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# Retinopathy in Diabetes mellitus is it due to Glycosylation or Lipid Peroxidation?

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## ABSTRACT

**Aim:** *To know the leading cause for diabetic retinopathy using simple serological parameters.*

**Materials and methods:** *Diabetic patients of age 38-70 with retinopathy were taken as cases and age matched healthy individuals with age of 37-69 was taken as controls for this study. Fasting and postprandial blood samples were collected for analyzing BG, HbA1C, Lipid parameters, MDA and Vitamin-C. SPSS Software 17 was used for statistical analysis.*

**Results:** *The results of cases were compared with a control, shows highly significance for each parameter. Vitamin-C showed significant negative correlation with the LDL and MDA. The LDL showed a significant positive correlation with HbA1C and MDA. TC and LDL showed significant positive correlation with MDA and Significant Negative correlation with the Vitamin-C.*

**Conclusion:** *Hyperglycemia is the cause for glycosylation which independently provokes lipid peroxidation by decreasing the antioxidant status that neutralize free radicals, so it is the glycosylation and lipid peroxidation plays a main role in the development of retinopathy in diabetes mellitus.*

**Key Words:** Retinopathy, Malondialdehyde, Lipid parameters, Vitamin-C, Diabetes mellitus

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## Introduction:

Diabetic patients have two- to four-folds increased risk for developing microvascular (Retinal, Neuronal, and Renal) and macrovascular complications. two important consequences for the development of complications include poor glycaemic control and formation of advanced glycosylated end products (AGE).[1,2] The toxic effects of hyperglycemia and its pathophysiological derivatives such as glycation products, oxidants, will exert directly on tissues or via sustained alteration in cell signalling pathway (such as changes in phospholipids or kinases) induced by the products of glucose metabolism.

| Parameters | Controls Mean±SD(n=50) | Cases Mean±SD (n=50) |
|---|---|---|
| Age (Years) | 52.8±9.1 | 53.1±9.6 |
| Duration (years) | NIL | 8.8±5.5 |

***Table-1 showing age of cases and controls and duration of diabetes of in cases***

It is well established that, oxidative stress plays a major role in the development of diabetic complications. [3] Further, lipid peroxidation and antioxidant enzymes in blood have been cited as markers for vascular injury/ microangiopathy in DM in several studies. [3]

Free radicals have important role in pathogenesis of diabetes mellitus. It is well documented that there is a link between oxidative stress and secondary complications of diabetes, which include lipid peroxidation tissue damage and late complications including cardiovascular disease, retinopathy, neuropathy, and nephropathy which affects the quality of life. [4]

In present study, we aimed to know the relation between glycosylated products, oxidants, and antioxidants in the development of retinopathy, and to know whether retinopathy is developed due to formation of glycosylated products (HbA1C) or due to lipid peroxidation products (MDA). The serological Glycosylated products, lipid peroxidation products and antioxidant (vitamin-C) were estimated and statically correlated with the antioxidant and to come out with the final product that relates to the development of retinopathy.

## Materials and Methods:

The study was carried out with the patients attending ophthalmology OPD. The study included a total number of 100 patients, of which 50 were type-II diabetic patients diagnosed with retinopathy with age range of 38-70 years were taken as cases, and 50 were healthy individuals with age of range 37-69 years were taken as controls.

Basic information like, age, weight, life style habits, hypertensions etc. were taken from the individuals by questionnaire. Written consent were obtained from every individual. The diabetic patients with smoking, alcoholics, hypertension, antioxidant therapy, and lipid lowering drugs intake were excluded from the study. Diabetic patient diagnosed with retinopathy were included in the study. The blood samples were collected from the patients with 12 hours fasting serum collected after centrifugation is used for estimation of Fasting blood glucose (FBS), Vitamin-C, MDA and lipid parameters. Fasting EDTA blood was used for the estimation of HbA1C estimation. Post prandial blood was collected after 2 hours of breakfast were used for estimating Post Prandial Blood Glucose (PPBS).

The FBS, PPBS, Lipid parameters like TC, HDL, and TG were analysed using Dade Behring Dimension, a fully automated chemistry analyser (Bayers technology) where as LDL and VLDL was estimated indirectly using friedwald equation and HbA1C was estimated using Randox Dyatona, a fully automated chemistry analyser using latex agglutination inhibition method. MDA was estimated using thiobarbituric acid method, where the pink colour chromogen formed was estimated at 530nm and Vitamin-C was estimated using titrimetric method Haris and Ray where the dye 2, 6 dichlorophenol indo phenol was titrated against Vitamin-C[6] . Results were tabulated and statistics was done using SPSS Software 17.0 and 'Pearson correlation' was used for correlating two parameters. A p-value of $<0.05$ was considered to be statically significant.

**Results:**

It is evident from the Table-2 that there is a highly significant ($P<0.001$) rise in the FBS, PPBS, HbA1C, lipid parameters and MDA levels and highly significant ($P<0.001$) fall of HDL and Vitamin-C in retinopathy patients compared to normal healthy controls. The FBS and 2 hrs. PPBS in the cases showed a significant rise compared to controls, which indicates that the patients are not under glycaemic controls. Significant alteration in the lipid parameters explains dyslipidaemia associated with DM. Serum MDA showed a five times significant rise indicates increased lipid peroxidation. The Vitamin-C showed a two times significant fall indicated decreased antioxidant status.

Correlation study revealed (Table – 3) no significant positive association between MDA to FBS and PPBS ($r=-0.18$, $p<0.05$; $r=-0.216$, $p<0.05$) and the correlation between HbA1C and LDL ($r=0.17$, $p<0.001$) doesn't showed any positive correlation (graph-I) which rules out, there is no relation between oxidants to blood glucose levels and glycosylated products (HbA1C) to increased lipid parameter mainly LDL.

| Parameters | Controls Mean±SD(n=50) | Cases Mean±SD (n=50) | P Value |
|---|---|---|---|
| **FBS mg/dl** | 98.4±5.9 | 212.8±71.8 | <0.001 |
| **PPBS mg/dl** | 127.9±13.5 | 295.1±88.5 | <0.001 |
| **HbA1C %** | 4.9±0.5 | 8.15±1.56 | <0.001 |
| **TC mg/dl** | 150.4±14.5 | 238.3±46.59 | <0.001 |
| **HDL mg/dl** | 51.9±5.6 | 39.7±4.9 | <0.001 |
| **LDL mg/dl** | 73.7±16.9 | 159.7±46.0 | <0.001 |
| **VLDL mg/dl** | 25.1±5.3 | 39.2±8.3 | <0.001 |
| **TG mg/dl** | 115.6±22.8 | 196.8±41.5 | <0.001 |
| **MDA n.mol/dl** | 209.5±35.2 | 1011.8±366 | <0.001 |
| **Vitamin-C mg/dl** | 1.02±.021 | 0.37±0.2 | <0.001 |

***Table-2 Mean±SD and p value of different parameters***

The correlation between MDA and Vitamin-C (r= -0.69, p<0.001) showed a significant negative correlation (graph-II) which rules out increase in lipid peroxidation products (MDA) levels relates to decreased antioxidant vitamin (vitamin-C) levels. The correlation between LDL and MDA (r=0.50, p<0.001) showed a significant positive correlation (graph-3). The correlation between LDL and Vitamin-C (r=-0.45 p<0.001) showed a significant negative correlation (graph-4) which points out the contributory role of hyperlipidaemia with decreased antioxidant levels. Similarly MDA levels showed a significant positive correlation with TC and LDL which express the role of dyslipidaemia towards lipid peroxidation and free radicals generation.

**Discussion:**

The two important consequences of hyperglycemia in DM are glycosylated products and oxidative stress.[2] But so far the studies correlating glycosylated products and oxidative stress are cumulative.[2] The FBS and PPBS in our study showed significant positive correlation (r=0.55, p<0.001: r=0.43, p<0.01) with HbA1C, which indicates, hyperglycemia (FBS, PPBS) is the cause for the glycosylation. As HbA1C is the index of mean plasma glucose for the preceding week to months.[5, 7, 8]

Increased HbA1c levels indicates risk for the development of microangiopathy in diabetes, and HbA1c above 12.6% indicates risk for microangiopathy.[8] The HbA1c was not correlated with MDA which indicates, hyperglycaemia is not the cause for lipid peroxidation (MDA).[2,9] But the studies of Naciye et al. and Madhur gupta et al. found correlation between HbA1c and lipid peroxidation [10, 9]. The levels of FBS, PPBS and HbA1c of our study were not correlated to the increased levels of MDA levels where we say, the increased FBS, PPBS and HbA1c is not effective for the increased lipid peroxidation (MDA)[2, 9].

| Parameters | FBS mg/dl | PPBS mg/dl | TC mg/dl | HDL mg/dl | LDL mg/dl | VLDL mg/dl | TG mg/dl | HbA1C % |
|---|---|---|---|---|---|---|---|---|
| **MDA n.mol/dl** | -0.181 | -0.216 | 0.475** | 0.083 | 0.507** | -0.093 | -0.101 | 0.01 |
| **Vitamin-C mg/dl** | 0.010 | 0.089 | -0.481** | -0.149 | -0.457** | -0.163 | -0.159 | -0.304† |

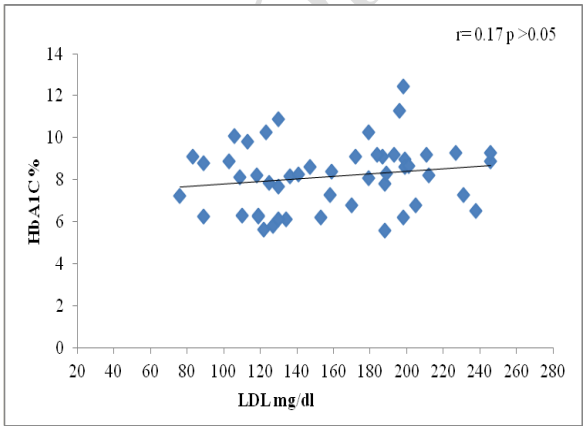
***Table-3 Pearson correlation (r) values for MDA and Vitamin-C with different parameters.***

Note: **p<0.001, †p<0.05

The increased levels of LDL showed a significant positive correlation (r=0.50 p<0.001) with MDA and negative correlation (r=-0.45 p<0.001) with Vitamin-C, which can be explained by the studies of Mosaad and et al. says compositional changes in LDL may lead to conformational changes possibly resulting in a different exposure of fatty acids to oxygen free radicals that enhances a faster rate of lipid peroxidation which cause increase in levels of MDA.[11]

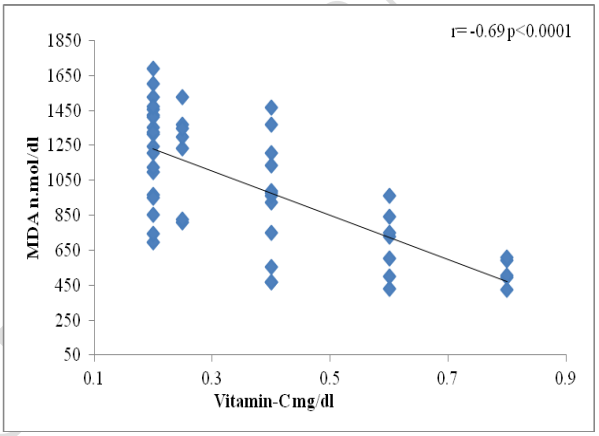
The MDA in our study showed a significant negative correlation (r=-0.69 p<0.001) between Vitamin-C, which states increased lipid peroxidation due to decreased antioxidant (Vitamin-C) levels. Ascorbic acid functions as an important biomolecule for cellular defence against oxygen toxicity and lipid peroxidation caused by free radicals mechanism.[12] However the decreased levels of Vitamin-C in DM is due to the common sharing of the glucose transporter (GLUT)

### *Graph-1 Correlation between HbA1c and LDL*



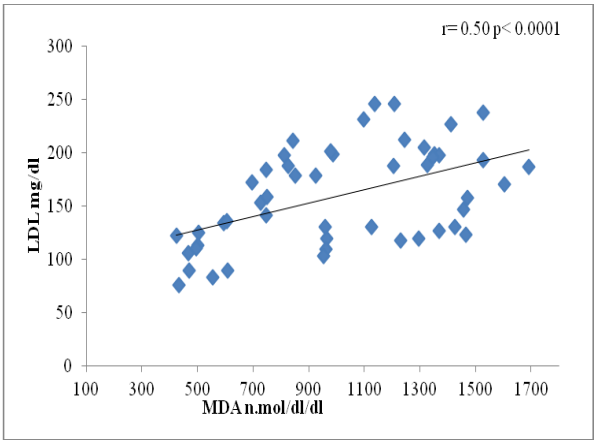

*r = Pearson correlation coefficient*

### *Graph-2 Correlation between MDA and Vitamin-C*



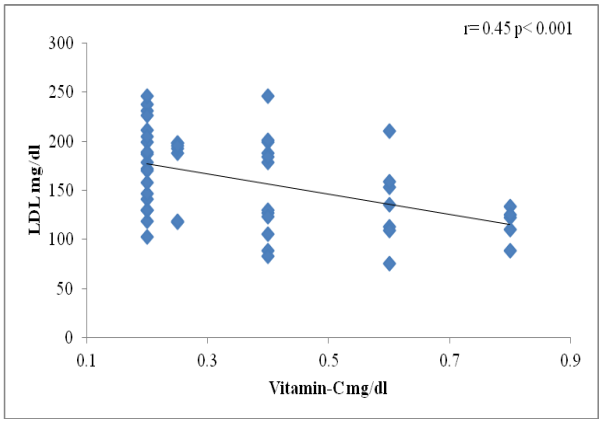

*r = Pearson correlation coefficient*

### *Graph-3 Correlation between LDL and MDA*




*r = Pearson correlation coefficient*

***Graph-4 Correlation between LDL and Vitamin-C***




*r = Pearson correlation coefficient*

between the glucose and Vitamin-C, So vitamin-C is significantly decreased in poorly regulated diabetes[13]. High extracellular glucose in DM may further impairs cellular uptake of Vitamin-C and accentuate the problems associated with its deficiency [14]. However in our study the Vitamin-C showed a slightly negative correlation (r=-0.304 p<0.05) with HbA1c, which explain the hyperglycemia to decreased Vitamin-C levels and decreased antioxidant levels[15]. Thus the reason for increased MDA levels in DM may be due to increased reactive oxygen products and decreased antioxidants. Insufficient neutralization of free radicals by antioxidants (Vitamin-C) causes oxidation of lipids, proteins, nucleic acids, glycolipids and glycoproteins [16].

Now it is evident from our study hyperglycemia is the cause for increased glycosylation which develops risk for the microangiopathy in the diabetes. Increased advanced glycation end-products also induce oxidative stress.[17] It is the hyperglycemia in the DM making the vitamin-C levels to decrease due to the sharing of the transporters and so decreased neutralization of oxidant levels that causing the lipid peroxidation and causing the rise in MDA levels to rise in the serum. The rise in serum MDA indicates the stress of oxidants on the plasma lipids. Thus the increase in lipid peroxidation product (MDA) in blood is associated with weakness of the defence antioxidant system in diabetes.[14] Thus we conclude that, it is the hyperglycaemia is the cause for the formation of glycosylation products. Thus the antioxidant levels was decreased significantly, which fails to neutralise the free radicals and make a role in the pathogenesis of diabetic retinopathy.[10] Thus we say that the hyperglycaemia is indirectly causing the decrease in the antioxidant levels and making insufficient neutralization of free radicals. Insufficient neutralization of free radicals cause oxidation of cellular lipids and causing dislipidaemia and raise in MDA levels which result development of microvascular complications.[16,18] Thus we say both the glycosylation and lipid peroxidation plays a main role in the development of retinopathy in diabetes mellitus.

**Conclusion:**

Due to high blood glucose levels in diabetes the levels of non enzymatic antioxidants like vitamin-C is decreased and oxidative stress is increased. Increase oxidative stress produces free radicals, which causes oxidation of lipids proteins nucleic acids, and further development of microvascular complications. Thus supplementation of Vitamin like Vitamin C and E with diet have a potential role in boosting antioxidants – related defences and will be important in mitigating the long – term complications of patients with diabetes.

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