

## RESEARCH ARTICLE

# Correlation of Protein Carbonyl and Protein Thiols in Oxidative Stress-induced Senescence of Red Blood Cell Membrane in Type 2 Diabetes Mellitus

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## ABSTRACT

**Introduction:** Diabetes mellitus (DM) is an endocrine dysfunction, which is associated with metabolic disease. Wherein chronic hyperglycemia presensitizes the oxidative process which in turn biochemically leads to advanced glycation. The aim is to study the changes caused by oxidative stress upon the red blood cell (RBC) membrane composition by protein carbonyl and protein thiols in type 2 DM (T2DM) in comparison with that of controls.

**Materials and methods:** The study includes two groups, consists of 100 cases of T2DM and 100 healthy subjects as controls. Blood samples were collected under strict aseptic precautions as per the criteria and estimation of fasting blood sugar (FBS), hemoglobin A1c (HbA1c), protein carbonyl with RBC membrane ghost preparation and estimation of protein thiols were done.

**Results:** It was found that there was significant increase of protein carbonyl in cases ( $1.20 \pm 0.08$ ), in comparison to normal controls ( $0.90 \pm 0.06$ ) with a statistical significance of ( $p < 0.001$ ) along with Protein thiols of RBC membrane which was significantly decreased ( $1.42 \pm 0.10$ ) in cases in comparison to normal controls. ( $2.12 \pm 0.12$ ) with a statistical significance of ( $p < 0.001$ ). In our study, the positive correlation of protein carbonyl and protein thiols was established with 74% of cases of type 2 DM falling into a HbA1C control group of 7 to 8% indicating the increased protein carbonyl and decreased protein thiols are early indicators of progressive diabetic changes.

**Conclusion:** Changes in RBC membrane of persons with T2DM acts as an early indicator by means of an increase in protein carbonyl and decrease in protein thiols, and this will further lead to progressive diabetic changes.

**Keywords:** Diabetes mellitus (DM), Oxidative stress, Protein carbonyl, Protein thiols, Reactive oxygen species.

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## INTRODUCTION

Diabetes is a significant health problem affecting people all over the world. In India, T2DM may increase from 50.8 million in 2010 to 87.0 million by 2030. The DM is a being a metabolic disease associated with chronic hyperglycemia and relative lack of insulin. Chronic hyperglycemia leads to damage and dysfunction of various organ systems—eyes, kidneys, nerves system, blood vessels, and heart.

Diabetes mellitus (DM) is a metabolic disease characterized by a state of chronic hyperglycemia resulting from defects in Insulin secretion, Insulin action or both. The chronic hyperglycemia is associated with long-term damage, dysfunction, and failure of normal functioning of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Chronic hyperglycemia leads to the generation of free radicals, especially reactive oxygen species (ROS) from glucose autooxidation and protein glycation. The teratogenic and carcinogenic effects of oxidative stress are because of modification in protein, lipid, and deoxyribonucleic acid (DNA), which ultimately causes cellular dysfunction.<sup>1</sup>

During DM, oxidative stress will lead to various complications. Increased levels of ROS in DM is because of their increased production and/or decreased destruction by antioxidants such as non-enzymatic or enzymatic reactions like catalase, reduced glutathione (GSH) and superoxide dismutase (SOD).<sup>2</sup> Oxidative stress is currently suggested as the mechanism underlying DM and its complications.<sup>3</sup> Oxidative stress leads to proteins, lipids and DNA modifications that cause cellular dysfunction and this could have teratogenic or carcinogenic consequences.<sup>4</sup> Over the last few decades, several age-related alterations of erythrocytes have been investigated,<sup>5</sup> the oxidative damage, initiated by ROS and other oxidants, upon the alteration of erythrocytes has been investigated.<sup>5</sup> In case of thalassemia, oxidative damage to the erythrocyte membrane plays an essential role in the aging (senescence) of pathological red cells,<sup>6</sup> of these oxidative damages to the erythrocyte membrane component is presently thought to play a pivotal role during the senescence of pathological red cells in thalassemia, sickle cell disease, etc.

A characteristic hallmark of many pathophysiological conditions is a decrease in the GSH: oxidized glutathione

(GSSH) ratio, The GSSG accumulates in the cells, it can undergo disulfide exchange reaction with protein thiols leading to S-glutathionylation. These S-glutathionylated protein has been investigated as a positive biomarker of oxidative stress in human disease such as DM.

In most of the pathological conditions, the accumulation of protein carbonyls has been observed. Protein carbonyl is the most commonly used marker and is also the most common indicator of protein oxidation. There is an appearance of carbonyl groups such as aldehyde/ketone groups of proteins which are caused because of oxidative modification. Protein carbonyls are more hydrophobic, and they resist proteolysis.<sup>4</sup> The study was taken up in both normal subjects as well as those with T2DM to assess the effects of oxidative stress on the erythrocyte membrane.

## MATERIALS AND METHODS

The study was conducted in Basaveshwara Medical College and Hospital, Chitradurga, a tertiary care center. After doing the biochemical tests of HbA1c in our central laboratory, 100 individuals with HbA1c levels more than 7% were taken as T2DM. One-hundred individuals with age and sex-matched healthy individuals with HbA1c <5.3% taken as controls. Both cases and control subjects were subjected to estimation of biochemical parameters. Venous blood samples were collected after taking aseptic precautions from the study subjects. Five mL of blood was collected in plain vacuum tubes, and 2 mL of blood was collected in ethylenediaminetetraacetic acid (EDTA) vacuum tubes. Samples were left for 20 minutes at room temperature and centrifuged at 3000 rpm for 4 to 5 minutes. Serum was used for the estimation of FBS by glucose oxidase (GOD)-peroxidase (POD) method.<sup>7</sup> Estimation of HBA1C was done by cation exchange resin method.<sup>8</sup> RBC membranes were prepared by Dodge et al. method,<sup>9</sup> protein carbonyl estimation was done by Levine et al. method<sup>10</sup> and protein thiols was estimated by Habeeb AFSA, methods enzymol.<sup>11</sup> The study was approved by the ethics committee, a written informed consent was obtained from all the participants in this study.

## Statistical Analysis

Statistical analysis of data was performed using Statistical Package for the Social Sciences (SPSS version 15.0), chi-square and Fisher exact test has been used to find the significance of protein carbonyl and protein thiols levels between cases and controls. R environment version 2.11.<sup>1</sup> were used for the analysis of the data and Microsoft Word and Excel have been used to generate graphs, tables, etc.

## RESULTS

According to our study, the observation which we got was there was a significant increase in protein carbonyl in cases ( $1.2 \pm 0.08$ ) when compared with controls ( $0.90 \pm 0.06$ ). Statistical significance amounted to the value of  $p < 0.001$ . Whereas in the case of protein thiols of RBC membrane there was a significant decrease ( $1.42 \pm 0.10$ ) in cases in comparison to normal controls ( $2.12 \pm 0.12$ ). statistical significance amounted to the value of  $p < 0.01$  as illustrated in Table 1. The present study revealed that in 74% cases of T2DM falling into a HbA1c control group of 7 to 8% were having a positive correlation, that of increased protein carbonyl and decreased protein thiols as early indicators for the changes seen in diabetic patients.

## DISCUSSION

Type 2 DM is a complex endocrine disorder, which is associated with aberrant metabolism of carbohydrate, fat, and protein due to absolute or relative deficiency of insulin. The biochemical processes of advanced glycation appear to be enhanced in the diabetic milieu as a result of not only hyperglycemia but also other stimuli such as oxidative stress, and lipid peroxidation leads to advanced glycation a biochemical process is enhanced in the diabetic milieu. Exposure of protein to oxygen radicals results in protein damage, this includes oxidative modification of many amino acids residue fragmentation, aggregation, and increased proteolytic susceptibility, like most biological membranes which are rich in proteins. Likewise, the plasma membrane of erythrocytes is also rich in proteins, which in itself is unique. These membrane proteins of erythrocytes thus attract or become targets for the ROS and reactive nitrogen species (RNS).<sup>12</sup>

**Table 1:** Comparison of FBS, HbA1c, protein carbonyl and protein thiols between type 2 DM patients and healthy controls

Parameters	Cases (n = 100) mean $\pm$ SD	Controls (n = 100) mean $\pm$ SD	p-value
Age	41.52 $\pm$ 5.47	41.52 $\pm$ 5.47	<0.001**
Fasting blood sugar (mg/dL)	197.50 $\pm$ 62.84	93.48 $\pm$ 7.54	<0.001**
HbA1C (%)	7.73 $\pm$ 0.67	5.34 $\pm$ 0.59	<0.001**
Protein Carbonyl (nmoles/mg of protein)	1.20 $\pm$ 0.08	0.90 $\pm$ 0.06	<0.001**
Protein Thiols (nmoles/mg of protein)	1.42 $\pm$ 0.10	2.12 $\pm$ 0.12	<s0.001**

\*\* Statistically Significant

This exposure of the protein to oxygen free radicals results in protein damage, a process of oxidative modification of many amino acid residues, fragmentation, aggregation, and increased protein susceptibility. They not only limit to protein but further cause damage to lipids and nucleic acids as well as leading to various diabetic complications<sup>13</sup> over a due course of time.

Biological systems are continually exposed to endogenous and exogenous ROS, and RNS and proteins are their major targets due to abundance, relatively long half-lives and high rate of reactions. However, the overall biology of oxido-nitrosative protein modifications remains complex and is not completely defined, but protein carbonylation is quite well characterized. Protein carbonylation is an irreversible, non-enzymatic modification of proteins. The carbonyl groups are introduced into proteins by a variety of oxidative/nitrosative pathways. ROS and RNS can react directly with the protein and generating products such as reactive carbonyl species (RCS) then react with the proteins.

Direct oxidation of proteins by ROS and RNS yields highly RCS derivatives resulting from oxidation of lysine, arginine, proline, and threonine amino acid residues. Carbonyl groups can also be generated by the reaction of the primary amino group of lysine with RCS such as ketoamines, ketoaldehydes, deoxyosones and eventually leading to the formation of advanced glycation end products (AGEs). Now it is well accepted that oxidation/nitration of proteins typically results in an increase in carbonyl contents. This increase is due to the oxido-nitration of lysine, arginine, proline and threonine residues. In short, protein carbonyl groups are the most reliable biomarker of oxidative/nitrosative stress.<sup>14</sup>

Cellular proteins are believed to be the targets of free radicals induced oxidation injury. Protein carbonyl content in the cells is one indication of oxidative damage to proteins and can be generated *via* nonspecific oxidation of amino acids or catalyzed oxidation of specified amino acid key to protein function by oxygen and glycation. Protein carbonyl is generated by oxidative modifications of proteins either by alpha-amidation ( $\alpha$ -amidation) pathway or by oxidation of glutamyl side chains, reactions with aldehydes like malondialdehyde produced during lipid peroxidation, oxidation of reducing sugars and reaction of oxidized product with lysine residues of proteins.<sup>15</sup>

Sagare et al., did a study on T2DM and its complications, by recruiting 50 T2DM cases and 50 age and sex-matched healthy subjects. They estimated protein carbonyl, glycated hemoglobin (HbA1c) and urine microalbumin. They found that there is a significantly increased level of protein carbonyls, HbA1c, and urine microalbumin in

T2DM cases when compared with controls. Our study results are also in accordance with these findings.<sup>16</sup>

In the present study, protein thiols content of RBC membrane was significantly decreased in cases when compared to controls, which exhibits the free radical injury due to the sulfhydryl groups and protein mixed disulfides with homocysteine and glutathione are found as low molecular weight disulfide pool in reduced form, which results in oxidation of cysteine residues and reversible formation of mixed disulfides among low molecular mass thiols (GSH), which can directly alter the orb regulate protein function for (redox function) and can have a role in protein oxidation, resulting in senescence of RBC membrane.<sup>17,18</sup>

## CONCLUSION

Our study revealed that there was an increase in protein carbonyl content in cases as compared with that of controls. One of the indications for oxidative damage is the presence of protein carbonyl content in the plasma membrane of erythrocytes. Oxidative damage leads to modification of proteins by oxidation of glutamyl side chains or by  $\alpha$ -amidation pathway, reaction with malondialdehyde, which is produced during reaction with that of oxidized products with lysine residue of proteins oxidation of reducing sugars and lipid peroxidation. Further studies have to be done to diagnose early changes in type 2 diabetics to identify high-risk subjects and to prevent further complications.

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