Association between Serum Malondialdehyde and Insulin in Type 2 Diabetes Mellitus in Eastern India

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ABSTRACT

Objectives: Oxidative stress and insulin plays a pivotal role in the pathogenesis of type 2 diabetes mellitus (T2DM). The aim of our study was to study the altered levels of serum insulin and malondialdehyde and putatively establish their association in T2DM individuals.

Methods: The preliminary case-control study included age, sex and body mass index (BMI) matched 56 T2DM cases and 42 healthy control subjects. Serum MDA was measured using a spectrophotometer while fasting serum levels of insulin was measured by commercially available immunoassay kits as well as routine biochemical parameters were analyzed in all study and control subjects.

Results: Serum MDA level was observed significantly higher among T2DM subjects with respect to controls (p < 0.0001). An increase in serum insulin levels were also found in T2DM cases as compared to controls and were statistically significant (p < 0.0001). Further, serum MDA level showed a significant weak positive correlation with fasting plasma insulin (r = 0.265; P = 0.047) level among T2DM subjects, but no significant correlation was observed in controls (r = 0.114; P = 0.471).

Conclusion: The association between serum MDA and insulin suggests that it may be used as a prognostic marker for the pathogenesis of T2DM.

Keywords: Diabetes Mellitus, Oxidative stress, Malondialdehyde, Insulin, Insulin resistance.
Introduction

Diabetes mellitus is a group of metabolic syndrome characterized by hyperglycemia which show defects in insulin secretion and/or insulin action as well as considered to be one of the leading causes of morbidity in developed countries and major threat to human health in the present era. The pathological mechanisms of hyperglycaemia seem to differ widely although pancreatic beta cells and reduced insulin sensitivity plays a vital role in the pathophysiology of type 2 diabetes mellitus (T2DM). The chronic hyperglycemia is may be linked with long-term damage, dysfunction, and failure of normal functioning of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels \cite{1,2}. The metabolic abnormalities and long term complications resulting from environmental, genetic and aetological factors such as obesity, sedentary lifestyle, unhealthy food habits etc are responsible for the T2DM patients worldwide\cite{3}. The prevalence of diabetes is drastically rising all over the world due to urbanisation, increased obesity rate, physical inactivity and approx 300 million people are likely to be effected by 2030 worldwide \cite{4}. The rise estimated in India is about 50.8 million people in 2010 to about 87 million by 2030 according to International Diabetes Federation (IDF) \cite{5}.

Insulin, one of the principal hormone known to regulate glucose uptake from the blood into primarily muscle and fat cells whose deficiency or insensitivity plays a pivotal role in the pathogenesis of diabetes mellitus \cite{6}. On the other hand, oxidative stress also plays an important role through metabolic dysfunction in T2DM as hyperglycaemia generates reactive oxygen species (ROS), which may cause damage to the cells in many ways such as increased flux of glucose and other sugars through the polyol pathway, increased intracellular formation of advanced glycation end products (AGEs), increased expression of the receptor for AGEs and its activating ligands, activation of protein kinase C isoforms, and over activity of the hexosamine pathway \cite{7}. It might be due to uncoupling of intracellular NADPH oxidase or due to diminished activity of antioxidant defense system known to be responsible for scavenging free radicals from the cells \cite{8,9}. Lipids are regarded as one of the primary targets of ROS. Moreover, ROS causes oxidation of complex lipids in vivo, synthesized by lipoxygenases as a response to cell injury, typically from hydrogen peroxide. Peroxidation of lipids produces highly reactive aldehydes which includes Malondialdehyde (MDA), predicted as a primary biomarker of free radical mediated lipid damage and oxidative stress \cite{10}. Oxidized lipids are able to generate MDA as a decomposition product and its mechanism involves formation of prostaglandins, like endoperoxides, from polyunsaturated fatty acid (PUFA) with two or more double bonds \cite{11}. Higher MDA level in plasma, serum, and many others tissues has been reported in diabetic patients but hardly any study has established the association of lipid peroxidation and insulin status in T2DM subjects \cite{12,13}. Thus, our study attempts to explore the association between lipid peroxidation and serum insulin levels in T2DM subjects and further to correlate their association with the pathogenesis of the disease.

Materials and Methods

The case control study includes 56 cases and 42 healthy controls which were age, sex and BMI matched. The study was done in the Department of Biochemistry, IIMSAR, Haldia, over a period of 6 months. The diabetic subjects were recruited from the outpatient department of Endocrinology, IIMSAR, while the control subjects were taken from the individuals coming to the outpatient department for a routine health check-up. A written informed consent from the patient and control was obtained after complete explanation and the study was approved by the institutional ethics committee. All the patients and controls were clinically examined and routine biochemical tests were analyzed for all subjects prior to selection. The patients on insulin treatment,
hypertension, ischemic heart disease, neurological disorders, renal failure, chronic liver disease, cancer, and immunological disorders were excluded from this study.

5 ml venous blood samples were obtained from the patients as well as controls after 10-12 hours of fasting. All the routine biochemical parameters were analyzed by automated clinical analyzers. The serum MDA level was measured spectrophotometrically by using 530 nm filter against water blank. The concentration of MDA in serum was determined from linear standard curve established by 1 to 8 nm of 1,1,3,3-tetramethoxypropane. The serum insulin level was measured by ELISA method.

Statistical analysis of different biochemical parameters was performed by Students’ t-test. All variables were expressed as mean ± SD (standard deviation). Means obtained from two normally distributed sample groups were compared by Student's unpaired two-tailed “t”-test and for nonparametric Mann-Whitney U “t” test. Correlation between two variables, was determined by Pearson's product moment correlation coefficient. A value of P < 0.05 was considered as statistically significant. All statistical analyses were performed by using Graph Pad prism software (version 5, 2007, San Diego, California, USA). Statistical analysis for sex distribution was evaluated by chi-square test by using statistical software STATA (version 8, Copyright 1984–2003, Stata Corporation, Texas, USA).

Results

The demographic and biochemical profile of the T2DM subjects and healthy controls is depicted in Table 1. There was no statistical significance in age, sex distribution or BMI in either of the two groups between T2DM and control subjects (Table 1). Fasting plasma glucose, HbA1c, serum cholesterol, and serum triglyceride levels were higher while serum HDL levels were lower in T2DM subjects compared to healthy controls which were found to be statistically significant (Table 1).

Serum MDA level was found significantly higher among T2DM subjects with respect to controls (3.31 ± 1.52 versus 1.91 ± 0.73 nmol/L; P < 0.0001) (Figure 1). Moreover, serum insulin levels were also increased in T2DM cases as compared to controls and were statistically significant (18.05 ± 7.39 versus 9.98 ± 3.86 µIU/mL; P < 0.0001) (Figure 2). As presented in Figure 3, serum MDA level showed a significant weak positive correlation with fasting serum insulin (r = 0.265; P = 0.047) level among T2DM subjects, but no significant correlation was observed in controls (r = 0.114; P = 0.471).

Table 1: Demographic and biochemical profile of the subjects

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 42)</th>
<th>T2DM (n = 56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (in years)</td>
<td>54.68 ± 5.6</td>
<td>55.09 ± 6.1</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>22/20</td>
<td>30/26</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.67 ± 1.12</td>
<td>25.96 ± 2.02</td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>89.23 ± 8.52</td>
<td>142.7 ± 35.67</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>1.26 ± 1.08</td>
<td>4.22 ± 2.14*</td>
</tr>
<tr>
<td>HbA1c</td>
<td>4.82 ± 0.58</td>
<td>7.42 ± 0.64</td>
</tr>
<tr>
<td>Serum total CHL (mg/dl)</td>
<td>168.5 ± 22.32</td>
<td>192.8 ± 32.6*</td>
</tr>
<tr>
<td>Serum HDL (mg/dl)</td>
<td>42.48 ± 3.12</td>
<td>36.22 ± 3.46</td>
</tr>
<tr>
<td>Serum TG (mg/dl)</td>
<td>118.4 ± 23.34</td>
<td>202.8 ± 89.2*</td>
</tr>
</tbody>
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FPG, fasting plasma glucose; CHL, cholesterol; TG, triacylglyceride; HDL, high density lipoprotein cholesterol. Age, BMI, and serum levels of biochemical parameters were expressed as the means ± SD. Statistically significant, * p < 0.001 vs Control.

![Figure 1: Serum MDA level among T2DM subjects with respect to controls](image-url)
Figure 2: Serum insulin levels in T2DM cases as compared to controls

Figure 3: Correlation between Serum MDA level with fasting serum insulin ($r = 0.265; P = 0.047$) level among T2DM subjects and in controls ($r = 0.114; P = 0.471$).

Discussion
Over the past decades, oxidative stress plays a pivotal role in cellular injury from hyperglycemia and leads to the progression of T2DM as high glucose level stimulates production of free radicals. The immune system becomes weak and the body could not counteract the generation of enhanced ROS which causes imbalance between ROS and their protection occurs which leads to production of oxidative stress [15,16]. ROS has various regulatory roles in cells and a certain amount of oxidative stress/ROS is essential for the normal metabolic processes [17]. ROS are formed by neutrophils and macrophages during the process of respiratory burst in order to eradicate antigens and also serve as stimulating signals of several genes which encode transcription factors, differentiation, and development as well as
stimulating cell-cell adhesion, cell signalling, involvement in vasoregulation, fibroblast proliferation, and increased expression of antioxidant enzymes [17-20].

The metabolic dysfunctions of diabetes cause mitochondrial superoxide overproduction in endothelial cells of both large and small vessels, as well as in the myocardium due to oxidative stress which acts as mediator of insulin resistance and diabetes mellitus which may contribute to micro- and macrovascular complications [2,21,22]. Few of the studies have found that T2DM patients have increased ROS production-induced higher oxidative damage in the circulation and reduced antioxidant defences mechanisms [23-26]. Increased ROS production in T2DM patient activates some pathways which includes hexosamine pathways, advanced glycation end-products (AGEs) formation, and PKCβ1/2 [27]. Hyperglycemia also provokes oxidative stress by several mechanisms such as glucose autoxidation, polyol pathway, AGE formation and PKCβ1/2 kinase. Elevated free fatty acids, leptin and other circulating factors in T2DM patients may also leads to overproduction of ROS. Insulin increases the production of hydrogen peroxide in adipocytes cultured in vitro and this H2O2 mimics the action of insulin [28]. Thus, a vicious cycle between hyperinsulinaemia and free radicals leads to the pathogenesis in early stages of T2DM. The contributory factor for deterioration of insulin action may be through insulin resistance induced elevated plasma free radicals along with hyperglycemia [29].

Several studies have shown an increased serum MDA levels and found an association of oxidative stress with obesity and insulin resistance in T2DM patients [30, 31]. Moreover, higher serum MDA have also been observed in nonobese T2DM cases and correlated with antioxidant status [32]. Our study shows that serum MDA level is higher in T2DM subjects as compared to controls which are in confirmation with other studies. Further, a strong positive correlation was found between serum MDA level and fasting serum insulin level which suggests that these two parameters are associated with each other and further could facilitate in the pathogenesis of T2DM subjects.

Even though our study on altered serum MDA and insulin in T2DM subjects is interesting, several limitations in our study need to be mentioned which include non estimation of MDA in erythrocytes and PBMC levels, known to be related in this context. Prediabetic subjects were also not included in this study where serum MDA might be an alarming factor in the pathogenesis of T2DM subjects. Further, antioxidant parameters were also not measured in this study. Moreover, another important limitation of the present study as well as other case control studies is its relation to the medication therapy of T2DM subjects. All the T2DM subjects in this study were on sulphonylureas or metformin either as monotherapy or in combination of both drugs. Further, many of these patients were also given intermittent or long term courses of vitamins and antihypertensive drugs. It is rather unknown whether these drugs have confounded our study results to some extent, but despite such differences in the drug history, it has been possible to obtain clear changes in the serum MDA levels as well as serum insulin levels in T2DM patients and further, a strong positive correlation between MDA and serum insulin suggests an association between these two parameters in the same cohorts. Thus serum MDA may be used as a prognostic marker for the pathogenesis of T2DM although a larger cross-sectional study needs to be done to find the association and conclude the fact.

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Conflict of Interest: None declared.

References


