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Association of Ischemia Modified Albumin in Terms of Hypoxic Risk with Carbonylated Protein, Glycosylated Hemoglobin and Plasma Insulin in Type 2 Diabetes Mellitus

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Abstract

Background: Diabetes mellitus is a metabolic disorder resulting in hyperglycemia. This occurs due to pancreatic β islet cell dysfunction characterized by inadequate insulin secretion, or it may occur due to insulin resistance. This progressive metabolic disorder leads to vascular complications. Oxidatively modified protein molecules vary over a wide range by glycosylation, disulphide formation or the content of carbonyl groups and are crucial for assessing the clinical relevance in various disease conditions like liver diseases and nephropathy. Protein modification indicators such as glycosylation, disulphide formation and the protein carbonyl formation. The present study was taken up to determine the Oxidative stress status in terms of Ischemia Modified Albumin (IMA) and Oxidation of proteins by Protein carbonyls that can predict the risk of protein damage in type II diabetes.

Materials and Methods: Sixty healthy individuals and equal number of patients with type 2 diabetes attending to R. L. Jalapa Hospital and Research Centre were recruited to the study. Protein carbonyls were estimated according to the method described by Levine et al. and IMA by albumin cobalt binding assay.

Results: Protein Carbonyl and IMA were significantly increased in type 2 diabetes patients (1.68±0.47 nmol/ml), (0.299±0.128) when compared to controls (0.70±0.34 nmol/ml), (0.071±0.067) with $p < 0.001$, CI 99.5. HbA_1C levels were significantly increased in type 2 diabetes (70.04±20.8 mmol/mol)

compared to controls (37.40±6.7 mmol/mol) with $p < 0.001$, CI 99.5. However, no statistical significant difference observed with respect to plasma insulin levels.

Conclusion: The present study showed that the hypoxic risk raises oxidative stress that markedly influences protein oxidation as protein carbonyls in type 2 diabetes. The prevailing condition might be the cause that significantly contributing to associated complications that lead to morbidity and, adversely affects the life quality and life expectancy in diabetes patients.

Key Words: Ischemia Modified Albumin, Oxidative stress, Protein Carbonyls, Type 2 Diabetes.

1. Introduction

Diabetes is a metabolic disease characterized by hyperglycemia. The implications of hyperglycemia are cellular damage, increased extra cellular matrix production and vascular dysfunction. The pathogenesis of diabetes occurred by the enhanced generation of reactive free radicals [1]. Free radicals are very reactive chemical species that can cause oxidative injury to the biomolecules like lipids, carbohydrates, proteins and nucleic acids. Under physiological conditions, there is a homeostasis between the generation of reactive oxidants /oxygen free radicals and antioxidant defense systems that neutralize free radical toxicity as a protective mechanism in organisms [2, 3]. Impairment in the oxidant/antioxidant equilibrium, particularly with enhancement of the former, contributes to the establishment of the oxidative stress condition is the most common basis of molecular, cellular and tissue damage mechanisms in a wide spectrum of human diseases [4, 5]. This underlying mechanism involving the oxidative stress in diabetes includes toxic effect by advanced glycation end products –receptor for advanced glycation end products interaction, non-enzymatic glycosylation with impairment in the tissue content and activity of antioxidant defense systems. Increased levels of oxidative damage to lipids have been detected in serum sample of diabetic patients and their presence correlates with the development of complications [6-15].

A variety of natural antioxidants exists to scavenge oxygen free radicals and prevent oxidative damage to biological membranes. One group of these antioxidants is represented by intracellular enzymes which include superoxide dismutase, glutathione peroxidase and catalase. In addition, numerous small nutrient molecules that are found in the body also present antioxidant capacity [16-18]. Several studies evaluated the status of free radical- induced lipid peroxidation and antioxidants in diabetic patients. From these, many studies assessed that antioxidants could act cooperatively in vivo, in a process that involves antioxidant regeneration so as to provide greater protection to the organism against radical damage than could be afforded by any single antioxidant acting alone. Nevertheless, few controversial reports have been reported concerning the antioxidant status in diabetic patients[19-21].

Protein oxidation, in contrast to lipid peroxidation does not have the features of chain reaction events. The plasma proteins destruction by peroxidation generally requires long periods. Therefore, the evaluation of protein oxidation in plasma is a valuable marker for estimating the intensity of oxidative stress induced by free radicals. Oxidatively modified protein molecules vary over a wide range by glycosylation, disulphide formation or the content of carbonyl groups and are crucial for assessing the clinical relevance in various disease conditions like liver diseases and nephropathy by virtue of altered functions or particularly ligand binding properties[22]. Among the few possible indicators, protein modification include glycosylation, disulphide formation, carbonylated groups as shown in the figure 1 and thus the information regarding the protein oxidation in various pathological conditions is limited and preparing observational reports on altered homeostasis between free radicals and antioxidants and protein damage by oxidation in type 2 diabetic patients is essential. Therefore, the present study was designed aiming to evaluate the new hypoxic indicator in terms of Ischemic Modified Albumin (IMA) and protein carbonyls for oxidation of proteins in carbonyl stress in type 2 diabetes.

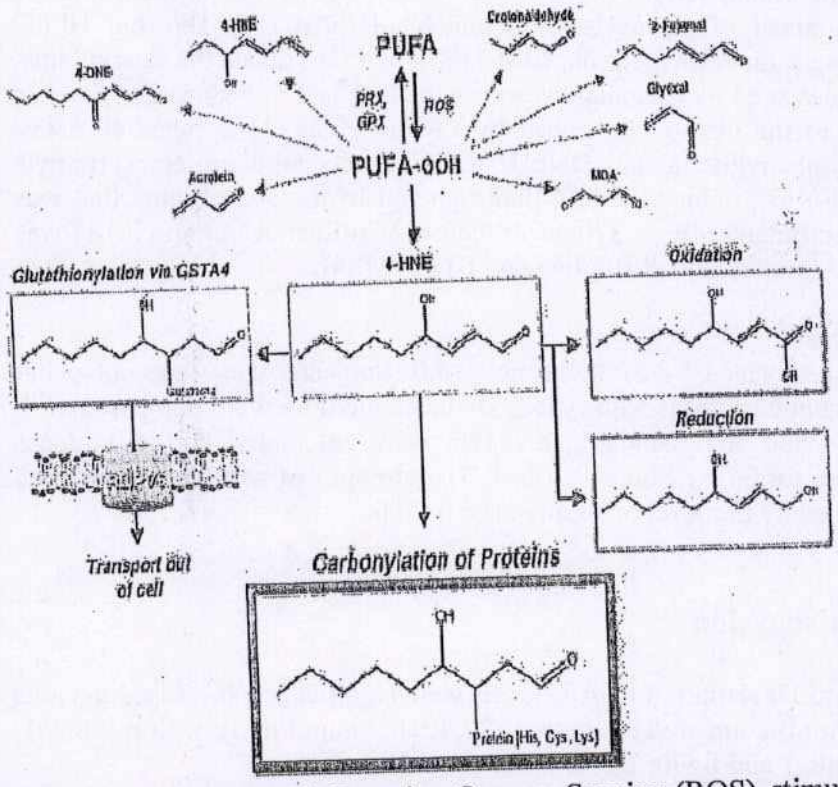

Figure 1: Mechanisms by which Reactive Oxygen Species (ROS) stimulate lipid peroxidation-induced protein carbonylation. Protein carbonylation can be induced by the oxidative modification of polyunsaturated fatty acids (PUFA), which then undergo lipid peroxidation reactions, generating products such as α, β- unsaturated aldehyde 4-hydroxynonenal (HNE). These molecules act as electrophiles in the covalently modification of proteins via non-enzymatic addition reactions.

Source: SarawutKumphune (2012). Oxidatively Modified Biomolecules: An Early Biomarker for Acute Coronary Artery Disease, Oxidative Stress and Diseases, Dr. VolodymyrLushchak (Ed.), ISBN: 978-953-51-0552-7. InTech, Available from: http://www.intechopen.com/books/oxidative-stress-and-diseases/oxidative modified biomolecules- an early biomarker for acute coronary artery disease

2. Material and Methods

In an observational study, sixty patients with type 2 diabetes mellitus and equal number of healthy individuals as volunteers visited to R. L Jalapa Hospital and Research Center, Kolar, India were enrolled in the study after obtaining patient informed consent form and institutional ethics committee clearance. The exclusion criteria were similar for both groups. Alcoholics, smokers, hypertension, diarrhea/vomiting/diuretics and renal disorders were excluded in the study.

2.1 Procedure

Three ml of overnight fasting venous blood was collected into EDTA vacutainer tubes, aliquated for measurement of glycosylated hemoglobin (HbA_1C) by Bio-Rad HPLC method. The remaining sample was centrifuged at 3500 g at 4^0C to obtain the clear plasma. Plasma insulin was measured by Chemiluminescence method, and protein carbonyls were estimated according to the method described by Levine et al[23] a sensitive assay contains 2, 4-dinitrophenylhydrazine (DNPH), which reacts with protein carbonyls forming a Schiff base to produce the 2, 4-dinitrophenyl hydrazone product that was measured spectrophotometrically at 370nm. Ischemia Modified Albumin (IMA) was estimated by using Albumin Cobalt Binding (ACB) assay[24].

2.2 Statistical Analysis

Statistical analysis performed by SPSS version 16.0. Subjects who were not under treatment with exogenous insulin with type 2 Diabetes mellitus were compared with healthy controls. Means and Standard Deviation were calculated and differences between means were tested by Student's t-test. The strength of association between variables was assessed by the level of significance $p< 0.05$.

3. Results and Discussion

3.1 Results

The Mean ± Standard Deviation of HbA_1C levels were significantly increased in cases (70.04±20.8 mmol/mol) compared to controls (37.40±6.7 mmol/mol), with $p < 0.001$, (as presented in Table 1 and figure 2).

Table 1: Level of IMA, Protein Carbonyl, HbA1c, insulin in the considered groups.

Groups	MEAN AND STANDARD DEVIATION			
	IMA (OD) Mean and SD	Protein Carbonyl (umol/ml) Mean and SD	Insulin (mcu/ml) Mean and SD	HbA1C (mmol/mol) Mean and SD
Controls	0.07±0.067	0.70±0.34	9.58±3.03	37.40±6.7
Type II Diabetes	0.30±0.128	1.68±0.47	10.89±5.37	70.04±20.8
p- Value	< .001**	< .001**	< .007^{NS}	< .001**

p Value < .005 = Statistically significant, .001** = highly significant, NS= Non significant

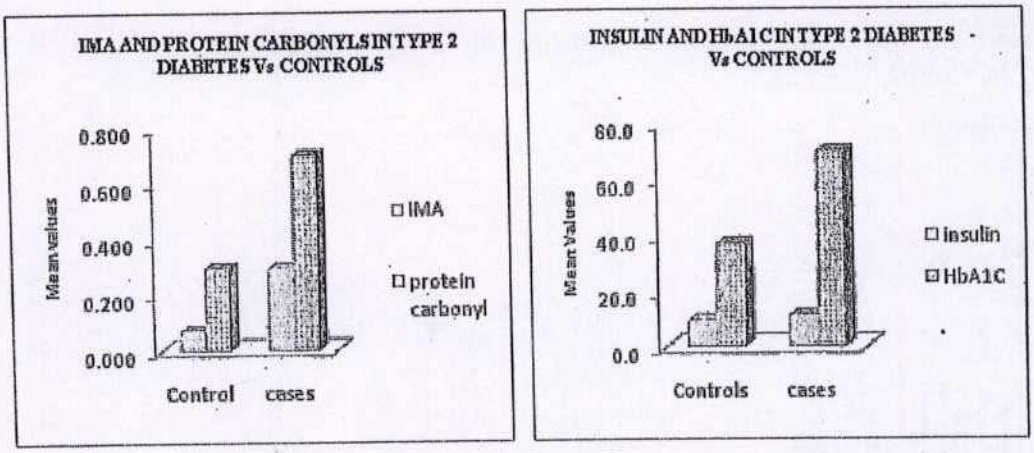

Figure 2: The Mean variations of IMA, protein carbonyls, HbA1c and Insulin in type 2 diabetes when compared with controls.

Protein Carbonyls and IMA were significantly increased in Type 2 Diabetes patients (1.68±0.47 nmol/ml), (0.30±0.13) when compared to controls (0.70±0.34 nmol/ml), (0.07±0.06) with p < 0 .001.

A series of variations with protein damage through protein carbonyls and IMA in type 2 Diabetes Vs. Control is shown in figure 3&4. A significant difference was observed between protein carbonyls, IMA and HbA_1C in controls with type 2 Diabetics. However, no statistical significant difference with respect to insulin levels is observed.

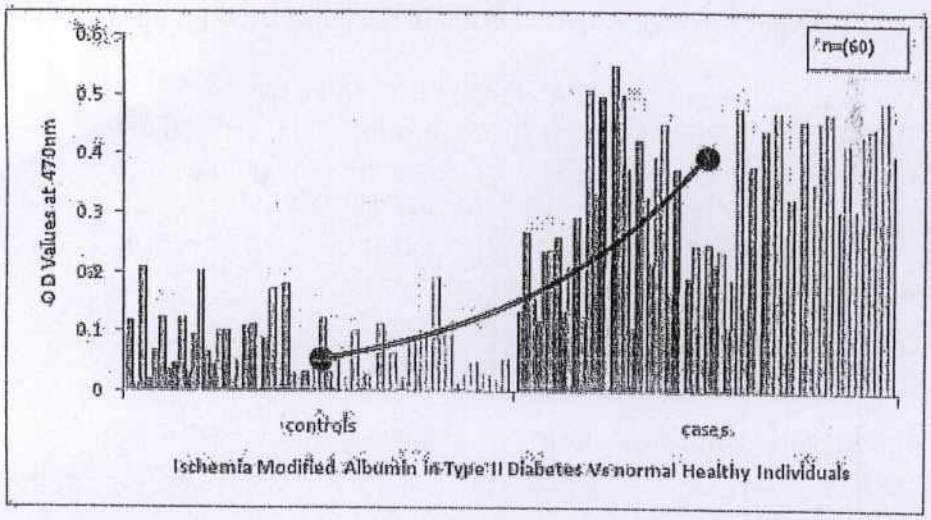

Figure 3: The series of variations with protein damage through protein carbonyls in type 2 diabetes Vs. Control

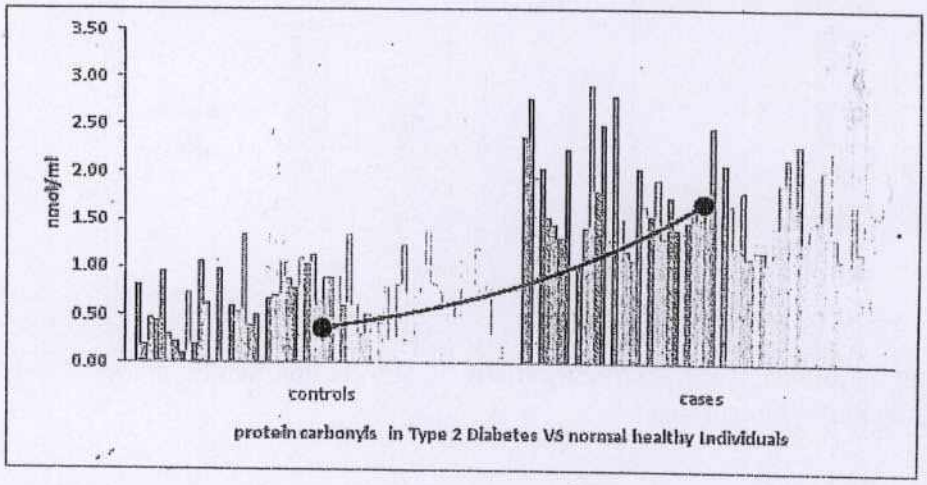

Figure 4: The series of variations with generation of hypoxic risk in Hyperglycemia condition via IMA in type 2 diabetes when compared to the Controls

3.2 Discussion

Diabetes mellitus is a chronic, systemic, metabolic disease defined by hyperglycemia and associated with metabolic derangements of carbohydrates, proteins and lipids. Hyperglycemia, through multiple mechanisms contributes to the onset and maintenance of oxidative stress, the latter represented by increased generation of free radicals and/or reduced participation of antioxidants (either enzymatic or non-enzymatic systems). Free radicals in turn are highly reactive and unstable due to their physico-chemical properties and therefore, their accurate measurement was found to

be difficult in vivo as well as in biological samples such as serum/plasma and urine. In recent years, reports available on the free radicals- induced oxidative stress have pointed to its implication in the pathology of type 1 Diabetes mellitus[25-27].

The present study highlights the relation between oxidative stress and the level of protein oxidation as protein carbonyls in type 2 diabetes patients. Prolonged exposure to hyperglycemia also increases by the production of reactive oxygen species through auto oxidation of glucose[28-29] and consequent non-enzymatic posttranslational modification of proteins resulting from chemical reaction between glucose and primary amino groups of proteins – glycation[30]. During acute ischemic conditions, the metal binding capacity of albumin at the amino terminal position of albumin for transition metals such as copper, nickel, and cobalt is reduced due to amino terminal dityrosine modification generating a metabolic variant of the protein commonly known as ischemia-modified albumin (IMA). Although the precise mechanism for IMA generation is yet unknown, in vivo generation of this marker might be interpreted as an efficient endogenous mechanism in response to ischemia.

Increased IMA levels have been reported in a variety of clinical conditions which have an ischemic element in their pathophysiology[24, 31]. Significantly increased IMA levels have been reported in diabetic nephropathy[32, 33]. The main factor involved in modifying metal binding domains of albumin molecule is the generation of reactive oxygen species due to ischemia- reperfusion injury as seen in diabetic nephropathy[34]. Plasma IMA levels are reported to correlate with parameters of oxidative stress like Advanced Oxidation Protein Products [AOPP] and thiol groups [35]. A significant positive correlation coefficient was observed between Protein carbonyls and plasma IMA levels ($r = 0.37$, $p < 0.001$) in the present study. In an earlier study, non-significant increase in IMA levels was observed in patients with type 2 diabetes not presenting micro or macro angiopathic complications[36]. Also, the same study also supported the involvement of oxidative stress and ischemic-hypoxia in pathogenesis of diabetic complications.

In the current study, our findings clearly show the relationship between increased risk of oxidative stress measured in terms of marker IMA and also the extent of protein oxidation by protein carbonyl occurred by overproduction of ROS and Reactive nitrogen species [RNS] as free radicals in type 2 Diabetics. To our knowledge, reporting the association between hyperglycemia, IMA and protein carbonyl content levels in type 2 Diabetes is the important characteristic that presents a positive correlation with HbA1c in diabetics.

4. Conclusion

The inference of the present study is that although the availability of conventional biomarkers is essential for detection of type 2 diabetes but also several studies reported that conventional biomarkers may not be accurate enough to detect the early phases of cellular injuries like ischemia. Our study revealed the fact that the role of oxidative modified proteins caused by oxidative stress through various mechanisms can be detected in terms of biomarkers like protein carbonyls as stable protein damage marker along with the IMA as an indicator of risk of ischemic conditions. Further

studies are necessary to authenticate the screening of these parameters in type 2 diabetes can be treated as beneficial in early understanding of long term type2 diabetic complications. Since ischemia, hypoxia, hyperglycemia and intensified oxidative stress are observed in diabetic patients; therefore, an association of the relevant respective parameters presents the situation and suggests the progression to complication in type2 diabetes mellitus.

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Competing interests

The authors declare no conflict of interest exists in the present study

Authors' Contributions

Dr C Dayanand: designed the study, manuscript finalization.
Pradeep Kumar vegi: performed the statistical analysis, wrote the first draft of the manuscript.
Dr V Lakshmaiah: Clinical interpretation and sampling.
Dr A V M Kutty: Critical Review

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