Original Article

DOI: 10.7860/JCDR/2018/36006.11920

A study on Association of Antioxidant Status of Red Blood Cells with Type 2 Diabetes

Biochemistry Section

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ABSTRACT

Introduction: Oxidative stress plays a role in the pathogenesis of type 2 Diabetes either by effecting insulin secretion or increasing resistance to insulin. Oxidative stress is dealt by the body with the help of several antioxidant systems. The antioxidant levels in disorders causing oxidative stress such as diabetes mellitus are found to be low. Total Antioxidant Capacity (TAC), Superoxide dismutase (SOD) and Glutathione peroxidase (GPx) levels are found to be depleted in diabetic groups when compared to control group comprising healthy individuals. The activities of SOD and GPx are significantly low in diabetic patients.

Aim: To determine the total antioxidant capacity and levels of antioxidants such as SOD and GPx in patients with type 2 diabetes and association between antioxidant levels and type 2 diabetics as well as type 2 diabetes and its associated complications.

Materials and Methods: The study population comprised of 150 individuals, 50 subjects belonging to each subgroup of type 2 diabetics, type 2 diabetics and its associated complications and healthy subjects. Study was conducted in a tertiary care centre in Mangalore, Karnataka during a study period of September to December 2016. Total antioxidant capacity of RBC, fasting blood levels of SOD and GPx were estimated by phosphomolybdenum method, NBT reduction method and Rotruck method respectively.

Results: The results of our study showed that total antioxidant capacity, SOD and GPx were lower among diabetics and diabetic patients with complications as compared to normal non-diabetic subjects. No significant differences in oxidative stress were found between patients with and without chronic complications of diabetes.

Conclusion: It is evident from the results of our study that there exists significant deficiency of markers of antioxidant defence in diabetics as well as diabetics with its associated complications. This indicates that there is a scope for antioxidant supplementation in individuals at risk of developing diabetes in general population and diabetic patients at risk of developing its chronic complications.

Keywords: Antioxidant defence, Glutathione peroxidase, Oxidative stress, Superoxide dismutase

INTRODUCTION

Diabetes Mellitus is a chronic metabolic disorder characterised by high levels of blood sugar (hyperglycaemia). The defect lies in insulin secretion or action resulting in impaired metabolism of carbohydrates, lipids and proteins which leads to long-term health complications [1,2]. According to 2015 estimates, it was noted that 415 million people had diabetes worldwide, out of which 90% of the cases comprised of type 2 diabetes mellitus [2-5].

In addition to physical inactivity, obesity and other major risk factors, oxidative stress also plays a role in the pathogenesis of type 2 Diabetes either by effecting insulin secretion or increasing resistance to insulin [6]. Long-term uncontrolled hyperglycaemia leads to damage, dysfunction and eventually failure of various organs such as eye (Diabetic Retinopathy), kidneys (Diabetic Nephropathy), nerves (Diabetic Neuropathy) and heart (Myocardial infarction) [2,7,8]. Hyperglycaemia is known to increase oxidative stress. Oxidative stress occurs as a result of imbalance between the systemic manifestation of reactive oxygen species and the body's ability to detoxify the reactive oxygen species or to repair the resulting damage [9].

Three major mechanisms responsible for hyperglycaemia-induced oxidative stress are identified. Reactive oxygen species produced by the proton electromechanical gradient generated by the mitochondrial electron transport chain resulting in increased production of superoxide is one [10]. In another mechanism, free glucose undergoes autoxidation in a transition metal catalysed process resulting in the production of superoxide anion and hydrogen peroxide [11]. Transition metal catalysed autoxidation of protein-bound Amadori products producing superoxide, hydroxyl radicals and dicarbonyl compounds is another known mechanism [12].

The oxidative stress is dealt by cells by means of several enzymatic and non-enzymatic antioxidant systems that work together. Studies have shown that the level of antioxidants in diabetics is reduced. There is evidence to suggest that plasma/serum antioxidant status is reduced along with specific antioxidants like ascorbic acid and vitamin E together with an increase in free radicals in type 2 diabetics. Catalase, SOD and GPx activity is found to be reduced in diabetics [10]. Total antioxidant capacity, SOD and GPx levels when compared to control group was found to be reduced among diabetic groups. It was found that activities of SOD and GPx were also low among two types of diabetic groups. [13]. Status of antioxidant enzymes and lipid peroxidation in type 2 diabetes mellitus with neuropathy showed an inverse relationship between GPx and glutathione reductase in patients with type 2 diabetics and control group [14].

The aim of this study is to evaluate the blood levels of several antioxidants in patients with type 2 diabetes and to understand the association between antioxidant levels and type 2 diabetics as well as type 2 diabetics with complications. Total antioxidant capacity of RBC and fasting blood levels of SOD and GPx (GSH-Px) was estimated and the above parameters were compared between the study groups comprising of type 2 diabetics, type 2 diabetics with its associated complications and healthy subjects. Even though there are several previous studies with some evidence of reduced levels of total antioxidant capacity as well as levels of SOD and GPx routine antioxidant supplementation is not followed by clinicians in individuals at risk of developing type 2 Diabetes Mellitus which

indicates that there is need for further substantial research to prove a definite association.

MATERIALS AND METHODS

- It is a case control type of study.
- The study was conducted over a time period of three months from September 2016 to December 2016 at a tertiary care centre in the coastal city of Mangalore, Karnataka.
- A total of 150 subjects had participated in the study, of which 50 each belonged to subgroups of type 2 diabetics, type 2 diabetics with its associated complications and healthy subjects.
- Patients diagnosed with type 2 diabetes mellitus, patients with type 2 diabetics and its complications and healthy subjects above 18 years willing to grant informed consent for participation in our study were included in the study.
- Subjects on drugs affecting lipid profile and antioxidant supplementations were excluded in the study.
- Informed consent for participation was taken from the subjects.
- Institutional ethical committee clearance was obtained.
- Taking aseptic precautions, approximately 3ml of venous blood was collected in EDTA tubes. Plasma was used to determine the Total antioxidant capacity and whole blood was used for estimating the levels of SOD and GPx. The samples were stored at -20°C until analysis. Estimation of total antioxidant capacity, superoxie dismutase and glutathione peroxidise was done by phosphomolybdenum, Nitrobluetetrazolium (NBT) reduction and Rotruck et al methods respectively [15-17].

STATISTICAL ANALYSIS

The collected information was summarised using the descriptive statistics such as mean, standard deviation, frequency and percentage. To compare the outcome measures between control, Type 2 diabetes and Type 2 diabetes mellitus with complication subjects, one-way ANOVA test was used. Multiple comparison between the groups was done by using Tukey's HSD test and Mann-Whitney U test. The p-value <0.05 was considered to be statistically significant.

RESULTS

interquartile range (IQR) is a stable measure of spread as it is not influenced by unusually large or small values. The median values for SOD levels in DM, DM with complications and control are 46.5, 28 and 16 respectively. Median and IQR are an alternative to mean and standard deviation.

Since the Kruskal Wallis p-value is <0.005, there was a difference in median SOD levels among the subjects.

DISCUSSION

Diabetes is a chronic metabolic state characterised by hyperglycaemia. Hyperglycaemia is known to cause oxidative stress by the synthesis of reactive oxygen species which is indicated by their elevated values. The reactive oxygen species are known to damage tissues by means of donation of electrons to molecules [18]. On the other hand, the anti-oxidant defence in diabetics is reduced leading to an imbalance between oxidative stress and body's defence against it [1,2]. Lipid peroxidation is a process where polyunsaturated fatty acids of the cell membranes are degraded into lipid hydroperoxides. Conjugated dienes and malondialdehyde produced during the process of lipid peroxidation are found to be elevated in type 2 Diabetes Mellitus. These products target carbohydrates, lipids, protein and DNA and damage cells [19,20].

In our study, levels of markers of anti-oxidant defence were quantified in normal, diabetic patients and diabetic patients with complications. TAC, SOD, GPx levels were evaluated in the above patients. The results of our study showed that TAC was lower among diabetics and diabetic patients with complications as compared with normal non-diabetic subjects. The values of GPx were also found to be lower among diabetics and diabetic patients with complications as compared to normal subjects [Table/Fig-1,2]. Comparison of SOD levels among three groups also showed lower levels of SOD among diabetics and diabetics with complications than normal subjects [Table/Fig-3]. No significant differences in oxidative defence markers were found between patients with and without chronic complications of diabetes.

Parameters	Control	Type 2 DM	Type 2 DM with complications	p-value
TAC in mM/l	1.351±0.520	1.0008±0.4596	1.1256±0.3616	<0.0001*
SOD in U/mgHb	44.9±11.3	30.7±7.78	31.9±22.9	<0.0001*
GPx in mcg/ml	140.86±24.066	110.20±32.233	116.82±31.028	<0.0001*

[Table/Fig-1]: Showing the concentration of TAC, SOD and GPx in control, Diabetes mellitus and Diabetes mellitus with complications (Values are expressed in Mean±SD). p-value <0.05 and hence there was a difference in TAC, SOD, and GPx.
DM: Diabetes mellitus; TAC: Total antioxidant capacity; SOD: Superoxide dismutase, Gpx: Glutathione peroxidase
*Indicates significant

TAC	Control and Type 2 Dm	Control and Type 2 Dm with Complications	Type Dm and Type 2 Dm with Complications
Mean Difference	0.350	0.2256	0.12478
p-value	0.001*	0.0367*	0.354
GPX			
Mean Difference	30.660	24.040	6.620
p-value	0.001*	0.001*	0.498
SOD			
Mean Difference	14.1198	13.0548	1.065
p-value	0.001*	0.003*	0.381

[Table/Fig-2]: Multiple comparison of TAC, GPx between the groups by using tukey HSD AND SOD using Mann-Whitney U Test:
DM: Diabetes mellitus; TAC: Total antioxidant capacity; SOD: Superoxide dismutase, Gpx: Glutathione peroxidase
Indicates significant

Control		Dm		Dm With Compli-cations		Kruskal Wallis P-Value
Median	IQR	Median	IQR	Median	IQR	
46.5	39.33 to 52.5	28	25.57 to 36.42	16	11.01 to 53.05	<0.001*

[Table/Fig-3]: Comparison of SOD between the groups using Kruskal -Wallis TEST
DM: Diabetes mellitus; SOD: Superoxide dismutase; IQR: Inter quartile range
*indicates significant

Lower levels of GPx and higher levels of glutathione reductase were found both in plasma and haemosylate in type 2 diabetics. Total antioxidant capacity of plasma that is responsible for antioxidant defence when assessed by Ferric Reducing Ability of Plasma (FRAP), serum uric acid and Gamma Glutamyl Transferase (GGT) was also higher in diabetic patients in the study, Markers of Antioxidant Defence in Patients with Type 2 Diabetics by Gawlik K et al., [21].

Total antioxidant capacity was depleted and the activities of SOD and GPx were significantly low in two types of diabetic patients compared to control in a study on Total antioxidant capacity, SOD and GPx in diabetic patients by Rahbani ME et al., [13].

In another study conducted by Kornhauser C et al., a decreased level of GPx and glutathione reductase was found in type 2 diabetics [22]. Similar results showing reduced levels of GPx and SOD was found in the study by Kumawat M et al., [14].

In the study by Ezeiruaku FC et al., the antioxidant enzymes GPx, SOD were significantly decreased in type 1 and type 2 diabetics as compared to non-diabetic group [23].

It was found that the antioxidant status in type 2 diabetics is reduced as decreased levels of GPx and SOD were found in type 2 diabetics in a study conducted by Briggs ON et al., [24]. The levels of GPx and SOD were found to be significantly low in type 2 diabetics in the study conducted by Holy B et al., [25].

LIMITATION

Lack of age and gender related correlation between the parameters compared among the control group is a limitation of the study. Further studies with larger sample size in each of the group is required to substantiate the results.

CONCLUSION

It is evident from the results of our study that there exists significant deficiency of markers of antioxidant defence in diabetics as well as diabetics with its complications. Hence, these markers could play a role in early identification of individuals at risk of diabetes mellitus and those individuals at risk of developing its complications. It also indicates that there is scope for research into the benefits of antioxidant supplementation in individuals at risk of developing diabetes in general population and diabetic patients at risk of developing its chronic complications.

REFERENCES

[1] Gezginci-Oktayoglu S, Basaraner H, Yanardag R, Bolkent S. The effects of combined treatment of antioxidants on the liver injury in STZ diabetic rats. Dig Dis Sci. 2009;54(3):538-46.
[2] American Diabetes Association Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010;33:S62.
[3] "Update 2015". IDF. International Diabetes Federation. p. 13. Retrieved 21 Mar 2016.
[4] Williams. Textbook of endocrinology (12th ed.). Philadelphia: Elsevier/Saunders. pp. 1371-1435. ISBN 978-1-4377-0324-5.
[5] Yuankai S, Hu Frank B. The global implications of diabetes and cancer. The Lancet. 2014;383(9933):1947-48.
[6] Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001;414(6865):813-20.
[7] Tesfaye S, Gill G. Chronic diabetic complications in Africa. Afr J Diabetes Med. 2011;19:04-08.
[8] Chintan AP, Nimish LP, Nayana B, Bhavna M, Mahendra G, Hardik T. Cardiovascular complication of diabetes mellitus. J Appl Pharm Sci. 2011;4:1-6.
[9] Montonen J, Knekt P, Jarvinen R, Reunanen A. Dietary antioxidant intake and risk of type 2 diabetes.Diabetes Care. 2004;27:362-66.
[10] Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature. 2000;404:787-90.
[11] Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: A new perspective on an old paradigm. Diabetes. 1999;48:1-9.
[12] Wolff SP. Diabetes mellitus and free radicals. Free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications. Br Med Bull. 1993;49:642-52.
[13] Rahbani-Nobar ME, Rahimi-Pour A, Rahbani-Nobar M. Total antioxidant capacity, superoxide dismutase and glutathione peroxidase in diabetic patients. Medical Journal of Islamic Academy of Sciences. 1999;12(4):109-14.
[14] Kumawat M, Singh N, Singh S. Status of antioxidant enzymes and lipid peroxidation in type 2 diabetes mellitus with neuropathy. Annals of Neurosciences. 2005;12(3):49-52.
[15] Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochemistry. 1999;259:337-41.
[16] Beaurchamp C, Fridovich I. Superoxide dismutase improved assay applicable to acrylamide gels. Analytical Biochemistry. 1971;44:276-87.
[17] Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as a component of Glutathione peroxidase purification and assay. Science. 1973;179:588-98.
[18] Sies H. Oxidative stress: oxidants and antioxidants. Exp Physiol. 1997;82:291-95. [PubMed].
[19] Freeman BA, Crapo JD. Biology of disease: free radicals and tissue injury. Lab Invest. 1982;47:412-26. [PubMed].
[20] Slater TF. Free-radical mechanisms in tissue injury. Biochem J. 1984;222:1-15. [PMC free article] [PubMed].
[21] Gawlik K, Naskalski JW, Fedak D, Pawlica-Gosiewska D, GrudzieńU, Dumnicka P, et al. Markers of antioxidant defence in patients with type 2 diabetes. Oxidative Medicine and Cellular Longevity. 2016;2016:2352361, 6 pages.
[22] Kornhauser C, Garcia-Ramirez JR, Wrobel K, Pérez-Luque EL, Garay-Sevilla ME, Wrobel K. Serum selenium and glutathione peroxidase concentrations in type 2 diabetes mellitus patients. Primary Care Diabetes. 2008;2(2):81-85.
[23] Ezeiruaku FC, Wankasi MM, George GS. The antioxidant enzymes (Superoxide dismutase; glutathione peroxidase; catalase) status in chronic and non chronic diabetic mellitus type 1 and type 2 subjects in Yenegoa, Bayelsa State, Nigeria. Journal of Biology and Genetic Research. 2016;2(1):34-44.
[24] Briggs ON, Brown H, Elechi-amadi K, Ezeiruaku F, Nduka N. Superoxide dismutase and glutathione peroxidase levels in patients with long standing type 2 diabetes in port harcourt, rivers state, Nigeria. International Journal of Science and Research (IJSR). 2016;5(4):1282-88.
[25] Holy B, Briggs ON. Clinical relevance of superoxide dismutase and glutathione peroxidase levels in management of diabetes type2. International Journal of Contemporary Medical Research. 2016;3(5):1380-82.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Feb 14, 2018**
Date of Peer Review: **Apr 20, 2018**
Date of Acceptance: **Jun 05, 2018**
Date of Publishing: **Aug 01, 2018**