

## Original article

# Correlation of protein carbonyl and MDA in diabetes and its complications

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### Abstract

**Background :** Diabetes mellitus(DM) is metabolic disorder characterized by hyperglycemia and abnormalities in lipid and protein metabolism. Oxidative stress along with protein carbonyl (PC) forms a very important causative factor for development of diabetes and its complications.

**Objectives :** The objective of the study was to assess correlation between protein carbonyl and diabetic complication. We have also correlated HbA1c and MDA levels with protein carbonyl.

**Methods :** This was a cross-sectional study. 135 subjects were included in the study. Group-I: 45 patients (24M/21F) of diabetes without complications; Group-II: 45 patients (23M/22F) of diabetics with complication (retinopathy, nephropathy, vasculopathy); Group-III: 45 age and sex match controls (24M/21F).

**Results :** The mean  $\pm$ SD values of PC, MDA and HbA1c in control group were  $1.15 \pm 0.14$ ,  $2.77 \pm 0.37$  and  $5.06 \pm 1.16$  respectively; in patients with DM without complications group values were  $2.01 \pm 0.41$ ,  $4.03 \pm 0.85$  and  $7.49 \pm 2.01$ ; in patients with diabetic complications group values were  $2.93 \pm 0.51$ ,  $5.71 \pm 0.73$  and  $8.67 \pm 2.91$ .

**Conclusion :** We found significant rise in serum level of PC and MDA in patient of DM and DM with complication as compared to controls. We also found a positive correlation between PC and HbA1c in DM group and in DM with complications group but not in control group. We suggest that PC and MDA could act as a stable oxidative stress marker in DM.

**Key words :** Protein carbonyl, malondialdehyde, HbA1c, oxidative stress, carbonyl stress

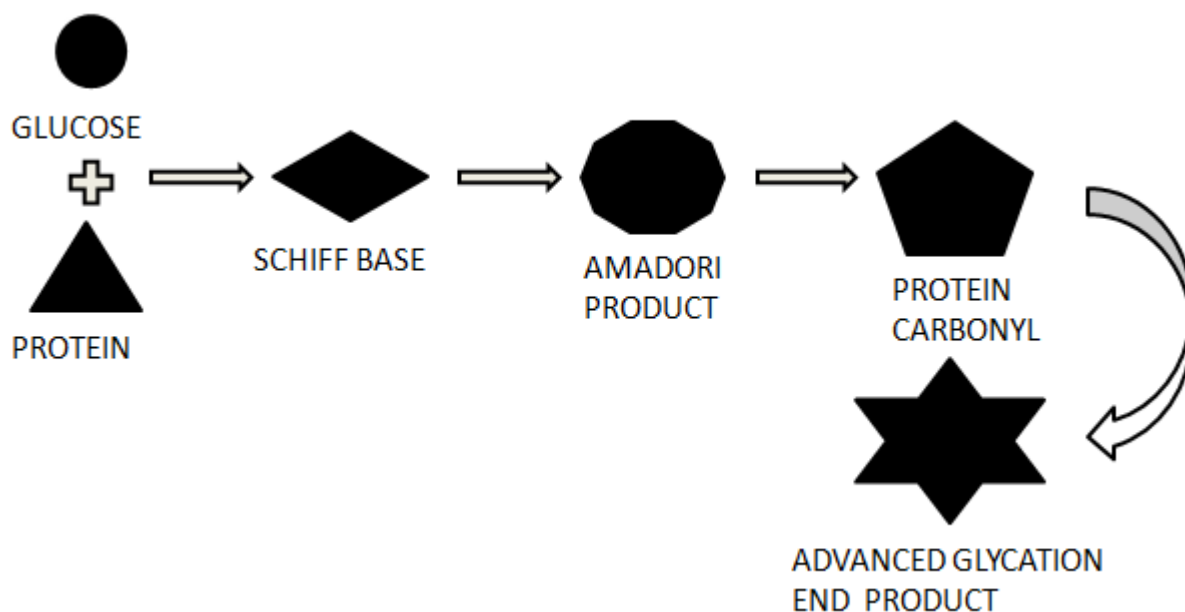
### Introduction

Diabetes mellitus (DM) is a group of a metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action or both. Diabetes is fast gaining the status of a potential epidemic in India.<sup>1,2</sup>

Oxidative stress along with protein carbonyl forms a very important causative factor for development of diabetic complications. Oxidative stress is a condition in which the cellular production of reactive oxygen species (ROS) exceeds the physiological capacity of the antioxidant defense system.<sup>3-6</sup> Hyperglycemia

is one of the most important factors that are responsible for oxidative stress and production of ROS in diabetes. Action of ROS on proteins, peptides, amino acids, aldehydes and ketones leads to formation of protein carbonyls. Protein carbonyl is formed as an intermediate in Maillard reaction<sup>7</sup> (Fig 1). Carbonylation results in irreversible modification of proteins, leading to the alteration of protein structure and function. Alteration of protein function further leads to cellular dysfunction and tissue damage.<sup>8</sup>

**Fig 1: Formation of AGE by Maillard reaction**



ROS degrade polyunsaturated lipids, forming malondialdehyde.<sup>9</sup>The inter-molecular cross-linking of collagen through MDA is important in the late complications of diabetes mellitus because it contributes to the stiffening of various tissues like cardiovascular tissue. The stabilization of long-lived proteins such as collagen through MDA cross-linking not only reduces its optimal functioning but reduces its already low turnover as they become resistant to the action of proteases and consequently allows further glycation by glucose and its oxidation products.<sup>10</sup>

It is difficult to measure ROS so we have measured protein oxidation in terms of protein carbonyl which is a stable product of irreversible non-enzymatic oxidation and lipid per-oxidation in terms of malondialdehyde (MDA), which is the end product of lipid per-oxidation.<sup>5,11</sup>

Role of carbonyl stress in diabetic complications has not been completely evaluated in Indian population and there is paucity in the literature regarding this. So, the present work was undertaken to study protein carbonyl as a marker of oxidative stress in diabetes and its progression.

#### **Objective**

The objective of the study was to assess correlation between carbonyl stress and diabetic complication. We have also correlated HbA1c and MDA levels with carbonyl stress.

#### **Materials and methods**

This was a cross-sectional study to assess correlation between carbonyl stress and diabetic complication. This study was carried out after approval from the institutional ethics committee and prior consent from all participants.

Total 135 subjects were included in the study. They were divided into three groups. Group-I: 45 patients

of Diabetics without complications, Group-II: 45 patients of Diabetics with complication (retinopathy, nephropathy, vasculopathy), Group-III: 45 age and sex match controls.

Patient on any anti-oxidant therapy, diabetes mellitus other than type 2, history of smoking, alcoholism and pregnant women, obese patients were excluded from the study.

Blood sample was collected in fluoride bulb (fasting and post prandial) for glucose estimation and in plain bulb for other parameters. Blood glucose was estimated by glucose oxidase peroxidase enzymatic method.<sup>12</sup> Serum protein carbonyl estimated by dinitrophenyl hydrazine method,<sup>13</sup> malondialdehyde by thiobarbituric acid method<sup>14</sup> and HbA1c by ion

exchange resin method<sup>15</sup>. Quality was checked by method standardization and calibration of methods.

### Statistical analysis

All the calculations were done using Microsoft Office Excel 2010 and statistical analysis was done using the Graph Pad Prism software, version 5.01. The collected data was analysed by applying Z- test and one – way ANOVA followed by Bonferroni post-test. P-value less than 0.05 ( $P < 0.05$ ) was considered to be statistically significant (S). P-value less than 0.001 ( $P < 0.001$ ) was considered to be statistically highly significant (HS). P-value more than 0.05 ( $P > 0.05$ ) was considered to be statistically non-significant (NS).

## Results

**Table 1 –**

Group	Controls	DM	DM with complication	p value <sup>a</sup>	p value <sup>b</sup>	p value <sup>c</sup>
Mean Age ± SD (Years)	56.64 ± 6.88	56.73 ± 6.26	57.04 ± 6.75	>0.05	>0.05	>0.05
Sex	24M/21F	24M/21F	23M/22F	>0.05	>0.05	>0.05
FBS (mg %)	82.22±11.96	169.64±40.43	188.57±37.80	<0.001	<0.001	<0.001
PPBS (mg%)	119.42±8.53	259.44±68.59	295.51±55.65	<0.001	<0.001	<0.001
HbA1c	5.06 ± 1.16	7.49 ± 2.01	8.67 ± 2.91	<0.001	<0.001	<0.001
Protein carbonyl (nmol/mg)	1.15 ± 0.14	2.01 ± 0.41	2.93 ± 0.51	<0.001	<0.001	<0.001
MDA (nmol/ml)	2.77 ± 0.37	4.03 ± 0.85	5.71± 0.73	<0.001	<0.001	<0.001

pvalue<sup>a</sup> - DM group vs control group.

pvalue<sup>b</sup>- DM with complication group vs control group.

pvalue<sup>c</sup>- DM group vs DM with complication group.

We found significant rise in serum level of Protein carbonyl in patient of diabetes mellitus ( $2.01 \pm 0.41$ nmol/mg) and diabetes mellitus with complication ( $2.93 \pm 0.51$  nmol/mg) as compared to controls ( $1.15 \pm 0.14$ nmol/mg) ( $p < 0.001$ ). This suggests potential role of carbonyl stress in pathophysiology of complications in diabetes patients. Significant rise was observed in serum level of MDA in patient of diabetes mellitus ( $4.03 \pm 0.85$ nmol/ml) and diabetes mellitus with complication ( $5.71 \pm 0.73$ nmol/ml) as compared to controls ( $2.77 \pm 0.37$ nmol/ml) ( $p < 0.001$ ).

In patients of DM, Coefficient of correlation between Serum MDA and protein carbonyl is 0.98 (95% Confidence Interval= 0.96 to 0.99, two tailed p value=  $< 0.001$ ). Hence Serum MDA and protein carbonyl are positively correlated in patients of DM. PC was also found to positively correlate with HbA1c (coefficient of correlation = 0.74).

In patients of DM with complication, Coefficient of correlation between Serum protein carbonyl and MDA is 0.73 (95% confidence interval= 0.56 to 0.84, two tailed p value=  $< 0.001$ ). Hence serum MDA and protein carbonyl are positively correlated in patients of DM with complication. We also found a positive correlation between PC and HbA1c (coefficient of correlation = 0.61).

On the other hand control group shows no correlation between PC and MDA or HbA1c.

### Discussion

Oxidative stress is now recognized to be prominent feature of DM and its progression. However, evidence for this association is inadequate because of lack of specific biomarkers and methods available to evaluate oxidative stress.

Malondialdehyde (MDA) is an accepted marker of lipid oxidative damage, whereas protein carbonyl is a marker of protein oxidative damage.

We found significant rise in serum level of PC and MDA in patient of DM and DM with complication as compared to controls indicating that an increase in oxidative stress might play a key role in pathogenesis of Diabetes mellitus and its complications.

Further we found that serum PC and MDA level were quite higher in patients of DM with complication as compared to those with DM only. This indicates that PC and MDA has role in progression of DM to its complication. The difference observed between DM without complications and DM with complications may be attributed to the severity of oxidative stress.

This study supports the hypothesis that poor glycemic control is an important factor in generation of increased protein oxidation in diabetic patients. Increase in plasma PC, and MDA levels in the diabetic patients with poor glycemic control may contribute to the development of diabetic complications.

Based on our findings, we suggest that oxidative stress plays a key role in the pathogenesis of DM as well as progression of DM to various complications already mentioned. The protein carbonyl resulted by oxidation makes the protein resistant to hydrolysis and functional inactivation of proteins in serum or plasma, cellular components, membrane proteins etc. since, protein is major constituents of all forms of the biological system the exact conformation and three dimensional folding are highly connected to the protein functions, the restore of nativity of protein is crucial.<sup>16</sup> However, more work is needed in Indian population to ascertain this.

### **Conclusion**

From the above study it can be concluded that protein carbonyl levels, like MDA and HbA<sub>1c</sub>, are raised in patients with diabetic complications. Apart from explaining the pathogenesis, protein carbonyl and MDA may supplement current diagnostic parameters of DM i.e. HbA<sub>1c</sub> and blood sugar levels. Protein carbonyl serves as a good marker of protein oxidative modification and therefore of 'carbonyl stress'. Further, along with HbA<sub>1c</sub>, PC and MDA may prove to be useful in monitoring progression of diabetic patients and institute appropriate therapy before they progress to overt complications.

### **Abbreviations**

AGE: advanced glycation end products

ANOVA: Analysis of variance

DM: diabetes mellitus

F : female

FBS : fasting blood sugar

HbA<sub>1c</sub> :glycated hemoglobin

HS : highly significant

M : male

MDA: malondialdehyde

NS : non-significant

PC: protein carbonyl

PPBS : post prandial blood sugar

ROS : reactive oxygen species

SD : standard deviation

We also suggest that, since protein carbonyl are intermediates in formation of advanced glycation end products (AGE), development and study of AGE inhibitors may be a promising area of research in treatment of diabetic complications.

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