ASSESSMENT OF THIOBARBITURIC ACID REACTIVE SUBSTANCE AND VITAMIN C IN PATHOGENESIS OF DIABETIC NEUROPATHY

Shilpashree Yeliyur Dhananjaya¹*, Tejaswi Heremarali Lokanathan²

Abstract
One of the most common complications of diabetes is diabetic neuropathy. The factor that is associated with the development of diabetic neuropathy is increased oxidative stress which in turn releases free radical and decreases antioxidant defense. This cross sectional study was carried out in the JSS Medical College, Mysore, Department of Biochemistry. Thirty patients with diabetic neuropathy and 30 unrelated age and sex matched healthy controls were included in the study. EDTA whole blood was used to estimate Glycated haemoglobin. Serum sample was used to estimate serum thiobarbituric acid reactive substance (TBARS) and Vitamin C levels. Mean serum TBARS levels were significantly greater in diabetic neuropathy. There was a statistically significant negative correlation between serum TBARS and Vitamin C levels in cases (r=−0.176). Highly significant negative correlation was found between Vitamin C and HbA1c in cases (r=−0.168). In this study we found that there is an inverse relationship between TBARS and vitamin C in diabetic neuropathy and it may be a result of poor Glycemic control.

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INTRODUCTION
The most common, long term and least recognized complication of both type 1 and type 2 diabetes mellitus is diabetic neuropathy \[2\]. About 10\% of the type 2 diabetic cases develop diabetic neuropathy\[^4\]. There are multiple factors acting simultaneously during the development of diabetic complications. One of the most common causes described is polyol pathway. The rise in blood glucose concentration enhances the activity of aldose reductase and sorbitol dehydrogenase which converts excess glucose to fructose and sorbitol respectively. When these sugars accumulate in the nerves they in turn results in decrease production of myoinositol, which is a key component of nerve conduction. In addition, during this interconversion there is a consumption 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 oxidative stress \[^6\].

TBARS is a highly toxic product formed by lipid peroxidation of fatty acid on the membrane by free radicals. Studies have shown that its concentration is considerably increased in diabetic neuropathy, correlating with poor glycemic control \[^7\]. Vitamin C is an aqueous phase antioxidant. It reacts with superoxide and hydroxyl radical and various hydroperoxides. Both ascorbate and ascorbyl radical have redox potential and react with most other biologically relevant radicals. Vitamin C offers the most effective protection against plasma lipid peroxidation \[^10\]. HbA\(_{1C}\) gives an input to a long term control of blood glucose. Glycation is the non-enzymatic addition of a sugar residue to amino group of proteins. Post-translationally glycated proteins are formed by a slow, non-enzymatic reaction between glucose and amino groups of proteins. One percent decrease in level of HbA\(_{1C}\) will decrease long term complications to an extent of 30\% \[^3\]. Very few studies were available among the population of Mysore regarding status of oxidative stress and antioxidant status in diabetic neuropathy. The present study was undertaken to evaluate the serum levels of oxidative stress marker (TBARS) and antioxidant Vitamin C and their correlation with Glycemic control.

MATERIALS AND 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 started after obtaining the approval of institutional ethical committee.
After explaining the details of the study a written 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 unrelated age and sex matched apparently healthy individuals were included as control participants.

**Collection of sample:** Fasting, un-haemolysed venous blood (5ml) was drawn from all the participants using universal precautions. 2ml of blood sample collected in EDTA Vacutainers was used for estimation of HbA\textsubscript{1c} in whole blood. 3ml of the fasting blood sample was collected in plain vacutainer and serum was carefully separated and stored at -20\degree C until biochemical analysis and was used to estimate blood glucose, TBARS and Vitamin C.

**Biochemical analysis:** GOD-PAP method was used for estimation of fasting blood glucose using Randox KIT-GL 3815 in the Randox Imola auto analyser \cite{13}. Assessment of oxidative stress was done by quantifying the TBARS by thiobarbituric acid reactivity \cite{5, 9}. 2,4 dinitrophenylhydrazine method for estimation of vitamin C \cite{15}. HbA\textsubscript{1c} was estimated by using RX SERIES HA 3830 KIT in the Randox Imola auto analyser \cite{12}.

**Statistical analysis:** SPSS for windows version-16 (2007) 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.
Table 1: Mean value of biochemical parameters in the study participants

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diabetic neuropathy</th>
<th>Healthy Controls</th>
</tr>
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<tbody>
<tr>
<td>FBS(mg/dl)</td>
<td>168.8±67.60</td>
<td>93.26±8.90</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>10.17±1.58</td>
<td>5.13±0.54</td>
</tr>
<tr>
<td>Vitamin C (mg/l)</td>
<td>5.55±1.80</td>
<td>12.29±3.80</td>
</tr>
<tr>
<td>TBARS(nmol/ml)</td>
<td>7.14±1.54</td>
<td>1.81±0.61</td>
</tr>
</tbody>
</table>

RESULTS

The mean values of FBS, TBARS, Vitamin C and HbA1c in cases and healthy controls are shown in table 1. The serum TBARS level was significantly elevated (p<0.001) and the Vitamin C level was significantly (p<0.001) decreased in case of diabetic neuropathy compared to controls. Increase in HbA1c level was also significant (p<0.001) in cases compared to healthy controls. Figure 1 shows the correlation between TBARS and Vitamin C in cases. There was a significant negative correlation between plasma TBARS and Vitamin C in cases (r=-0.176). There was a positive correlation (Figure 2) between plasma TBARS and HbA1c (r=0.276) indicating that as HbA1c increases, TBARS also increases. Correlation study revealed inverse relationship (Figure 3) between Vitamin C and HbA1c (r=-0.168).
DISCUSSION
In the present study, the serum level of TBARS, Vitamin C was evaluated and its relationship with HbA1c was studied. The values were compared between diabetic neuropathy and healthy controls. We observed an increase in the level of TBARS and significant decrease in antioxidant Vitamin C in diabetics with neuropathy, suggesting an imbalance of oxidative stress and antioxidant status in diabetic neuropathy. We also observed a positive correlation between TBARS and HbA1c in cases. Findings of the present study are in agreement to previous studies done by peers in the same field of research \[1\]. This elevation of TBARS levels may result from hyperglycemic state that induces overproduction of oxygen free radicals in diabetic neuropathy \[10, 14\]. Auto oxidation of glucose due to hyperglycemia, glycation of proteins and lipids non-enzymatically, increased activity of sorbitol pathway, oxidation of advanced glycation endproducts (AGEs) these are the mechanisms that contribute to increased lipid peroxidation in diabetic patients leading to complications \[8\]. Decrease in Vitamin C levels may be attributed to increased consumption of the antioxidant against elevated lipid peroxidation. Other likely mechanism for low Vitamin C is inhibition of ascorbic acid carrier that also transports glucose in case of hyperglycemia of diabetes \[11\]. The correlation of TBARS and Vitamin C with HbA1c shows a positive and negative correlation respectively suggesting that good glycemic control is essential for proper balance of oxidative and antioxidant status in diabetic neuropathy.

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

CONFLICTS OF INTEREST- None

REFERENCES