BioBacta

Journal of Bioscience and Applied Research
www.jbaar.org

Interconnection between oxidative stress and type 2 diabetes mellitus

Vaishali S. Pawar[1*], Ajit Sontakke[2], and Satyajeet K. Pawar[3]

1. MD Biochemistry, Assistant Professor, Department of Biochemistry, KVV(DU), KIMS, Karad, Maharashtra, India.
   Orcid ID: 0000-0003-3022-1536, E-mail: drvspawar269@gmail.com
2. MD Biochemistry, Professor & HOD, Department of Biochemistry, KVV(DU), KIMS, Karad, Maharashtra, India. E-mail: ajit712@yahoo.com
3. MD Microbiology, Associate Professor, Department of Microbiology, KVV(DU), KIMS, Karad, Maharashtra, India
   Orcid ID: 0000-0001-9638-2864, E-mail: drskpawar@gmail.com

Corresponding Author:
Name- Dr. Vaishali S Pawar
Address- Krishna Institute of Medical Sciences, Malkapur, Karad, Maharashtra, India. Pin- 415124
Phone numbers- 9423867394
E-mail address - E-mail: drvspawar269@gmail.com

DOI: 10.21608/jbaar.2023.325677

Abstract

Total antioxidant capacity (TAC) and malondialdehyde (MDA) are two important biomarkers used in the context of diabetes mellitus (DM) to assess oxidative stress and damage. This study aimed to compare TAC and MDA levels in diabetic and non-diabetic individuals and find out the correlation between them. Estimation of TAC and MDA levels was done in a total of 200 individuals (100 non-diabetic and 100 diabetic individuals) by using standard spectrophotometric methods. This case-control study was done from May 2022 to Dec 2022 in a tertiary care hospital. For statistical analysis, version 20 of SPSS software was used. MDA and fasting plasma glucose (FPG) levels were significantly higher (P=0.000) and TAC levels were significantly lower (P=0.000) in diabetic than non-diabetic individuals. A significant negative correlation was observed between MDA and TAC in both groups. No significant correlation was found between MDA, TAC, and FPG levels. With the rise in the duration of diabetes significant increase was found in MDA and FPG levels. Also, there was a significant decrease in TAC levels. The combination of increased MDA levels, elevated FPG levels, and decreased TAC with increasing duration of diabetes indicates a state of heightened oxidative stress in DM patients.

Keywords: Oxidative stress, Total antioxidant capacity, Malondialdehyde, Type 2 diabetes mellitus

Received: July 25, 2023. Accepted: October 27, 2023. Published: November 12, 2023

INTRODUCTION

Diabetes mellitus (DM) is a rising challenge in India and there are estimated 77 million people above the age of 18 years are suffering from type 2 DM. [1]

DM is a chronic metabolic condition represented by elevated blood glucose levels. Commonly, oxidative stress is regarded as a promoting factor in the pathogenesis of type 2 diabetes. [2] Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms. ROS are highly reactive molecules that can cause damage to cells and tissues if not neutralized by antioxidants. [3]

Total antioxidant capacity (TAC) is a measure of the overall antioxidant capacity of a biological sample, including blood or tissues. It reflects the combined activity of various antioxidants such as enzymes (e.g., superoxide dismutase, catalase, etc) and non-enzymatic molecules (e.g., vitamins C & E, glutathione, etc), in neutralizing ROS. TAC levels indicate the ability of an individual's body to counteract oxidative stress. In DM, TAC levels are often decreased, indicating reduced antioxidant defense capacity. [4-6]

On the other hand, malondialdehyde (MDA) is a biomarker of lipid peroxidation, which is one of the consequences of oxidative stress. Lipid peroxidation refers to the oxidative damage to lipids in cells by ROS. [3] MDA is a by-product of lipid peroxidation and is considered a reliable marker of oxidative damage. In DM, MDA levels are often elevated due to increased oxidative stress and lipid peroxidation. [4-6] The decline in TAC and elevation in MDA can contribute to the development and progression of diabetic complications.

This study was carried out to assess the interconnection between total antioxidant capacity and malondialdehyde levels in type 2 diabetes mellitus along with the duration of DM.

MATERIAL AND METHODS

Study population and design:

This case-control study was performed at a tertiary care hospital starting from May 2022 till Dec 2022. Ethical clearance for the study was obtained from the Institutional Ethics Committee [Ref. No. KIMSDU/IFC/03/2019 Protocol no. 0575/2018-2019]. With the help of a study by Rani & Mythili [5] and from the formula: $n= (SD_1^2 + SD_2^2) (Z_{1-\alpha/2} + Z_{1-\beta})^2 /d^2$, the sample size was calculated which was a minimum of 60 in each group. A hundred individuals diagnosed with type 2 DM and 100 non-diabetic individuals from the outpatient department were included in the study.

Inclusion criteria: For the diabetic group- Age >40years, known case of type 2 DM based on the criteria suggested by the American Diabetes Association (fasting plasma glucose ≥126mg/dl, postprandial plasma glucose ≥200mg/dl in two separate test samples)[7], DM for >10 years on treatment. For the non-diabetic group- age >40 years, age sex matched with the diabetic group, no evidence of DM or diabetic complications.

Exclusion criteria: Age< 40 years, Type 1 diabetic patient or any other type of diabetes, patients with liver disease, thyroid disorders or other endocrine diseases, diabetic complications, pregnant and lactating females, persons using antioxidant medications, tobacco users, and smokers.

Patients were selected consecutively according to selection criteria. The participants were explained about the study and after taking written consent blood was collected. General characteristics like age, sex, etc., history, and clinical examination findings from their file were filled in a form for every individual.

Sample collection and preparation:

A fasting venous blood sample (5ml) was collected from each patient, 1ml in a fluoride oxalate vacutainer tube and 4ml in a plain vacutainer tube. Plasma and serum were separated and used for investigations. Plasma was used for the estimation

of fasting plasma glucose (FPG) and serum was used for the measurement of total antioxidant capacity (TAC) and malondialdehyde (MDA).

Biochemical assay:

FPG was measured by enzymatic colorimetric method using glucose oxidase peroxidase test [8] using an auto analyzer. The results were reported as mg/dl. Serum total antioxidant capacity (TAC) was assessed by the ferric-reducing ability of plasma (FRAP) assay [9] using a spectrophotometer. The results were reported as mmol/L. Serum malondialdehyde was assessed by Thiobarbituric acid reactive substances (TBARS)assay, Kei Satoh method [10] using a spectrophotometer. The results were reported as µmoles/L.

Statistical analysis:

Data analysis was done with the help of IBM SPSS Statistics Version 20 (SPSS Inc, Chicago, Illinois, USA). The normal distribution of the data was assessed using Kolmogorov-Smirnov test. Continuous variables were stated as mean ± standard deviation (SD) and compared by independent sample t-test. Categorical variables were stated as numbers (percentages) and compared by the Chi-square significance test or Fisher's exact test. Analysis for correlation was done using the Spearman correlation test which was specified as Spearman's correlation coefficient. Statistical significance was measured as a P-value ≤ 0.05.

RESULTS

In the present study, a total of 200 individuals were studied. General characteristics and biochemical parameters in non-diabetic and diabetic individuals are displayed in Table 1. Both non-diabetic and diabetic groups were age and sex-matched. In the non-diabetic group mean age of individuals was 61.98±7.20 years and in the diabetic group it was 64.13±8.22 years. Serum TAC levels were significantly lower (P =0.000) in the diabetic group (1.75±0.38 mmoles/L) than non-diabetic group (2.39±0.15 mmoles/L). Serum MDA and fasting plasma glucose levels were significantly higher (P =0.000) in the diabetic group than in the non-diabetic group. (Table1)

Table (1): General characteristics and biochemical parameters in non-diabetic and diabetic individuals

Parameters	Non-diabetic group	Diabetic group	P value
Gender			
Male	56	59	0.387
Female	44	41	
Age(years)	61.98±7.20	64.13±8.22	0.56
MDA(µmoles/L)	1.66±0.55	3.92±1.85	0.000*
TAC(mmoles/L)	2.39±0.15	1.75±0.38	0.000*
FPG (mg/dl)	89.55±9.48	174.33±58.62	0.000*

*P<0.05. MDA- malondialdehyde, TAC- total antioxidant capacity, FPG- fasting plasma glucose.

TAC correlated negatively with MDA in the non-diabetic group (P=0.006) and diabetic group (P=0.001) (Table 2). Figure 1 shows a scatter diagram with a negative correlation between TAC and MDA in the diabetic group. No significant correlation was found between MDA, TAS, and FPG (Table 2).

Depending upon the duration of diabetes, diabetic individuals were divided into 3 groups: duration 11-20 years, 21-30 years, and 31-40 years. On comparing, it was found that TAC levels were significantly decreased (P=0.023) whereas MDA and FPG levels were significantly increased (P=0.001 & P=0.043 respectively) with the increase in duration of DM (Table 3).

Table 2: Correlation between biochemical parameters in non-diabetic and diabetic individuals

Correlation between parameters		Non-diabetic group	Diabetic group
MDA and TAC	Spearman's ρ (rho)	-0.274	-0.333
	P- value	0.006*	0.001*
MDA and FPG	Spearman's ρ (rho)	0.146	0.165
	P- value	0.146	0.102
TAC and FPG	Spearman's ρ (rho)	0.190	0.147
	P- value	0.059	0.145

*P<0.05. MDA- malondialdehyde, TAC- total antioxidant capacity, FPG- fasting plasma glucose.

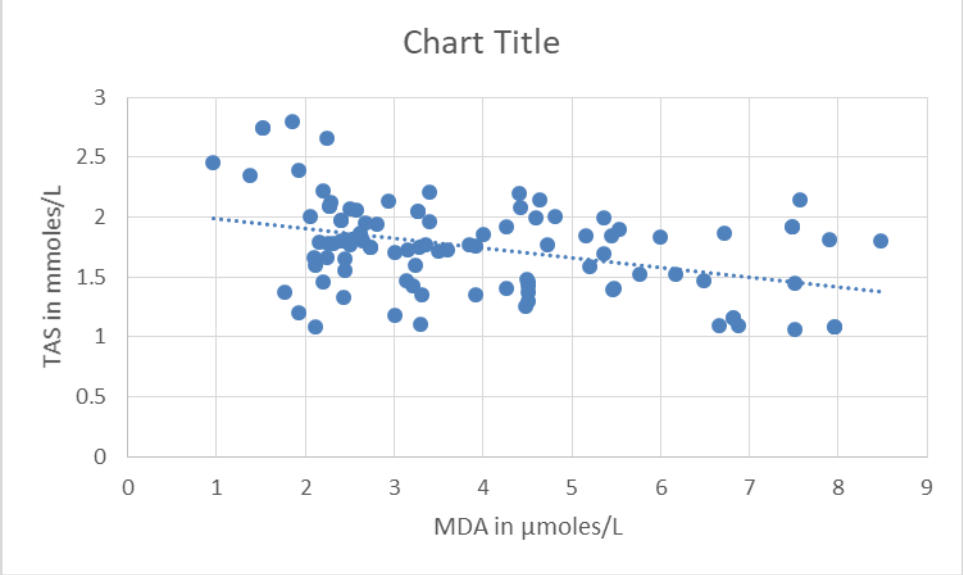

Fig 1: Scatter diagram showing Spearman's correlation between TAS and MDA in the diabetic group

Table 3: Duration of diabetes and biochemical parameters in diabetic individuals

	Duration of diabetes(years)			
	11-20 (n=58)	**21-30(n=36)**	**31-40(n=6)**	**P value**
MDA(µmoles/L)	3.34±1.78	4.72±1.67	4.94±1.67	0.001*
TAC(mmoles/L)	1.81±0.41	1.69±0.31	1.36±0.10	0.023*
FPG (mg/dl)	167±59.83	176.97±53.13	235±55.67	0.043*

*P<0.05. MDA- malondialdehyde, TAC- total antioxidant capacity, FPG- fasting plasma glucose.

DISCUSSION

In the present case-control study, we estimated total antioxidant capacity as a measure of the body's defense mechanism against oxidative stress, malondialdehyde as a marker of oxidative damage along with fasting plasma glucose levels in non-diabetic and diabetic individuals. Our result showed that the diabetic group was exposed to higher oxidative stress than the non-diabetic group as revealed by a decrease in TAC and an increase in MDA. These findings were consistent with previous studies by Najafi et al.[4], Rani & Mythili [5] and Pieme et al.[6]in which diabetic patients displayed lower levels of antioxidants and higher levels of ROS markers like MDA. Vincent et al. [11] have shown that chronic hyperglycemia in DM stimulates the overproduction of ROS which consequently attacks lipids in cells and causes increased production and release of lipid peroxidation products like MDA. [5, 12] Also, chronic hyperglycemia in DM impairs the body's antioxidant defense mechanisms leading to a decrease in TAC. [11] As a result, the body becomes less efficient in eliminating ROS, allowing oxidative stress to persist. The decrease in TAC further exacerbates lipid peroxidation and the accumulation of MDA.

However, the study by Srivatsan et al. [13] showed an increase in Vitamin C, E, and Superoxide dismutase among diabetics suggesting that it reflected the intense adaptive response of the antioxidants to the augmented oxidative stress in the diabetic stress. Also, a study by Savu et al. [14] showed increased activity of antioxidants in diabetic patients. Total antioxidant capacity measurement is more significant than measuring individual antioxidants may it be exogenous or endogenous antioxidants as it provides combined information about antioxidant status.

The study by Pieme et al.[6]showed a significant positive correlation between fasting blood glucose and catalase activity, while a significant negative association was demonstrated between fasting blood glucose and glutathione. However, our study does not show a correlation between FPG and TAC. Our study showed a continuing increase in oxidative stress with chronicity of diabetes as revealed by an increase in MDA and FPG with a decrease in TAC with increasing duration of DM. This suggests the duration of diabetes is an important factor in DM because it indicates a progressive deterioration and an increased risk of diabetic complications. This oxidative stress contributes to the development and progression of diabetic complications by damaging cells, tissues, and organs throughout the body.

CONCLUSION

The combination of increased MDA levels, elevated FPG levels, and decreased TAC indicates a state of heightened oxidative stress in DM patients. It is

important to note that these changes are general trends observed in DM and individual variation may exist. Regular monitoring of blood glucose levels and assessment of oxidative markers, including MDA and TAC, can provide valuable information about the progression of DM. Proper management of blood glucose levels, along with interventions to reduce oxidative stress and enhance antioxidant defense are key strategies in the management of DM

Acknowledgments: The authors would like to thank the authorities of Krishna Vishwa Vidyapeeth (Deemed to be University) and KIMS, Karad for encouraging and supporting the research.

Conflict of Interest: Nil

Authors' Contribution:

VSP and AS participated in the research design, VSP did the experimental study, VSP and SP did data analysis, and VSP, AS, and SP participated in the writing of the paper; all authors read and approved the final manuscript.

REFERENCES

1. World Health Organization. Diabetes-India. [cited 2023 March 17]. Available from: URL: https://www.who.int/india/health-topics/mobile-technology-for-preventing-ncds.
2. Maritim AC, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol*. 2003;**17**(1):24-38. [PubMed ID: 12616644]. https://doi.org/10.1002/jbt.10058.
3. Satyanarayana U. Biochemistry (6e-E-book). New Delhi: Elsevier Health Sciences;2021
4. Najafi A, Pourfarzam M, Zadhoush F. Oxidant/antioxidant status in Type-2 diabetes mellitus patients with metabolic syndrome. *J Res Med Sci*. 2021 Jan 28;**26**:6. [PubMed ID: 34084185]. [PubMed Central ID: PMC8103957]. https://doi.org/10.4103/jrms.JRMS_249_20 .
5. Rani AJ, Mythili SV. Study on total antioxidant status in relation to oxidative stress in type 2 diabetes mellitus. *J Clin Diagn Res*. 2014 Mar;**8**(3):108-10. [PubMed: 24783095]. [PubMed Central ID: PMC4003597]. https://doi.org/10.7860/JCDR/2014/7603.4121.
6. Pieme CA, Tatangmo JA, Simo G, Biapa Nya PC, Ama Moor VJ, Moukette Moukette B, et al. Relationship between hyperglycemia, antioxidant capacity and some enzymatic and non-enzymatic antioxidants in African patients with type 2 diabetes. *BMC Res Notes*. 2017 Mar 29;**10**(1):141. [PubMed ID: 28356165]. [PubMed Central ID: PMC5372257]. https://doi.org/10.1186/s13104-017-2463-6.
7. Care D. Position statements & ADA statements. *Diabetes Care*. 2004;**27**(1):S106-9.
8. Lab Sakha [Internet]. Erba-Glucose.pdf. [cited 2023 March 17] Available from: https://www.labsakha.com/wp-content/uploads/2019/02/Erba-Glucose.pdf.
9. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem*. 1996 Jul 15;**239**(1):70-6. [PubMed ID: 8660627]. https://doi.org/10.1006/abio.1996.0292.
10. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin Chim Acta*. 1978 Nov 15;**90**(1):37-43. [PubMed ID: 719890]. https://doi.org/10.1016/0009-8981(78)90081-5.
11. Vincent AM, Russell JW, Low P, Feldman EL. Oxidative stress in the pathogenesis of diabetic neuropathy. *Endocr Rev*. 2004 Aug;**25**(4):612-28. [PubMed ID:

15294884].
https://doi.org/10.1210/er.2003-0019.

12. Benrebai M, Abidli N, Nasr SM, Benlatreche C. Oxidative stress status in type 2 diabetic patients in Eastern Algeria. *World Appl Sci J.* 2008;**4**(5):714-19.
13. Srivatsan R, Das S, Gadde R, Manoj-Kumar K, Taduri S, Rao N, et al. Antioxidants and lipid peroxidation status in diabetic patients with and without complications. *Arch Iran Med*. 2009 Mar;**12**(2):121-7. [PubMed ID: 19249880].
14. Savu O, Ionescu-Tirgoviste C, Atanasiu V, Gaman L, Papacocea R, Stoian I. Increase in total antioxidant capacity of plasma despite high levels of oxidative stress in uncomplicated type 2 diabetes mellitus. *J Int Med Res.* 2012;**40**(2):709-16. [PubMed ID: 22613434].
https://doi.org/10.1177/147323001204000235.