals designated for the control group

Exclusion criteria:

- ✓ Patients diagnosed with type 1 diabetes mellitus
- ✓ Patients who are not willing to provide their written informed consent
- ✓ Smokers, individuals with alcohol dependency, and pregnant or lactating women
- ✓ Patients currently undergoing treatment with antioxidants such as vitamins A, E, and C, or lipid-lowering medications.
- ✓ Patients with psychiatric disorders
- ✓ Patients suffering from chronic conditions such as kidney, liver, or cardiac diseases, tuberculosis, leprosy, recent trauma, or those who have had surgery.

A total of 140 participants were recruited, consisting of 70 individuals diagnosed with type 2 diabetes mellitus (test group) and 70 healthy individuals (control group). A venous blood sample of 5 ml was collected from all diabetic patients and healthy individuals after obtaining their medical history. Subsequently, the blood samples were sent to the central laboratory for the assessment of parameters related to blood glucose levels (HbA1c%, FBS mg/dl, and PPBS mg/dl), as well as markers for oxidative stress, including TAC mmol/l and MDA mmol/l.

Methods for the determination of biochemical parameters:

- ✓ Malondialdehyde (MDA) was measured as thiobarbituric acid reactive substances (TBARS).
- ✓ Total antioxidant capacity was evaluated through the ferric reducing ability of plasma (FRAP).
- ✓ Blood glucose levels were determined using the glucose oxidase and peroxidase enzymatic method.¹⁵
- ✓ HbA1c was measured using A High-performance Liquid Chromatography.¹⁶

Statistical Analysis:

The data obtained were entered into MS Excel and analyzed using an unpaired test, while the correlation between the variables was evaluated using Pearson's correlation analysis with SPSS software version 23.0. The results were presented as mean $\pm$ SD (standard deviation), and a P value of <0.05 was deemed statistically significant.

RESULTS

The current study sought to assess the level of oxidative stress in diabetic patients compared to healthy individuals. A total of 140 participants were enlisted for the research. Among these 140 participants, 70 were diagnosed with type-2 diabetes mellitus, while the remaining individuals were healthy. The majority of participants were male in both the diabetic group (n=42) and the control group (n=39), with the remainder being female. In the diabetic group, 55% of patients were aged between 41 and 50 years, 30% fell within the 31 to 40 age range and remaining 15% were in the 51 to 60 years age range. The body mass index (BMI) for the control and diabetic groups was recorded at 21.9 ± 0.2 and 25.0 ± 0.1 , respectively. The sociodemographic characteristics of the diabetic group were as follows: 37 participants were vegetarians, 58 were married, 47 had a family history of diabetes, 61 were educated, 38 resided in urban areas, and the rest lived in rural regions. **Figure-1**

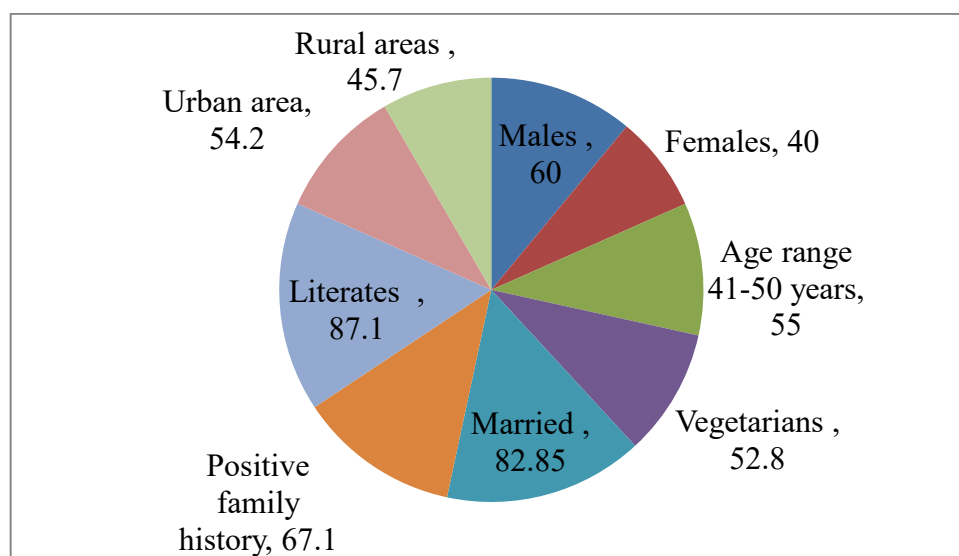


Figure-1: Sociodemographic profile of diabetic patients

A notable increase in mean fasting blood sugar (128.4 ± 2.27 mg/dl), postprandial blood sugar (208.7 ± 6.51 mg/dl), and HbA1c (8.38 ± 0.46 %) levels was observed in the diabetic group compared to the control group (FBS: 85.4 ± 3.20 mg/dl; PPBS: 137.1 ± 3.41 mg/dl; HbA1c: 5.21 ± 0.11 %), which was statistically significant ($p < 0.05$).

Table-1: Blood glucose level in control and diabetic group

Variables	Control group Mean $\pm$ SD	Diabetic group Mean $\pm$ SD	Mean difference	P value
FBS(Mg/dl)	85.4 ± 3.20	128.4 ± 2.27	-42.6 ± 1.44	0.000
PPBS(Mg/dl)	137.1 ± 3.41	208.7 ± 6.51	69.8 ± 1.96	0.000
HbA1c (%)	5.21 ± 0.11	8.38 ± 0.46	-3.02 ± 0.12	0.000

P value $< 0.05^{**}$ statistically significant

When a comparison was made between the groups, diabetic patients showed a significant increase in mean MDA (6.08 ± 0.48 mmol/l) levels and a decrease in mean TAC levels (0.57 ± 0.09) compared to the control group (MDA: 3.35 ± 0.259 mmol/l; TAC: 1.53 ± 0.06 mmol/l), which was statistically significant. **Table-2 and Figure-3**

In the diabetic group, a positive correlation was observed between HbA1c and MDA levels (R value: 0.9607; P value: 0.000), alongside a negative correlation between HbA1c and TAC levels (R value: -0.8944; P value: 0.000). These correlations were determined to be statistically significant ($p < 0.05$). **Tables 3 and 4, Figures 4 and 5.**

Table-2: MDA and TAC level in control and diabetic group

Variables	Control group Mean $\pm$ SD	Diabetic group Mean $\pm$ SD	Mean difference	P value
MDA(mmol/l)	3.35 ± 0.259	6.08 ± 0.48	2.73 ± 0.22	0.000
TAC(mmol/l)	1.53 ± 0.06	0.57 ± 0.09	0.96 ± 0.03	0.000

P value $< 0.05^{**}$ statistically significant

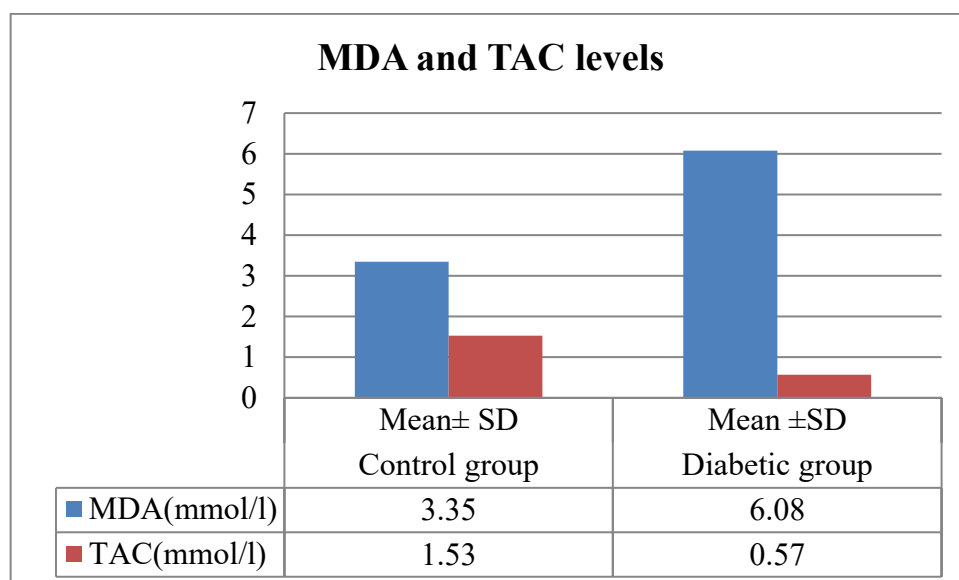


Figure-3: Graphical representation of MDA and TAC level in control and diabetic group

Table-3:Correlation between HbA1c and MDA in diabetic patients

	HbA1c (%)	MDA(mmol/l)	R value	P value
Diabetic patients	3.38 ± 0.466	6.08 ± 0.48	0.9607	0.000

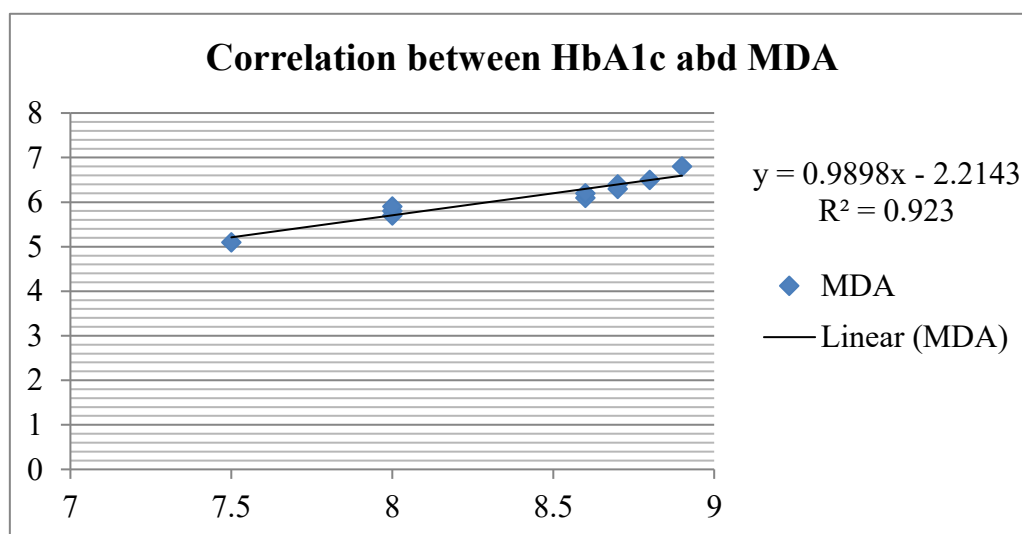


Figure-4: Showing correlation between HbA1c and MDA level in diabetic patients

Table-4: Correlation between HbA1c and TAC in diabetic patients

	HbA1c (%)	TAC(mmol/l)	R value	P value
Diabetic patients	8.38 ± 0.46	0.57± 0.09	-0.894	0.000

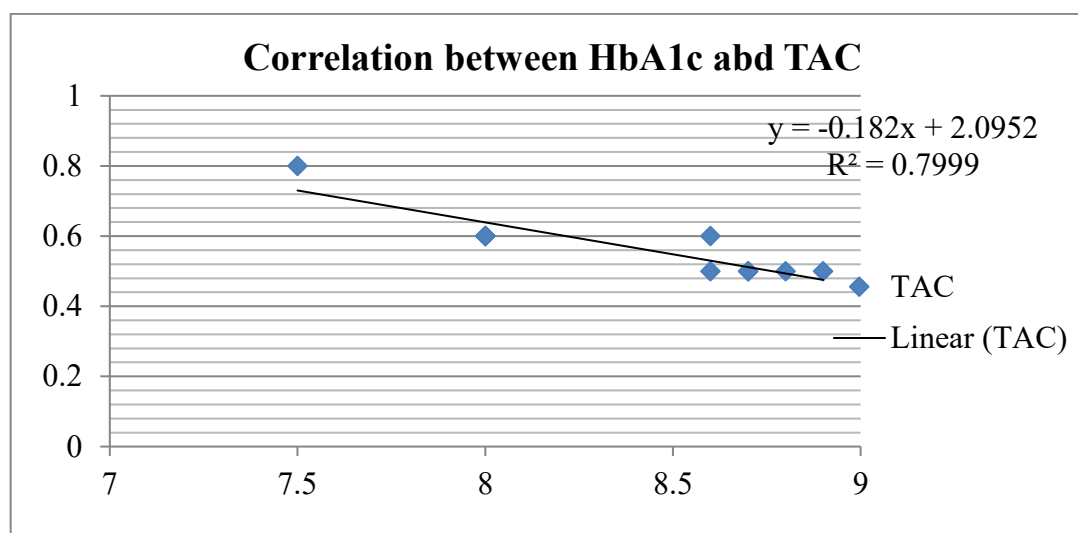


Figure-5: Showing correlation between HbA1c and TAC level in diabetic patients

DISCUSSION

The main goal of this study was to assess blood glucose levels and oxidative stress in diabetic patients compared to healthy individuals. Additionally, the study aimed to determine if there was a correlation between blood glucose and oxidative stress levels. A total of 140 participants were involved in this research. Among these, 70 were healthy individuals, while the remaining participants were diagnosed with type 2 diabetes mellitus. In the diabetic group, 60% were male, 55% were aged between 41-50 years, 87.1% were literate, 54.2% resided in urban areas, 52.8% followed a vegetarian diet, 82.85% were married, and 67.1% had a family history of diabetes mellitus. **Figure-1**

Previous research has indicated a higher prevalence of diabetes among males, which aligns with the findings of this study^{17, 18, 19}; however, another study suggested that females are more affected.²⁰ According to earlier studies, the majority of the diabetic population falls within the 41-50 age range, which is consistent with the results of this study.²¹ The prevalence of diabetes tends to rise with age. After a certain age, insulin production decreases, leading to insulin resistance^{22, 23}. This study sought to explore the relationship between blood glucose and oxidative stress levels, as oxidative stress typically increases with age; thus, participants aged 30 to 60 were included. In diabetic patients, BMI (kg/m²) was found to be higher compared to healthy individuals, these findings supported by previous research.¹⁷ The WHO's guidelines on diabetes prevention and control (2009) noted a high prevalence of diabetes among the Indian population in urban areas, which was similarly observed in this study,²⁴ although this was contradicted by earlier studies.^{20, 25}

In the current research, an increase in the average blood sugar levels was observed in the diabetic group, specifically FBS: 128.4 ± 2.27 mg/dl; PPBS: 208.7 ± 6.51 mg/dl; and HbA1c: 8.38 ± 0.46 %, when compared to the control group (FBS: 85.4 ± 3.20 mg/dl; PPBS: 137.1 ± 3.41 mg/dl; HbA1c: 5.21 ± 0.11 %), which was statistically significant ($p < 0.05$). Previous research has shown a notable increase in average blood sugar levels among diabetic patients compared to healthy individuals, and similar findings were observed in this study.²⁶

Additionally, diabetic patients demonstrated a significant increase in mean MDA (6.08 ± 0.48 mmol/l) levels and a decrease in mean TAC levels (0.57 ± 0.09) compared to healthy subjects (MDA: 3.35 ± 0.259 mmol/l; TAC: 1.53 ± 0.06 mmol/l). In the diabetic group, this study identified a strong positive correlation between HbA1c and MDA levels (R value: 0.9607; P value: 0.000), as well as a negative correlation between HbA1c and TAC levels (R value: -0.8944; P value: 0.000). Diabetic patients exhibited a significant increase in mean MDA and a decrease in mean TAC levels in previous studies, consistent with the findings of the current research.^{27, 19} As oxidative stress increases, MDA levels rise while TAC levels fall. In line with the results of this study, earlier research also reported a strong positive correlation between HbA1c and MDA, and a negative correlation between HbA1c and TAC levels ($p < 0.05$).²⁶

Total antioxidant capacity encompasses all antioxidants present in serum/plasma, foods (dietary total antioxidant capacity), and other bodily fluids.²⁸ TAC serves as a cumulative measure rather than a straightforward sum of quantifiable antioxidants.²⁹ The assessment of total antioxidant capacity is an important indicator of the risk associated with free radical activity in individuals with type 2 diabetes.³⁰

The reduction in TAC levels among diabetic patients can be linked to the overproduction of free radicals, the auto-oxidation of glucose, non-enzymatic glycation, and the unfavorable socioeconomic conditions of these individuals. Such socioeconomic challenges result in a diminished consumption of dietary antioxidants, including vitamin C, vitamin E, beta-carotene, and sulfur-containing amino acids like methionine. Furthermore, this situation leads to a scarcity of essential micronutrients such as selenium, zinc, copper, and manganese, which subsequently hampers the adequate production of antioxidant enzymes like superoxide dismutase and glutathione peroxidase.³¹ A decline in TAC levels coupled with an elevation in MDA levels signifies oxidative stress, which contributes to poor glycemic regulation and heightens the risk of developing complications associated with diabetes.

CONCLUSION

The current research concludes that individuals with diabetes mellitus demonstrate elevated blood glucose and MDA levels, alongside reduced TAC. Furthermore, the study established a significant positive correlation between oxidative stress and plasma glycemic levels, evidenced by a notable increase in MDA levels concurrent with rising blood glucose levels. Timely identification of oxidative stress in diabetic patients can facilitate effective glycemic control and limits diabetes-related complications by the administration of antioxidants like vitamins C, E, and A, in conjunction with antidiabetic medications.

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REFERENCES

1. Banerjee M, Vats P. Reactive metabolites and antioxidant gene polymorphisms in Type2 diabetes mellitus. *Redox Biolog.* 2014; 2:170-177.
2. Zimmet P, Alberti KG, Shaw J. Global and societal implications of diabetes epidemic. *Nature.* 2011; 414:782-787.
3. Alexandra Kautzky-Willer, Jürgen Harreiter, Giovanni Pacini. Sex and Gender Differences in Risk, Pathophysiology and Complications of Type 2 Diabetes Mellitus. *Endocr Rev.* 2016.37 (3):278-316.
4. Rahimi R, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. *Biomed Pharmacother.* 2005; 59(7):365-373.
5. Whiting DR, Guariguanta L, Weil C. Shaw JEIDF Diabetes Atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res Clin Pract* 2011; 94:311–21.
6. Tiwari BK, Pandey KB, Abidi AB, Rizvi SI. Markers of oxidative stress during diabetes mellitus. *J Biomark.* 2013; 37:87-90.
7. Joshi SR, Parikh RM. India - diabetes capital of the world: now heading towards hypertension. *J Assoc Physicians India.* 2007; 55: 323–4.
8. American Diabetes Association. 2018. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018. *Diabetes Care.* 41(1), s13-s27.
9. Johansen J.S., A.K. Harris D. J. Rychly, A. Ergul, Oxidative stress and use of anti oxidants in diabetes; linking basic science to clinical practice, *cardiovasc. diabetol.* 2005; 4(5):1-5.
10. Faramawy EL, S.M., Rizk, R.A. Spectrophotometric studies on antioxidants-doped liposomes. *J. Am. Sci.* 2011; 7(7): 363–369.
11. Ayepola, O.R., Brooks, N.L., Oguntibeju, O.O.. Oxidative Stress and Diabetic Complications: The Role of Antioxidant Vitamins and Flavonoids. 2014.<<http://dx.doi.org/10.5772/57282>>.

12. Yoshikawa T, Naito Y. What Is Oxidative Stress? Journal of Japan Medical Association. 2002;45(7):271–276.
13. Devasagayam T. Free radicals and antioxidants in human health: current status and future prospects. J Assoc Physicians India. 2004;52(1) :794–804.
14. Akinosun OM, Bolajoko EB. Total Antioxidant Status in Type 2 Diabetic Patients: Experience at University College Hospital (Uch) Ibadan, Nigeria. Nigerian Journal of Clinical Practice. 2007; 10(2):126-129.
15. Akinosun OM, Bolajoko EB. Total Antioxidant Status in Type 2 Diabetic Patients: Experience at University College Hospital (Uch) Ibadan, Nigeria. Nigerian Journal of Clinical Practice. 2007; 10(2):126-129.
16. J E Davis, Jay M Mcdonald, Leonard Jarett; A High-performance Liquid Chromatography Method for Hemoglobin A_{1c}. *Diabetes* 1 February 1978; 27 (2): 102–107.
17. Meghna Borah, Rohini Kanta Goswami. Sociodemographic and clinical characteristics of a diabetic population at a tertiary care center in Assam, India. Journal of Social Health and Diabetes.2017;5(1):37-42.
18. International Diabetes Federation. IDF Diabetes Atlas, 5th ed., 2013; Brussels: Belgium.
19. Chaganti nagesh ,nilamnigam ,srawankumar , anil kumar. Study on effect of vitamin-c as add on therapy with antidiabetic drugs in newly diagnosed type-2 diabetes mellitus patients. J adv sci res, 2021; 12 (1): 66-71.
20. Jaya Prasad Tripathy, J. S. Thakur, Gursimer Jeet, Sohan Chawla, Sanjay Jain, Arnab Pal, Rajendra Prasad and Rajiv Saran. Prevalence and risk factors of diabetes in a large community-based study in North India: results from a STEPS survey in Punjab, India. Diabetol Metab Syndr 2017; 9(8):2-8.
21. Chaganti Nagesh, Anil Kumar, Srawan Kumar, Nilam Nigam. Study On Effect OfVitamin-C As Add On Therapy With Antidiabetic Drugs In Type-2 Diabetes Mellitus Patients Associated With Sod2 Gene Polymorphism. J Adv Sci Res, 2021; 12 (2): 171-177.
22. Bhalerao SD, Somannavar M, Vernikas SS, Ravishankar R, Goudar SS. Risk factors for type 2 diabetes mellitus in rural population of North Karnataka: A community-based cross-sectional study. Int J Pharma Med Biological Sci. 2014; 3(1):1-4.
23. D'Souza AM, Kundapur R, Kiran NU. A Cross-sectional study to determine the prevalence of diabetes mellitus and its household awareness in the rural field practice areas of a medical college in Mangalore-a pilot study. Nitte Uni J Health Sci. 2015;5(3):43.
24. World Health Organization. Prevention and control of Diabetes Mellitus: Report of an Inter country workshop, Dhaka, Bangladesh: WHO; 2009.
25. Little M, Humphries S, Patel K, Dodd W, Dewey C. Factors associated with glucose tolerance, pre-diabetes, and type 2 diabetes in a rural community of south India: a cross-sectional study. Diabetol Metab Syndr. 2016; 8(21):1-11.
26. Suvarna Devi C, Nishitha ganugapeta, Sai Sravan Kumar Reddythala, M. Anuswaru. Estimation of Total Antioxidant Capacity in Patients of Type-2Diabetes Mellitus in Correlation with HbA1c.journal of cardiovascular disease research. 2024. 15(05):431-438.
27. Chaganti Nagesh, Anil Kumar, Srawan Kumar, Nilam Nigam. Study On Effect OfVitamin-C As Add On Therapy With Antidiabetic Drugs In Type-2 Diabetes Mellitus Patients Associated With Sod2 Gene Polymorphism. J Adv Sci Res, 2021; 12 (2): 171-177.
28. Wang Y, Yang M, Lee SG, Davis CG, Koo SI, Chun OK. Dietary total antioxidant capacity is associated with diet and plasma antioxidant status in healthy young adults. J AcadNutr Diet. 2012; 112(10):1626-1635.
29. Pourvali K, Abbasi M and Mottaghi A. Role of Superoxide Dismutase 2 Gene Ala16Val Polymorphism and Total Antioxidant Capacity in Diabetes and its Complications. Avicenna J Med Biotech. 2016; 8(2):48 56.
30. Akinosun OM, Bolajoko EB. Total Antioxidant Status in Type 2 Diabetic Patients: Experience at University College Hospital (Uch) Ibadan, Nigeria. Nigerian Journal of Clinical Practice. 2007; 10(2):126 129.

31. Low PA. The role of oxidative stress and antioxidant treatment in experimental diabetic neuropathy. *Diabetes*. 1997; 46(2):538-542.