Correlation of Protein Carbonyl and Malondialdehyde in Oxidative Stress Induced Senescence of RBC Membrane in Type 2 Diabetes Mellitus

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Abstract

Diabetes mellitus is a group of metabolic disease characterised by a state of chronic hyperglycemia. The biochemical process of Advanced Glycation appears to be enhanced in the Diabetes melieu as a result of not only hyperglycemia but also other stimuli such as oxidative stress and lipid peroxidation.A case control comparative study was done with Type 2 Diabetes mellitus and normal controls at BMCH RC, chitradurga. According to the criteria, blood sample were collected under aseptic precautions and evaluation of fasting blood sugar, HbA1C, Protein carbonyl along with RBC membrane ghost preparation and estimation of malondialdehyde(MDA) were done.

Index terms— diabetes mellitus, oxidative stress, reactive oxygen species, protein carbonyl and malondialdehyde (MDA).

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The aim of the study is to establish a link between the oxidative stress induced by changes with protein carbonyl content and MDA damaging the RBC membrane composition in Type 2 DM in comparison to normal controls.

The correlation of Malondialdehyde (MDA) and Protein carbonyl levels in relation to control of Type 2 Diabetes mellitus based on HbA 1 C level indicate that there is an autooxidation of glucose which results in persistent production of malondialdehyde(MDA) and ROS which can release advance glycation end products (AGE) and advanced lipoxidation end products(ALE) along with increased carbonylation of proteins leading to protein damage, oxidative modification of aminoacid residues ,aminoacid fragmentation and increased proteolytic susceptibility. Protein carbonyl can be generated by via non specific oxidation of aminoacid by via nonspecific oxidation of aminoacid or via catalysed oxidation of specific aminoacid key to protein function by oxygen and glycation.

A case control comparative study was done with Type 2 Diabetes mellitus and normal controls at BMCH & RC, chitradurga. According to the criteria, blood sample were collected under aseptic precautions and evaluation of fasting blood sugar, HbA 1 C, Protein carbonyl along with RBC membrane ghost preparation and estimation of malondialdehyde(MDA) were done. It was found that there was significant increase of protein carbonyl in serum of Type2 DM cases (1.20±0.08) in comparison to control groups (0.90±0.06) with a statistical significance of ($p<0.001$) along with Malondialdehyde (MDA) of RBC membrane which was also significantly increased (4.23±0.21) in Type 2 Diabetes Mellitus in comparison to normal control (3.28±0.19) with a statistical significance of $P<0.001$. In our study, the positive correlation of membrane Malondialdehyde(MDA) and protein carbonyl was established with 74% of cases of Type 2 Diabetes Mellitus falling into the HbA1C control group of

7-8% indicating that protein carbonyl, Malondialdehyde (MDA) levels are early indication of progressive diabetic changes.

1 Introduction

iabetes mellitus is the major health problem affecting people all over the world. It is one of the most extensively investigated human diseases. Diabetes Mellitus is a metabolic disease characterized by a state of chronic hyperglycemia resulting from defects in insulin secretion, insulin action or both. The vast majority of diabetes falls into two broad categories. During diabetes mellitus, persistent hyperglycemia produces free radicals especially reactive oxygen species (ROS), glucose autooxidation and protein glycosylation. Increase in the levels of ROS in diabetes mellitus is due to their increased production and/or decreased destruction by non enzymatic or enzymatic reactions like catalase, reduced glutathione (GSH), superoxide dismutase (SOD) antioxidants. 1 The impairment caused by increased ROS is thought to result in random damage to proteins , lipids and DNA. 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. 2 Over the last few decades several age related alterations of erythrocytes have been investigated, 3 of these oxidative damage to the erythrocyte membrane components is presently thought to play a key event during senescence of pathological red cells in thallesemia, sickle cell anaemia etc. The oxidative damage is probably initiated by reactive oxygen species (ROS) and other oxidants endogenously. 4 The study was undertaken to evaluate the effect of oxidative stress on erythrocyte membrane in Type 2 Diabetes mellitus and compare them with normal subjects.

2 II.

3 Materials and Methods

The study was approved by 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 recruited from the institute's 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 DM were taken as controls. Both cases and controls were subjected to estimation of biochemical parameters. Fasting plasma glucose was estimated by using commercially available kit in automated analyzer. 5 The estimation of glycosylated hemoglobin was done by cation exchange resin method 6 , RBC membrane were prepared by Dodge et al 7 , protein carbonyl estimation was done by Levine et al 8 method and MDA was estimated by Ohkawa et al method. 9 III.

4 Statistical Analysis

Statistical analysis of data was performed using SPSS (Version 15.0). Chi-square and Fisher Exact test has been used to find the significance of protein carbonyl and MDA levels between cases and controls. R environment Ver 2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc., IV.

5 Results

A Comparative study consisting of 50 Diabetic Mellitus patients and 50 controls was undertaken to investigate the oxidative stress parameters in type 2 DM cases when compared to controls. The mean age of the diabetics was 41.52 ±5.47years whereas it was 55.58±12.84 years respectively. Both among the cases and controls the sex distribution was same i.e. 80% and 20% males and females respectively. The maximum number of the age group of 41-45 i.e. 32%. The mean FBS levels among cases and controls were 197.50±8462 and 93.48±7.54 mg/dl and respectively. There is significant difference between levels of protein carbonyl and MDA levels among diabetics and controls. The mean protein carbonyl in cases and controls were 1.20±0.08 and 0.90±0.06 nmols/mg of protein respectively ($p<0.001$). The mean MDA in cases and controls were 4.23±0.21 and 3.28±0.19 nmols/mg of protein respectively ($p<0.001$).

V.

6 Discussion

Diabetes Mellitus is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. The biochemical process of advanced glycation appears 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. Protein carbonyl content in the cells is one of the indications of oxidative damage to protein and can be generated via nonspecific oxidation of aminoacids exposure of protein to oxygen radicals results in protein damge, this includes oxidative modification of many amino acid residue fragmentation, aggregation and increased proteolytic susceptibility. Like most biological membranes the plasma membrane of erythrocytes is rich in protein owing to this unique feature membrane proteins of erythrocytes

are primary target for ROS & RNS. 10 The protein carbonyl content was increased in cases in comparison to controls. Cellular proteins are believed to be the targets of free radical induced oxidation injury. Protein carbonyl content in the cells is one indication of oxidative damage to proteins and can be generated by via non specific oxidation of aminoacids or via catalysed oxidation of specific aminoacid key to protein function by oxygen and glycation. Persistent hyperglycaemia in diabetes mellitus leads to increased formation of free radicals through various mechanisms. These free radicals attack and damage lipids, proteins and nucleic acids resulting in various late diabetic complications. 11 In the present study MDA content of cases was significantly raised in comparison to controls which exhibits the free radical injury due to peroxidative breakdown of phospholipids, fatty acids and accumulation of MDA resulting in senescence of RBC membrane.

7 VI.

8 Conclusion

The present study suggested that excess free radicals are generated due to persistant hyperglycemia, which induces changes in membrane lipid peroxidation and oxidation of proteins and fragmentation which are potential risk factors for the development and progression of oxidative damage resulting in senescence of RBC membranes.

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Figure 1:

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[*] , * . P<0.001)=significant.

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