

**LIPID PEROXIDATION MEASURED AS SERUM MALONDIALDEHYDE  
AND VITAMIN-C AS OXIDATIVE-ANTIOXIDATIVE BIOMARKERS IN  
TYPE II DIABETIC PATIENTS**

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## Abstract

**Background:** Oxidative stress (OS) has been implicated in the initiation, progression and pathology of type 2 diabetes mellitus (DM) and its associated complications.

**Methods:** Serum samples were screened for lipid peroxidation marker, malondialdehyde (MDA), Vitamin C (Vit-C), and lipid parameters from 35 type 2 diabetic patients without complications and 30 non-diabetic healthy controls.

**Results:** There were significant differences in all biochemical parameters in diabetic group compared to control group with a significant elevation in all the parameters but Vit-C and high density lipoproteins (HDL) found to be decreased significantly ( $p < 0.05$ ). Pearson's correlation analysis revealed that MDA was found to be negatively correlated with Vit-C ( $r = 0.28$ ,  $p < 0.05$ ).

**Conclusion:** Type 2 DM is associated with lipid abnormalities and OS causing increased lipid peroxidation and depleted antioxidant levels. As these changes might lead to diabetic complications, our findings suggest the supplementation of antioxidants as a therapeutic measure to type 2 DM patients.

**Keywords:** Diabetes, Dyslipidemia, Oxidative stress, Vitamin C, Malondialdehyde

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## 1. Introduction

Every fifth diabetic in the world is an Indian <sup>1</sup>, making India a diabetes capital of the world. By 2025, India alone would have 57 million diabetics mainly of type 2 diabetes constituting 90% of the diabetic population <sup>2</sup>. Diabetes

occurs in all populations and age groups but is increasing in prevalence in the elderly and in blacks, Hispanics, Native Americans, and Asians <sup>3</sup>. Irrespective of the ethnic background the CV risk among diabetic subjects is greater by a factor of 2 to 4 compared to non-

diabetic subjects<sup>4</sup>. About 65% of deaths in individuals with type 2 diabetes are related to heart disease or stroke<sup>5</sup>. An exceptionally high risk has been observed among patients with type 2 diabetes<sup>6</sup>.

OS induced by reactive oxygen species (ROS), which is generated by hyperglycaemia, is one of the major foci of recent research related to diabetes mellitus<sup>7</sup>. The OS in DM is greatly increased due to prolonged exposure to glycaemia and impairment of the oxidant/antioxidant balance. Lipids are among the primary targets of OS<sup>8</sup>. Diabetes mellitus is characterized by hyperglycaemia together with biochemical alterations of glucose and lipid peroxidation. There is emerging evidence that diabetes leads to depletion of the cellular antioxidant defence system and increased levels of ROS which has the ability to react with all biological molecules like lipids, proteins, carbohydrates, DNA etc and exert cytotoxicity on cellular components<sup>9</sup>. Thus increased ROS and impaired antioxidant defense contributes for initiation and progression of micro and macro vascular complications in diabetics<sup>10</sup>. A variety of natural antioxidants exist to scavenge oxygen free radicals and prevent oxidative

damage to biological membranes. One group of these antioxidants is enzymatic, which includes superoxide dismutase, glutathione peroxidase and catalase. In addition to enzymatic antioxidants, there are major natural antioxidants, derived from natural sources by dietary intake that includes vitamin A, vitamin C, vitamin E and carotenoids<sup>11</sup>. Vitamin C, structurally identical to glucose is an important antioxidant capable of scavenging oxygen-derived free radicals<sup>12</sup>. There are several studies that have evaluated free radical induced lipid peroxidation and the antioxidants in diabetic patients<sup>13-15</sup>.

Diabetes increases the risk of cardiovascular (CV) disease. An epidemiological study by Stratton et al suggests an independent effect of hyperglycemia on CV risk<sup>16</sup>. Nonglycemic factors, such as lipid abnormalities are associated with increased CV risk<sup>17</sup>. Some complications of diabetes mellitus are associated with increased activity of free radical-induced lipid peroxidation and accumulation of lipid peroxidation products<sup>18</sup>. Hence in the present study we focused on the evaluation of MDA as oxidative damage marker, Vit-C as an antioxidant marker, measured (TC, TG,

HDL) and calculated (LDL, and VLDL) lipid variables in diabetic study group and non-diabetic control group. We also studied whether any correlation exists between MDA and Vit-C in the patient group.

## 2. Material and methods

Our study was done with 35 type2 diabetic patients (30-72 yr age; 18males, 17females) with fasting blood sugar >126mg/dl, without any micro and macro vascular complications along with 30 age and sex matched healthy controls having no history of diabetes. Patients with renal, hepatic disease, any chronic or acute inflammatory illness, smokers, alcoholics, and patients on hypolipidemic agents were excluded from the study. After obtaining institutional ethical clearance, patients were enrolled from the out patient department of Endocrinology, S V Institute of Medical Sciences (SVIMS University), A.P, India. Informed consent was obtained from all the participants recruited in the study.

### 2.1 Analytical methods

Venous blood samples were drawn after an overnight fast in plain bottles; samples were allowed to clot and

centrifuged for serum for immediate analysis or storage in vial at  $-40^{\circ}\text{C}$  for further analysis. Serum samples were analyzed for TC, TG, and HDL using commercial kits on Beckmann Synchron CX9 autoanalyzer. VLDL and LDL were calculated by using the formulae  $\text{TG}/5$  and Friedewald formula respectively. Vit-C was estimated colorimetrically and serum TBARS levels were determined for the assay of MDA<sup>19</sup>.

### 2.2 Statistical analysis

Values are expressed as mean $\pm$ SD. Unpaired t test was used for comparing individual means between patient and control groups. Difference in means were considered significant statistically at  $p<0.05$ . Association between MDA and Vit-C was studied by Pearson's correlation analysis. Statistical analysis was done on Microsoft excel and SPSS 11.5 for windows.

## 3. Results

Mean  $\pm$  SD values of all the studied biochemical variables were shown in table 1 and depicted in figures 1&2. There were significant differences in all biochemical variables between study and control groups, with significant rise

of TC, TG, LDL, VLDL, MDA and depletion of HDL, Vit-C levels in diabetic patients vs controls. In addition, we also found negative correlation of lipid peroxidation marker, MDA with antioxidant maker, Vit-C. These data showed that there were marked changes in lipids, lipid peroxidation and antioxidant status in diabetes patients compared to healthy controls.

#### 4. Discussion

Results obtained in the present study shows dyslipidemia in diabetes group verses control group with significant elevations in TC, TG, LDL, VLDL and significant decrease in HDL. The lipid abnormalities observed in our study are well in line with the study of Rani et al<sup>15</sup>, and Pasupathi et al<sup>13</sup> that may contribute to CV risk in diabetes. The abnormally high concentration of serum lipids in diabetes is mainly a result of the increase in mobilization of free fatty acids from peripheral depots, due to loss of the inhibitory actions of insulin over hormone-sensitive lipase<sup>20</sup>.

Statistically significant differences were demonstrated related to serum MDA and Vit-C in patients with type 2DM. Serum MDA measured as TBARS in patients with type 2DM were significantly higher

than compared to healthy controls ( $2.6 \pm 0.4$  vs  $1.7 \pm 0.7$   $\mu\text{mol/L}$ ) confirming increased lipid peroxidation associated with an increased production of ROS. This observation of significant rise in MDA was similar to previous observations<sup>13-15, 21</sup>. This elevation of MDA levels may result from hyperglycemic state that induces overproduction of oxygen free radicals in diabetes<sup>22</sup>. Hyperglycaemic-induced glucose autooxidation, non-enzymatic glycation of proteins and lipids, increased sorbitol pathway activity, oxidation of advanced Glycation end-products (AGEs) and cyclooxygenase dependent formation of prostaglandin H<sub>2</sub> (PGH<sub>2</sub>) includes the mechanisms that contribute to increased lipid peroxide formation in diabetic patients<sup>23</sup>. Increased levels of oxidative damage products in serum of diabetic patients correlates with the development of vascular complications<sup>24</sup>.

We also found a significant decrease in Vit-C levels in diabetic group compared to controls ( $0.2 \pm 0.09$  vs  $0.4 \pm 0.08$  mg/dl). Decrease in antioxidant Vit-C was also reported in previous DM studies<sup>13, 14, 20</sup>. Vit-C serves as an antioxidant both by scavenging peroxy radicals and regenerating reduced Vit-E defending against increased OS in

diabetes<sup>25</sup>. Decrease in Vit-C levels could be due to its increased utilization in the antioxidant defence against elevated lipid peroxidation due to OS. Other likely mechanism for low Vit-C is inhibition of ascorbic acid carrier that also transports glucose by the hyperglycemia of diabetes<sup>26</sup>. When checked whether any association exists between serum MDA and Vit-C levels, we found a negative correlation between them ( $r = 0.28$ ,  $p = <0.05$ ) indicating the involvement of Vit-C in counteracting the effects of OS. Therefore, it is evident that increased OS in diabetes could contribute to depletion of Vit-C<sup>27</sup>, and enhanced OS may be attributed to hyperglycemia, depleted antioxidant status, and dyslipidemia through increased availability of substrates for oxidation.

In vivo evidence of the effects of vitamin C and vitamin E on lipid peroxidation is sparse. Improvement of lipid profile and reduction of diabetic complications was reported after supplementation of Vit-C in type 2DM<sup>28</sup>. A randomized controlled trial by Huang *et al* revealed that individual supplementation of Vit-C and Vit-E reduced lipid peroxidation and better results can be seen upon combined supplementation of both vitamins<sup>29</sup>.

The plasma concentration of vitamin C is considered to be strongly correlated with transient consumption of foods such as fruit, supplements, and vegetables<sup>30</sup>. Bonina *et al* reported therapeutic benefit of red orange extract in protecting against diabetic complications due to uncontrolled lipidperoxidation<sup>31</sup>. There is evidence on the mechanistic<sup>32</sup> and molecular<sup>33</sup> aspects of OS in the pathogenesis of type 2DM. Role of free radicals, antioxidants and OS has been reviewed extensively with respect to onset, progression<sup>34</sup> and pathogenesis<sup>35</sup> of diabetic complications including atherosclerosis<sup>36</sup>. There is also clinical and experimental evidence<sup>37</sup> for OS as an exacerbating factor for DM which has been studied in relationship to diet<sup>38</sup>, antioxidants and lifestyle<sup>39, 40</sup>. The findings reported in our study and the information existing in the discussed literature suggests altogether OS as a therapeutic target in DM<sup>41</sup>.

To conclude, increase in oxidative damage measured as high MDA levels and decrease in antioxidant status measured as low Vit-C levels along with deranged lipid profile and decreased antioxidant status could lead to the development of complications associated with DM. Therefore, it

appears reasonable to suggest supplementation of Vit-C to treat elevated OS and lipidperoxidation that may predispose diabetic patients to complications associated with diabetes. Future research focusing on the therapeutic role of antioxidant supplementation may prove beneficial in diabetes.

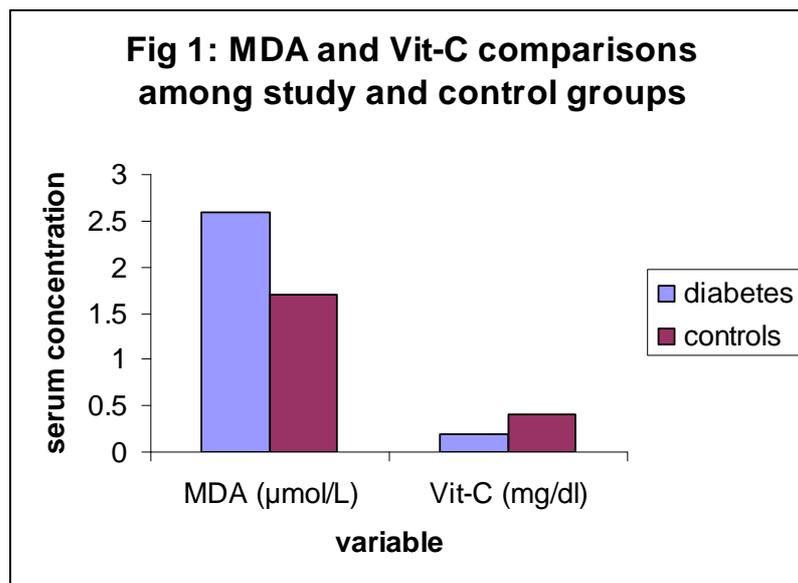
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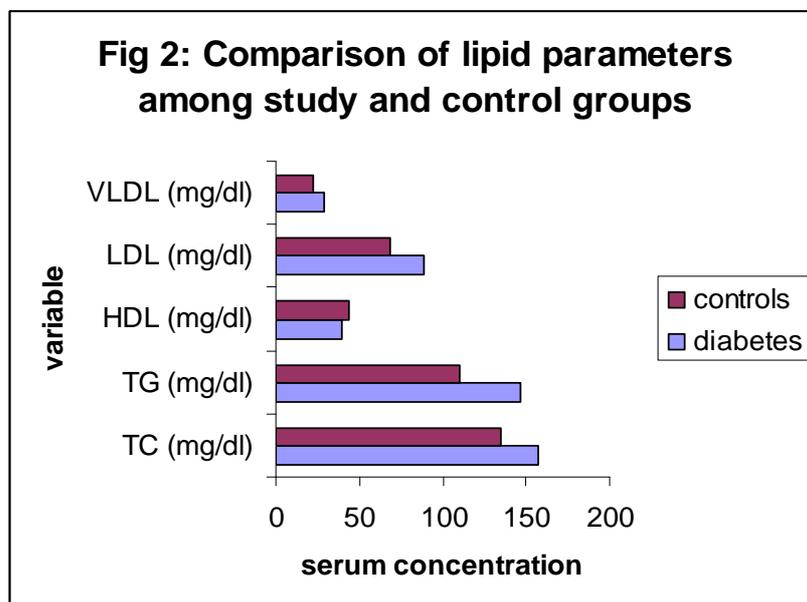
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**Fig 1:** when group means of MDA and Vit-C were tested for statistical significance, MDA was found to be elevated and Vit-C was found to be reduced significantly in diabetes group verses healthy controls.



**Fig 2:** lipid abnormalities were depicted in diabetes group with significant elevations in total cholesterol, Triglycerides, Low density lipoproteins, and Very low density lipoproteins. High density lipoproteins were showed to be decreased significantly in diabetes group verses healthy controls.

**Table 1:** Mean  $\pm$  SD, p values of biochemical variables

variable	diabetes	controls	p value
MDA ( $\mu\text{mol/L}$ )*	2.6 $\pm$ 0.4	1.7 $\pm$ 0.7	<0.001
Vit-C (mg/dl)*	0.2 $\pm$ 0.09	0.4 $\pm$ 0.08	<0.001
TC (mg/dl)*	157 $\pm$ 29.3	135 $\pm$ 20.5	<0.001
TG (mg/dl)*	147 $\pm$ 76.4	110 $\pm$ 27.9	<0.001
HDL (mg/dl)*	40 $\pm$ 6.1	44 $\pm$ 6.0	<0.05
LDL (mg/dl)*	89 $\pm$ 27.5	68 $\pm$ 22.2	<0.001
VLDL (mg/dl)*	29 $\pm$ 15.2	22 $\pm$ 5.5	<0.05

\*(statistically significant)