

Research Article

Lipid peroxidation and superoxide dismutase activities in patients with type 2 diabetes complicated with peripheral neuropathy

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Received: 07 December 2015

Accepted: 06 January 2016

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ABSTRACT

Background: Diabetic neuropathy is one of most common complication of diabetes. The suggested factor for the development of diabetic neuropathy is increased lipid peroxidation which is a result of enhanced free radical generation and decreased antioxidant defence.

Methods: This cross sectional study was carried out in the department of Biochemistry, JSS Medical College, Mysore. Thirty patients with diabetic neuropathy, 30 diabetics without any complications & 30 age and sex matched controls were included in the study. Glycated haemoglobin, superoxide dismutase were estimated in whole blood by latex agglutination inhibition assay and INT reduction method respectively. Serum thiobarbituric acid reactive substance (TBARS) was estimated by thiobarbituric acid assay. SPSS version 16 for windows was employed for statistical analysis.

Results: Mean serum TBARS levels were significantly greater in diabetic neuropathy. There was significant negative correlation between serum TBARS and SOD in cases ($r=-0.176$). Highly significant negative correlation was found between SOD and HbA1c in cases ($r=-0.168$).

Conclusions: There exists an inverse relationship between TBARS and antioxidants in diabetic neuropathy and is a result of poor glycaemic control.

Keywords: Oxidative stress, Diabetic neuropathy, Antioxidants, TBARS

INTRODUCTION

Diabetic neuropathy is one of the most common, least identified long term complication of type 1 and type 2 diabetes mellitus.¹ Previous studies have shown that 25% of the diabetic patients are at risk of developing diabetic neuropathy in their life time.²

The cause for the diabetic complication is multifactorial and they act simultaneously. Polyol pathway activation is one of the most common causes described in development of diabetic complication. Hyperglycaemia increases the actions of aldose reductase and sorbitol dehydrogenase this converts excess glucose to fructose and sorbitol respectively. When these sugars pile up in

the nerves they diminish the synthesis of myo-inositol, which is a decisive component of nerve conduction. In addition during this inter conversion there is a desolation of nicotinamide adenine dinucleotide phosphate stores which is necessary for detoxification of reactive species. This increase in reactive species serves as an indicator of increased lipid peroxidation.³

Lipid peroxidation of fatty acid on the membrane by free radicals produces a highly toxic end product i.e. TBARS. Studies have shown that its concentration is considerably increased in diabetic neuropathy, correlating with poor glycaemic control.⁴

Natural SOD has been utilized in a wide variety of pathological states as beneficial agents. SOD drives the reaction to convert superoxide to hydrogen peroxide (H₂O₂). SOD reduces O₂⁻ by successive oxidation and reduction of the transition metal ion at the active site in a ping-pong type mechanism with remarkably high reaction rates and converts (O₂⁻) into less harmful H₂O₂.⁵

Long-term control blood glucose status can be assessed by glycated haemoglobin. Non-enzymatic addition of a sugar residue to amino group of proteins is known as glycation. Glycated proteins are formed post-translationally from the slow, non-enzymatic reaction between glucose and amino groups of proteins. Decrease in level of HbA_{1c} by 1% will decrease long term complications to an extent of 30%.⁶

Very few studies were available among the population of Mysore regarding status of lipid peroxidation and antioxidant status in diabetic neuropathy. The present study was undertaken to evaluate the serum levels of lipid peroxidation marker (TBARS) and antioxidant enzyme superoxide dismutase and their correlation with glycaemic control.

METHODS

This cross sectional study was done during the period between February 2012 to January 2013, in the department of Biochemistry, JSS Medical College, Mysore. The study was conducted after obtaining the approval of Institutional ethical committee. After explaining the details of the study an informed consent was taken from all the participants. Thirty participants in the age group 40-80 years were selected from type 2 diabetic neuropathy diagnosed by diabetic neuropathy scoring system who visited the outpatient and inpatient department of Medicine of JSS Hospital, Mysore. Participants with acute or chronic infections, fever, anaemia, malignancy, acute and chronic nephritis, cirrhosis, congestive heart failure were excluded from the study. None of the participants were on antioxidant supplementation. Thirty diabetics without any complication, 30 unrelated age and sex matched healthy individuals were included as control.

Collection of sample

Fasting, un-haemolysed venous blood (6ml) was drawn from all the participants using universal aseptic precautions. 3ml of blood sample collected in EDTA vacutainers was used for estimation of HbA_{1c} & SOD in whole blood. 3ml of the fasting blood sample was collected in plain vacutainers and serum was carefully separated and stored at -200°C until biochemical analysis and was used to estimate blood glucose and TBARS.

Biochemical analysis

Fasting blood glucose was estimated by GOD-PAP

method using RANDOX KIT-GL 3815 in the RandoxImola auto analyser.⁷ Assessment of lipid peroxidation was done by quantifying the TBARS by thiobarbituric acid reactivity.^{8,9} Vitamin C was measured by 2,4 dinitrophenylhydrazine method.¹⁰ HbA_{1c} was estimated by using RX SERIES HA 3830 KIT in the Randox Imola auto analyzer.¹¹

Statistical analysis

SPSS for windows version-16 was employed for statistical analysis. Comparison between cases and controls was calculated using analysis of variance (ANOVA), independent sample's t test and Pearson correlation coefficient test.

RESULTS

The mean values of FBS, TBARS, SOD and HbA_{1c} in cases, diabetics without any complication and healthy controls are shown in Table 1. The serum TBARS level was significantly elevated (p<0.001) and the SOD level was significantly (p<0.001) decreased in case of diabetic neuropathy compared to controls. Increase in HbA_{1c} level was also significant (p<0.001) in cases compared to healthy controls.

Table 1: The mean value of Biochemical parameters in the study participants.

Parameters	Diabetic neuropathy	Diabetic without complication	Healthy Controls
FBS (mg/dl)	168.8±67.60	124.86±22.09	93.26±8.90
HbA1c (%)	10.17±1.58	7.7±1.02	5.13±0.54
SOD (U/l)	5.55±1.80	144±41.09	12.29±3.80
TBARS (nmol/ml)	7.14±1.54	5.05±2.64	1.81±0.61

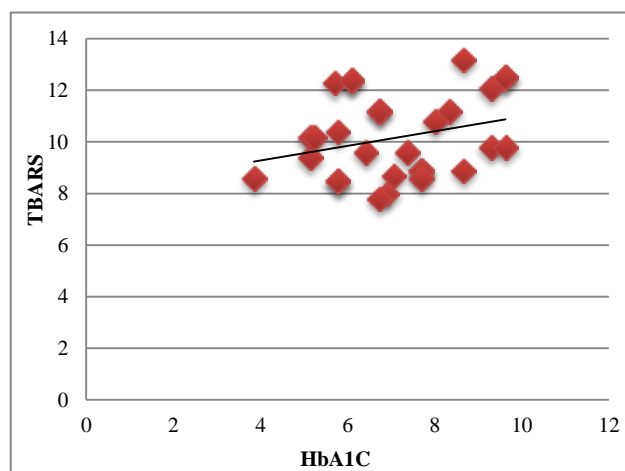


Figure 1: Correlation between HbA_{1c} and TBARS.

Figure 1 shows the correlation between TBARS and SOD in cases. There was a significant negative correlation between plasma TBARS and SOD in cases ($r = -0.424$). There was a positive correlation (Figure 2) between plasma TBARS and HbA_{1c} ($r=0.276$) indicating that as HbA_{1c} increases, TBARS also increases. Correlation study revealed inverse relationship (Figure 3) between SOD and HbA_{1c} ($r = -0.114$).

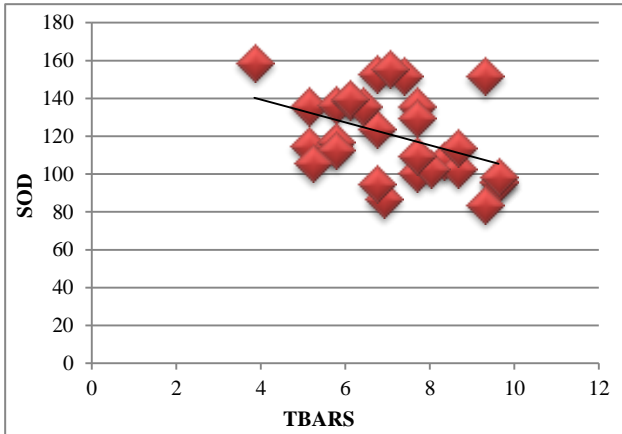


Figure 2: Correlation between TBARS and SOD.

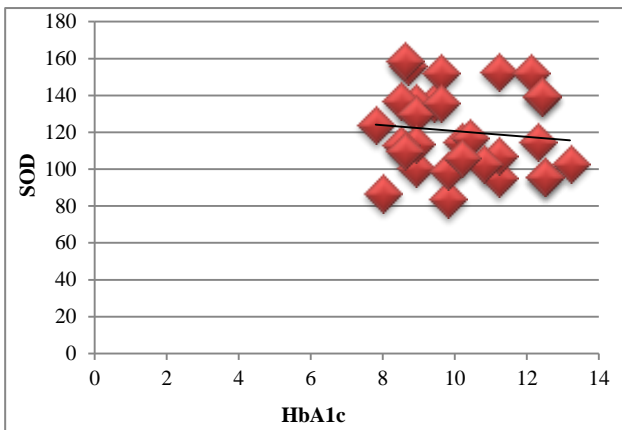


Figure 3: Correlation between HbA_{1c} and SOD.

DISCUSSION

In the present study, the serum level of TBARS, SOD was evaluated and its relationship with HbA_{1c} was studied. The values were compared between diabetic neuropathy, diabetics without any complication and healthy controls.

We observed an increase in the level of TBARS and significant decrease in antioxidant enzyme SOD in diabetic neuropathy, suggesting an imbalance of lipid peroxidation and antioxidant status in diabetic neuropathy. We also observed a positive correlation between TBARS and HbA_{1c} in cases. Findings of the present study are in agreement to previous studies done by peers in the same field of research.^{12,13} This elevation

of TBARS levels may result from hyperglycaemic state that induces overproduction of oxygen free radicals in diabetic neuropathy.¹⁴

The suggested factor responsible for development of diabetic neuropathy are hyperglycemia leading to auto oxidation of glucose, glycation of proteins and lipids non-enzymatically, increased activity of sorbitol pathway, oxidation of advanced glycation end products (AGEs).¹⁵

Decrease in SOD levels may be attributed to increased consumption in the antioxidant defence against elevated lipid peroxidation.¹⁶

The correlation of TBARS and SOD with HbA_{1c} shows a positive and negative correlation respectively suggesting that good glycaemic control is essential for prevention complication like diabetic neuropathy.

CONCLUSIONS

The result of the present study suggests that lipid peroxidation is greatly increased in cases of diabetic neuropathy and is inversely related to glycaemic control. This is due to decreased antioxidant activity increased lipid peroxidation which worsens the condition and becomes vicious cycle leading to nerve damage in diabetic neuropathy. Hence good glycaemic control is essential for diabetic neuropathy.

ACKNOWLEDGMENTS

My sincere acknowledgement to JSS University, Mysore for funding this research Project. I am thankful to Dr. Suma MN, Professor and Head, Department of Biochemistry, JSS Medical College, Mysore for her constant support during the research work. My sincere thanks to Dr. Devaki RN, Professor and Head, Chamarajanagar Institute of Medical Sciences, Chamarajanagar for her continuous guidance during the research.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Dhanajaya SY, Lokanathan TH. Lipid peroxidation and superoxide dismutase activities in patients with type 2 diabetes complicated with peripheral neuropathy. *Int J Res Med Sci* 2016;4:457-60.