"PROTEIN CARBONYL & MICROALBUMINURIA IN TYPE 2 DIABETES MELLITUS"

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ABSTRACT:

INTRODUCTION: Diabetes is a devastating disease throughout the world. It is associated with several mechanisms, one of which is oxidative stress. Oxidative stress plays an important role in the pathogenesis and the complications of diabetes. A hyperglycemia results in overproduction of oxygen free radicals, which contributes to the progression of diabetes. The development of complications during diabetes is associated with oxidative stress. Measuring protein carbonyl associated with nephropathy as oxidative protein damage is the primary aim of our study.

METHODS: We measured protein carbonyl, urine microalbumin & glycated hemoglobin in type 2 Diabetes mellitus & studied for correlation among these parameters towards development of nephropathy. Fifty diabetic and fifty age matched healthy subjects were included in the study. Both the groups were evaluated for protein carbonyl, urine microalbumin & glycated hemoglobin.

OBSERVATIONS: Plasma protein carbonyls were significantly ($p< 0.001$) elevated in diabetes (0.091 ± 0.13 mmol/L) when compared with healthy subjects (0.037 ± 0.012 mmol/L). Glycated hemoglobin has shown proportional increase with blood glucose. Urine microalbumin is found to be increased in patients with diabetes & positively correlated with protein carbonyl.

RESULTS: Increased formation of protein carbonyl, a marker of oxidative stress produced under hyperglycemia of diabetes mellitus may be one of the probable cause for evolution of nephropathy in diabetes mellitus.

KEYWORDS: Diabetes mellitus – Glycated hemoglobin – oxidative stress- protein damage

INTRODUCTION: Development of complications like angiopathy and diabetic nephropathy are frequently seen in type 2 Diabetes mellitus (DM). [1] Increased oxidative stress is widely accepted cause in the development & progression of diabetic complications. Excessively high levels of free radicals have been reported as damaging cause for cellular proteins, membrane lipids, nucleic acids & eventually cell death. [2]

Oxidative stress & associated oxidative damage are currently ackwoleged as components of molecular & cellular damage mechanism which is not only involved in vascular dysfunction but also genesis of DM.[3] Exposure of proteins to ROS causes major physical change in protein structure, affecting downstream functional consequences such as inhibition of enzyme, binding activities, increased susceptibility for aggregation and proteolysis, increased or decreased uptake by cells, and altered immunogenicity. [4, 5]

Protein carbonyl content is actually the most general indicator & by far the most commonly used marker for protein oxidation. Accumulation of these protein carbonyls have been observed in many pathological condition. [6]

Protein carbonylation occurs at the early stages & remains in circulation for longer period compared with other parameters of oxidative stress such as glutathione disulfide & MDA.

It is a measure of chemical & non enzymatic oxidative modification. Therefore the evaluation of protein oxidation in plasma is respected marker of free radical intensity. [7] Glycosylated Hemoglobin (GHb) is more prone for oxidation than non glycosylated Hemoglobin. In poorly controlled DM increased GHb is observed that become more prone for oxidation resulting in increased formation of protein carbonyl product. [8]Carbonyl stress alters the structure & functions of cellular matrix proteins that may contribute for development of nephropathy in diabetes. [9]

The present study was carried out to evaluate carbonyl stress in type 2 DM and correlation of Protein carbonyl with risk measures like microalbuminuria & GHb as a glyacemic index to assess their involvement in development of nephropathy.

MATERIALS & METHODS:

The present study was carried out on the subjects of Out Patient Department of our Hospital. Age matched subjects were included for study. Present study comprised of two groups

Group A: Known diabetic patients – n= 50

With fasting blood Glucose ≥ 126 mg/dl

Glycosylated Hb ≥ 7.0

DM Patients with atherosclerosis, chronic infections, and acute renal failure were excluded from the study.

Group B:Control group/ healthy subjects n = 50

Fasting blood glucose ≤ 110 mg/dl

Glycosylated Hb ≤ 5.6

The healthy subject did not show any inflammatory conditions, abnormalities in lipid & carbohydrate metabolism or kidney disorders in routine medical checkups.

5 ml Fasting blood samples of patients & control were collected in plain bulb & Sodium fluoride bulb. Precaution was taken to avoid any traces of hemolysis. After an hour clear serum was separated by centrifugation at 3000 rpm for 5 min & serum samples were analysed for Total protein concentration by Biuret method & Protein carbonyl assay by spectrophotometric DNPH method of Reznick & Packer et al. [10] Plasma samples were analysed for blood glucose by routine GOD-POD method & Glycosylated Hemoglobin in hemolysate was determined by particle enhanced immunoturbidimetric method using commercial kits supplied by DiaSys diagnostic systems. First morning samples of urine from the same patients were also collected in sterile plastic container containing 6 N HCL as preservatives for analysis of microalbumin by latex turbidimetry method using commercial kit by Euro diagnostic systems.

Student's t test was employed for statistical analysis of data to compare between study & control group & the data was expressed as mean ± SD. Pearson's correlation coefficients were used to observe correlation between two parameters.

RESULTS: The biochemical parameters of control & study group are given in table no. 1. Increase in fasting blood glucose is observed when compared to normal healthy group shows statistical significance. Observed rise in HbA1C in DM compared to control group remain statistically significant. Protein carbonyl content in serum of diabetic patients shows highly significance increase as compared with control group, when measured in terms of total protein i.e. nmol per mg of proteins & in terms of actual serum content i.e. mmol/l. Significant increases in urine microalbumin levels were found in diabetic patients when compared with healthy subjects.

Table No.1 – Biochemical parameters in study groups

Demographic Criteria	Control group (n= 50)	Diabetic patients (n= 50)
Mean age in yrs	54.62 ± 8.27	59.37 ± 9.70
Blood sugar fasting mg/dl	92.03 ± 8.8	197.66 ± 73.96 **
HbA1C %	5.37 ± 0.57	7.97 ± 1.07 **
Total protein mg/dl	6.82 ± 0.62	6.50 ± 0.694^{NS}
Protein carbonyl mmol/L	0.037 ± 0.012	0.091 ± 0.13**
Protein carbonyl n mol per mg of protein	0.380 ± 0.17	1.519 ± 0.46**
Urine Microalbumin mg/L	18.91 ± 12.10	41.60 ± 38.42*

a) * $p < 0.05$ - significant compared to control
b) ** $p < 0.001$- highly significant compared to control
c) NS – non significant

Statistically significant mild positive correlation was observed between protein carbonyl & urine microalbumin whereas correlation between protein carbonyl & GHb and GHb & microalbumin have failed to show any statistical correlation.

DISCUSSION & CONCUSION:

DM is chronic, systemic metabolic disorder defined by Hyperglycemia & characterized by alteration in metabolism of carbohydrates, protein & lipids. Oxidative stress in a type 2 DM is the outcome of either increase in the rate of free radical production or impairment in the antioxidant mechanisms. [11]

Proteins are vulnerable to oxidative stress; oxidation of proteins can lead to a whole array of amino acid modifications. Oxidative damage causes several specific modifications in protein, since proteins have unique biological functions, functional consequences result from their modification like long-lived proteins gets accumulated, suffer damage by glycation and oxidation, looses protein solubility, and impairs function. [12]

Protein carbonyl is generated by oxidative modifications of proteins either by α-amidation pathway or by oxidation of glutamyl side chains, this leads to formation of a peptide in which the N-terminal amino acid is blocked by a α-ketoacyl derivative. However, direct oxidation of lysine, arginine, proline and threonine residues may also yield carbonyl derivatives. [13] Besides this, protein carbonyl may also be formed by reactions with aldehydes like Malondialdehyde (produced during lipid peroxidation) or with reactive carbonyl derivatives generated due to oxidation of reducing sugars or reaction of oxidized product with lysine residues of proteins. Accumulation of plasma protein carbonyls, termed as "carbonyl stress," causes renal damage. Carbonyl stress has also been implicated in the development of diabetic complications like

nephropathy. [14] Our results suggest increase in protein carbonyl level in type 2 DM as compared with control congregates with findings of Agnieszka et al. [15]

Diabetic nephropathy is a leading cause of end-stage renal failure, which could account for disabilities and high mortality rates in patients with diabetes. Enhanced oxidative stress in hyperglycemia may modify endothelial function by variety of mechanism. [16] This endothelial dysfunction could contribute to the pathogenesis of microalbuminuria either directly by causing increase in glomerular pressure & the synthesis of a leaky glomerular basement membrane or indirectly by influencing glomerular mesangial & epithelial cell function in a paracrine fashion. [17] Importantly the molecular pathways by which endothelial dysfunction causes microalbuminuria has yet to be worked out. So, high amount of carbonyl production resulting from oxidative stress may cause complication of diabetes including nephropathy. Carbonyl stress due to ROS may contributes to diabetic nephropathy was reported by Vicentini et al. [18] Our results also supports this assumption as there is mild but positive correlation observed between protein carbonyl & microalbuminuria.

Glycated hemoglobin is regarded as an intermediate index of diabetic control, showing rapid response to the short-term improvement of blood sugar. As reported in earlier studies [19, 20, and 21] we also found increased GHb concentration as compared to control. In poorly controlled diabetes mellitus, glucose oxidation through the pentose phosphate pathway leads to the excessive formation of NADPH, which in turn can promote lipid peroxidation in presence of cytochrome P-450 system. Alternatively, inactivation or inhibition of antioxidant enzymes by glycosylation in poorly controlled diabetes mellitus may give rise to increased lipid peroxidation. (22) Dalle-Donne et al (6) have reported that protein carbonyl groups are introduced in proteins by secondary reaction of the nucleophilic side chains of cysteine, and lysine residues and aldehydes produced during lipid peroxidation. Microalbuminuria in type 2 diabetic patients might be promoted by an insufficient counter-regulation of the antioxidant system in the event of increased glyco-oxidation/glycation of proteins which is induced due to carbonyl stress. (23)

Thus, monitoring of changes in protein oxidation has shown practical application in a type 2 diabetes mellitus & its complications. Further research on large sample is needed to find out protein carbonyl levels in diabetic patients with & without microalbuminuria and glycemic control criteria for further categorization & assessment of risk in development of nephropathy.

REFERENCES :

1. Zimmet, P.Z., McCarty, D.J., and de Courten, M.P. The global epidemiology of non-insulin-dependent diabetes mellitus and the metabolic syndrome. Journal of Diabetes and its Complications.1997; 11: 60-68.

2. Maritim AC, Sanders RA, Watkins JB. Diabetes, oxidative stress, and antioxidants: A Review. J Biochem Mol Toxicol. 2003; 17: 24-38.

3. Brownlee, M. Biochemistry and molecular cell biology of diabetic complications.
Nature. 2001; 414: 813–20.

4. David S Small, Jeff S Coombes, Nigel Bennett, David W Jhonson, Glenda C Globe. Oxidative stress, anti-oxidant therapies and chronic kidney disease: Review Article. Nephrology; NEP-2011-0573.R1
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5. Requena, JR., Levine, RL. and Stadtman, ER.Recent advances in the analysis of
oxidized proteins. Amino Acids. 2003; 25: 221–6.

6. Dalle-Donnea, Ranieri Rossib, Daniela Giustarinib , Aldo Milzania, Roberto Colombo Isabella
Review - Protein carbonyl groups as biomarkers of oxidative stress. Clinica Chimica Acta 2003; 329: 23–38.

7.Ramakrishna V, Jailkhani R: **Evaluation of oxidative stress in insulin dependent diabetes mellitus (IDDM) patients.** *Diagnostic Pathology* 2007; **2**(22)**:** 1746-1596.

8. Sen S.Kar M, Roy A,Chakraborty A S. Effect of nonenzymatic glycation on functional & structural properties of hemoglobin. Biophysic chemisty 2005; 113: 289-98.

9. Jakus V. The role of free radicals, oxidative stress and antioxidant systems in diabetic vascular disease. BRATISL LEK LISTY 2000; 101 (10): 541- 551

10. Reznick AZ, Packer L.et al: Oxidative damage to proteins: Spectrophotometric method for carbonyl assay. Methods Enzymol 1994; 233: 357- 363.

11. Maiese K, Morhan SD, Chong Z Z: Oxidative stress biology and cell injury during type 1 and type 2 diabetes mellitus. *Curr Neurovasc Res* 2007; 4(1)**:**63-71.

12. Hawkins CL, Morgan PE, Davies MJ. Quantification of protein modification by oxidants. Free Radic Biol Med. 2009; 46(8):965-88.

13. Levine R.L., Garland D., Oliver C.N., Amici A, Climent I, Lenz A.G. et al. Determination of carbonyl content in oxidatively modified proteins. Method Enzymol ; 1990: 186: 464-478.

14. Origin Dubourg L, de Precigout V, Aparicio M, Clerk M: Evaluation and significance of "carbonyl stress" in long-term uremic complications. Kidney Int.1999; 55:389–399.

15. Agnieszka Piwower , Maria Knapik−Kordecka, Maria Warwas: Connection Between Ischemia−Modified Albumin Levels and Markers of Diabetic Nephropathy and Oxidative
Protein Damage in Type 2 Diabetic Patients: Adv Clin Exp Med 2009; 18(4):353–360.

16.U Çakatay.Protein oxidation parameters in type 2 diabetic patients with good and poor glycaemic control. Diabetes & Metabolism. 2005; 31(6) **-** 551-557.

17. Ryuichi Kikkawa, MD, Daisuke Koya, MD, and Masakazu Haneda, MD: Progression of Diabetic Nephropathy. American Journal of Kidney Diseases*,* 2003; 41(3): S19-S21.

18. Vicentini, Juliana MS; Valentini, Juliana MS; Grotto, Denise MS; Paniz, Clóvis MS; Roehrs, Miguel MS et al. Association among Microalbuminuria and Oxidative Stress Biomarkers in Patients with Type 2 Diabetes. Journal of Investigative Medicine. 2011, 59 (4) : 649-654.

19. Rohlfing CL, Wiedmeyer HM, Little RR, England JD, Tennill A, Goldstein DE. Defining the relationship between plasma glucose and HbA(1c): analysis of glucose profiles and HbA(1c) in the Diabetes Control and Complications Trial. Diabetes Care. 2002. 25(2), 275-8.

20. Vivian Fonseca, Silvio E. Inzucchi, and Ele Ferrannini. Redefining the Diagnosis of Diabetes Using Glycated Hemoglobin. Diabetes Care.2009; 32 (7), 1344-1345.

21. Padala Ravi Kumar, Anil Bhansali, Muthuswamy Ravikiran, Shobhit Bhansali,Pinaki Dutta, J. S. Thakur. et al. Utility of Glycated Hemoglobin in Diagnosing Type 2 Diabetes Mellitus: A Community-Based Study. ENDOCRINE CARE; 2010: 95 (6), 2832.

22. Sundaram RK, Bhaskar A, Vijayalingam S, Viswanathan M, Mohan R, Shanmugasundaram KR. Antioxidant status and lipid peroxidation in type II diabetes mellitus with and without complications. Clin Sci (Lond), 1996; 90, 255-60.

23. Kanti Bhooshan Pandey and Syed Ibrahim Rizvi : Age-dependent oxidative stress biomarkers in type 2 diabetic patients. Journal of human diseases, 2011; 1: 1-9.

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