

Biochemical markers for early senescence of erythrocyte membrane in type 2 diabetes mellitus

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Abstract

Diabetes mellitus is an ice berg of disease resulting due to impairments in insulin secretion, insulin action or both. Hyperglycemia caused due to the above defects leads to complications like neuropathy, nephropathy, retinopathy, peripheral vascular disease and coronary artery disease as a result of stimuli for oxidative stress on cell-membrane. The aim of the study is to establish a relation between the stress induced by oxidants bringing changes with the erythrocyte membrane. The protein content, protein thiols, protein carbonyl levels in relation to control of type 2 DM based on HbA1C level indicates that there is an auto-oxidation of glucose which results in persistent production of thiols and carbonyl leading to protein damage, modification due to amino acid residues, fragmentation of amino acid stress and raised proteolytic susceptibility. Protein thiols and carbonyl are the results of oxidation of amino acid which is non-specific or by specific amino acid oxidation.

A case control comparative study was done with type 2 DM and normal control at BMCH & RC, Chitradurga. The maximum number were of the age group of 41-45 i.e. 32%. The mean FBS levels among cases and controls 197.50 mg/dl and 93.48 mg/dl respectively. There was significant difference between levels of protein content (4.56 ± 0.19), protein carbonyl (1.20 ± 0.08) and protein thiol levels (1.42 ± 0.10) among diabetics in comparison to protein content (5.40 ± 0.31), protein carbonyl (0.90 ± 0.06), and protein thiols (2.12 ± 0.12) in controls. It was found that there was significant increase of protein carbonyl, decrease protein content and decrease in protein thiols in diabetic patients.

Keywords: Diabetes mellitus, Oxidative stress, Reactive oxygen species, Protein content, Protein carbonyl, Protein thiols.

Introduction

Diabetes mellitus is a disease associated with wide array of metabolic abnormalities, which can also concern erythrocyte function. Erythrocyte of diabetic patients have reduced life span,¹ altered membrane dynamic properties² and increased membrane thermo stability.³

Diabetes mellitus is a burning health issue affecting people all over the world. It is one of the most extensively studied human diseases. Diabetes mellitus is an endocrine disease characterized by a state of long standing hyperglycemia resulting from defects in insulin production, insulin action or both. Diabetes is a long term disease leading to a number of complications- cardiovascular, renal, ocular, and neurological and others. The vast majority of diabetes falls into two main entities. Type 1 diabetes is caused by an absolute deficiency of insulin secretion. It mainly affects young lean individuals. Type 2 diabetes being more common is caused by a combination of insulin resistance and less than adequate compensatory insulin secretion. Type 2 diabetes mainly, although not exclusively tends to involve middle aged obese individuals. In diabetes, chronic persistently elevated blood sugar levels lead to the generation of free radicals specially ROS, affecting all tissues, through glucose autooxidation and protein glycosylation. Increase in the levels of ROS in diabetes is due to their increased generation and/or decreased degradation by non enzymatic or enzymatic reactions

like catalase, reduced glutathione (GSH), superoxide dismutase (SOD) antioxidants. The increase in the levels of ROS results in damage to DNA, lipids and proteins. Increase in ROS levels may also contribute a stress signal that triggers specific redox sensitive signalling pathways. These free radicals may modify erythrocyte membrane proteins or lipids which results in disorientation of membrane lipids and proteins including phospholipids.⁴

Formation of glycated hemoglobin is irreversible and its level in blood depends on both life span of RBC's (average 120 days) and blood glucose concentration. The glycosylated hemoglobin assay is a very useful index that is unique as it gives a retrospective insight of glucose control over time in patients with diabetes. Glycated hemoglobin concentration represents the integrated values of glucose over preceding 6 to 8 weeks since the rate of formation of glycated hemoglobin is directly proportional to the concentration of glucose in blood. Other advantage of glycated hemoglobin values for assessing glucose control is because these are free of day to day glucose fluctuations and are unaffected by exercise or recent food ingestion.⁵

HbA_{1c} levels reflect the average blood glucose concentration. It is currently considered the best index of metabolic control for diabetic patients in clinical setting.

Thiols are sulfhydryl containing organic antioxidants which constitute the major portion of the total body antioxidants and they play a crucial role in defence against reactive oxygen species. Total thiols comprising of both intracellular and extracellular thiols either in the free form as oxidized or reduced glutathione, or thiols bound to proteins. Among thiols that are bound to major proteins, albumin makes the major portion of the protein bound thiols which bind to -SH groups at cysteine. Thiols play a key role in detoxification, signal transduction, apoptosis and various other functions at molecular level.⁶

Oxidative damage to membrane proteins results in the increase in Protein carbonyl content in the cells. This can occur through nonspecific oxidation of aminoacids or through exposure of protein to oxygen radicals results in protein damage, this includes oxidative modification of much amino acid residue fragmentation, aggregation and increased proteolytic susceptibility. As erythrocyte membrane is rich in proteins, it acts as a primary target for ROS and RNS. Considerable evidence indicates that maintenance of protein redox status is of fundamental importance for cellular function therefore changes in redox homeostasis of proteins are considered to be among the molecular mechanisms leading to endothelial dysfunction. Protein carbonyl groups may be induced in membrane proteins by secondary reaction of nucleophilic side chains of cysteine, histidine and lysine residues and reactive aldehydes produced during the peroxidation of membrane lipids.⁷

Over the past few decades many alterations of erythrocyte senescence have been investigated, off these oxidative damage to the erythrocyte membrane components is presently thought to play a key event during ageing of pathological red cells in various haemolytic anaemias. This oxidative damage is probably initiated by reactive oxygen species (ROS) and other oxidants endogenously.⁸

Materials and Methods

The study was conducted in the department of Biochemistry and department of Internal Medicine, BMCH and RC Chitradurga from January 2013 to December 2014. After taking due clearance from the Ethics committee; a written informed consent was

obtained from all participants in this study. A total of 100 patients with type 2 diabetes mellitus were selected from the institute's Internal Medicine department. The diagnosis of type 2 diabetes mellitus was confirmed by glycosylated hemoglobin (>7). Hundred age and sex matched apparently healthy individuals with normal plasma glucose and with no symptoms suggestive of diabetes mellitus were taken as controls. Fasting plasma glucose was determined by using commercially available reagents in automated analyser.⁹ The estimation of glycosylated hemoglobin was done by cation exchange resin method, RBC membrane were prepared by Dodge et al,¹⁰ protein content by Lowry et al,¹¹ protein carbonyl estimation was done by Levine et al¹² method and protein thiols by Habeeb AFSA Method¹³

Patients with type 1 diabetes mellitus, chronic kidney disease and liver disease were excluded from the study.

Data from both groups were tabulated in Microsoft Excel 2007 and statistical analysis of data was performed using SPSS (Version 20.0). Chi-square and Fisher Exact test has been used to find the significance of protein, protein carbonyl and protein thiols between cases and controls.

Results

A comparative study consisting of 100 diabetes mellitus patients and 100 controls was undertaken to investigate the indicators of oxidative stress in type 2 diabetes mellitus cases when compared to controls. The mean age of the diabetics was 47.12 ±5.47 years whereas it was 53.58±12.84 years in controls respectively. Both among the cases and controls the sex distribution was same i.e. 80% and 20% males and females respectively. The maximum number were of the age group of 41- 45 i.e. 32%. The mean FBS levels among cases and controls 197.50 mg/dl and 93.48 mg/dl respectively. There was statistically significant difference between levels of protein content (4.56±0.19), protein carbonyl (1.20±0.08) and protein thiol levels (1.42±0.10) among diabetics in comparison to protein content (5.40±0.31), protein carbonyl (0.90±0.06), and protein thiols (2.12±0.12) in controls.

Table 1: Comparison of protein content, protein carbonyl and protein thiols in type 2 diabetic cases and controls groups studied

Variables	Cases	Control	Difference	P value
PROTEIN (mg/ml)	4.56±0.19	5.40±0.31	0.84	<0.001**
PROTEIN CARBONYL (nmoles/mg of protein)	1.20±0.08	0.90±0.06	0.30	<0.001**
PROTEIN THIOLS (nmoles/mg of protein)	1.42±0.10	2.12±0.12	0.70	<0.001**

Significantly more with P=<0.001**

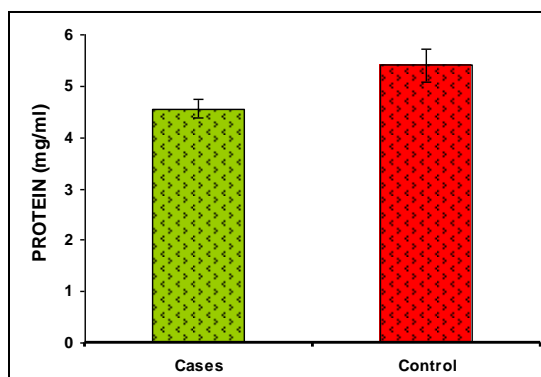


Fig. 1

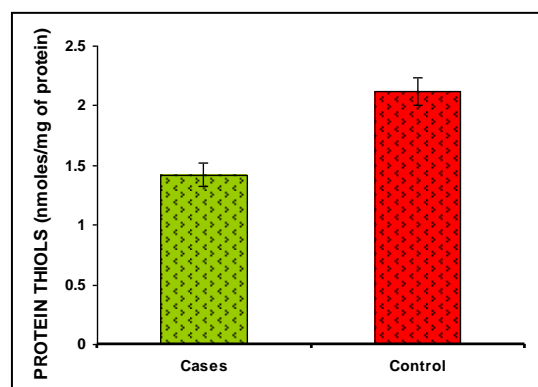


Fig. 2

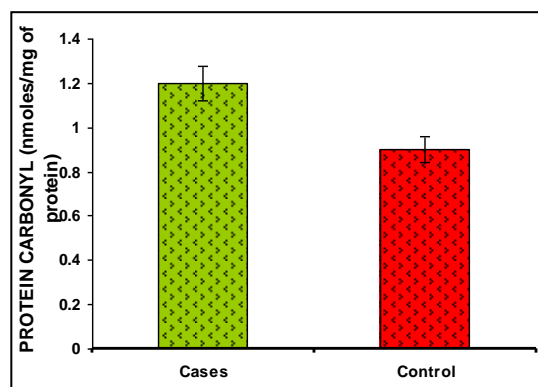


Fig. 3

Discussion

Oxidative stress may be defined as a measure of the steady level of reactive oxygen radicals in biological system. Increased oxidative stress may result from over production of precursors to reactive oxygen radicals and or decreased efficiency of inhibitory and scavenger systems. The stress then may be amplified and propagation by an autocatalytic cycle of metabolic stress, tissue damage and cell death. Oxidative stress and oxidative damage to tissues are common end points of chronic diseases such as atherosclerosis, rheumatoid arthritis and diabetes. Oxidative stress is currently suggested as mechanism underlying diabetes and diabetic complications.¹⁴

Glucose under physiological conditions produces oxidants that possess reactivity similar to hydroxyl free radicals. Increase free radical produced and elevated levels of lipids and several peroxidation products have been reported in diabetic patients.

Free radical induced oxidation injury mainly damages cellular proteins. Protein carbonyl content in the cells is an important marker of oxidative damage to proteins and can be generated by non specific oxidation of amino acids or by catalysed oxidation of specific amino acid key to protein function by oxygen and glycation. Carbonyl formation in proteins is dependent on metal ions such as Fe^{2+} and Cu^{2+} . These can bind to the cation binding site in proteins and with help of H_2O_2 or O_2 they can change the side chains of amino-acids to carbonyl groups. By involving in the Fenton reaction,¹⁵ copper catalyse the production of hydroxyl radical, which oxidises lipids, proteins and DNA.¹⁶ Additionally accumulation of carbonyl groups on protein results in series of chemical modifications, and result in formation of advanced protein oxidation products (APO) or advanced glycation end products (AGE).¹⁷ Elevated levels of protein carbonyl group content are reported in various diseases¹⁸

Thiols are sulfhydryl containing organic compounds. Of the available antioxidants in the body, thiols constitute the major chunk of the total body antioxidants and play a significant role in defence against ROS. Thiols are the facile targets for free radicals hence in the oxidative stress conditions the membrane thiols are reduced. Decreased levels of thiols has been noted in various medical disorders including diabetes mellitus. A characteristic hallmark of many pathophysiological conditions is a decrease in the GSH:GSSG ratio. The GSSG accumulates in the cells, it can undergo disulfide exchange rxn with protein thiols leading to S-Glutathionylation, these S-Glutathionylated protein has been investigated as positive biomarker of oxidative stress in human diseases such as diabetes.⁶

In the present study glycated hemoglobin levels are significantly increased ($P < 0.001$) in cases as compared to controls. These findings are in accordance with studies of Meena Verma,¹⁹ Hattice Pasaglu,²⁰ and Rama Srivastan.²¹ The protein carbonyl content was increased in cases in comparison to controls; this is in accordance with the study done by D. Konukoglu,²² Odetti et al²³ and Telci et al.²⁴ In the present study the protein thiols are decreased significantly ($P < 0.001$) in cases which is in accordance with Rama Srivastan et al. Thus thiols are investigated as positive biomarker of oxidative stress in human diseases such as diabetes.

Conclusion

Our study suggested that increased free radicals are produced due to persistent hyperglycemia, which induce changes in membrane protein content, protein carbonyl, protein thiols in comparison to normal controls as a result of changes due to oxidation of

proteins and fragmentation which are potential risk factors for the development and progression of oxidative damage resulting in senescence of erythrocyte membranes.

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