Original Research Article

A study on ischemia modified albumin, carbonylated protein and its association with glycated hemoglobin in type II diabetes mellitus

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Abstract

Aim: Diabetes mellitus is a metabolic disorder resulting to hyperglycemia. This occurs due to β islet cell dysfunction of pancreas 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 and are crucial in assessing the clinical relevance in various disease conditions. Protein modification indicators include glycosylation, disulphide formation and the protein carbonyl formation etc. The present study was taken up to determine the Oxidative stress in terms of Ischemia Modified Albumin (IMA) and Oxidation of proteins by Protein carbonyls which can predicts the risk of protein damage in type II diabetes. **Materials and Methods:** Sixty healthy individuals and equal number of patients with Type II diabetes attending to R. L. Jalapa hospital and Research Centre were recruited into the study. Protein carbonyls estimated according to Levine *et al.* method and IMA by Albumin cobalt binding assay. **Results:** Protein Carbonyl and IMA were significantly increased in Type II 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. HbA1C levels were significantly increased in type II 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 risk of rising Oxidative stress hints to the protein oxidation in type II diabetes contributing significantly to associated complications that leads to morbidity and adversely affects the quality and length of life.

Key Word: Oxidative stress, Ischemia Modified Albumin, protein Carbonyls, Type II Diabetes.

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INTRODUCTION

Diabetes is associated with metabolic alterations and characterized by hyperglycemia. The squeal of hyperglycemia such as cellular damage, increased extra cellular matrix production and vascular dysfunction have been implicated in the pathogenesis of vascular disease in diabetes by the rigorous free radicals[1]. Free radicals are very reactive chemical species that can cause oxidative injury to the bio molecules like lipids, carbohydrates, proteins and nucleic acids. Under normal physiological conditions, there is a homeostasis between generations of oxygen free radicals or oxidative stress and antioxidant defense systems that neutralize free radical toxicity as protective mechanism in organisms[2,3]. Impairment in the oxidant/antioxidant equilibrium, particularly enhancement in numerator creates oxidative stress condition, the most common basis of molecular, cellular, and tissue damage mechanisms in a wide spectrum of human diseases [4, 5]. The underlying mechanism involves increased oxidative stress in diabetes that 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.

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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, 7-15]. A variety of natural antioxidants exist to scavenge oxygen free radicals and prevent oxidative damage to biological membranes. One group of these antioxidants is intracellular enzymes which include super oxide dismutase, glutathione peroxidase and Catalase etc. In addition, numerous small nutrient molecules that are present in the body are also having antioxidant capacity [16-18]. There are several studies evaluated the status of free radical induced lipid peroxidation and the antioxidants in diabetic patients. From these, many studies assessed individual antioxidants that act cooperatively in vivo to provide greater protection to the organism against free radical damage that could be provided by any single antioxidant acting alone. Even though, few controversial reports have been reported concerning the antioxidant status in diabetic patients [17, 19-21]. Protein oxidation in contrast to lipid peroxidation does not have the features of chain reactions events. The plasma proteins destruction by peroxidation generally has a quite long period. Therefore, the evaluation of protein oxidation in plasma is a valuable marker of free radical intensity/stress. Oxidatively modified protein molecules vary over a wide range and are crucial in assessing the clinical relevancein various disease conditions by virtue of altered functions or particularly ligand binding properties. From the few possible indicators for protein modification include glycosylation, disulphide formation, carbonylated groups. Since, the information regarding the protein oxidation in various other pathogenic conditions is limited and preparing observational reports on altered homeostasis between free radicals and antioxidants and protein damage by oxidation in type II diabetic patients is essential. Therefore, the present study is designed with the aim to evaluate the altered homeostasis in terms of Ischemic Modified Albumin (IMA) and protein carbonyls for oxidation of proteins in carbonyl stress in type II diabetes.

MATERIALS AND METHODS

Sixty patients with type II diabetes mellitus and equal number of normal subjects as volunteers visited to R. L Jalapa Hospital and Research Center, Kolar, India were considered into the study after obtaining patient informed consent form and institutional ethics committee clearance. The exclusion criteria for selection of subjects were similar for both the controls and case groups. Alcoholics, smokers, hypertensive patients suffering from diarrhea/vomiting/diuretics and renal disorders were excluded in the study.

Procedure: Three ml of fasting venous blood was collected in to EDTA vaccutainer, aliquated for measurement of glycosylated hemoglobin (HbA_1C) by Bio-Rad HPLC method. The remaining sample is centrifuged at 3500 g at 4^0C to obtain the clear plasma. Plasma insulin is measured by Chemiluminescence method and protein carbonyls were estimated according to method described by Levine *et al* [16]. Which is highly sensitive assay contains 2, 4-dinitrophenylhydrazine (DNPH), which reacts with protein carbonyls forming a Schiff base to produce the 2,4-dinitrophenyl hydrazone product measured spectrophotometrically at 370nm. Ischemia Modified Albumin (IMA) is estimated by using Albumin Cobalt Binding (ACB) assay.

Statistical Analysis: Statistical analysis was performed by SPSS version 16.0. Subjects with NIDDM /age onset diabetes/adult onset diabetes/type II diabetes were compared with healthy controls. Means and standard deviationwere calculated and differences between means were tested by student's t-test. The strength of association between pairs of variables was assessed by Pearson correlation coefficient. The level of significance was set at $p < 0.05$.

RESULTS

The mean ± Standard deviation of Protein Carbonyls and IMA were significantly increased in Type II 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 cases (70.04±20.8 mmol/mol) compared to controls(37.40±6.7 mmol/mol) with $p < 0.001$, CI 99.5. Which were presented inas given in Table 1 and figure 4. A positive correlation was observed between Protein carbonyls, IMA and HbA_1C in controls with type II diabetics. However, no statistical significant difference with respect to insulin levels is observed.

Table 1: Indicating the Mean and SD of IMA, Protein Carbonyl,HbA1c, insulin, parameters in type II diabetes and controls groups

	MEAN AND STANDARD DEVIATION			
Groups	**IMA (OD)**	**Protein Carbonyl (umol/ml)**	**Insulin (mcu/ml)**	**HbA1C (mmol/mol)**
Controls	**0.071±0.067**	**0.70±0.34**	**9.58±3.03**	**70.04±20.8**
Type II Diabetes	**0.299±0.128**	**1.68±0.47**	**10.89±5.37**	**37.40±6.7**
p- Value	**<0.001****	**<0.001****	**<0.007^{NS}**	**<0.001****

P Value <0.005 = Statistically significant, 0.001 = highly significant, NS= Non significant**

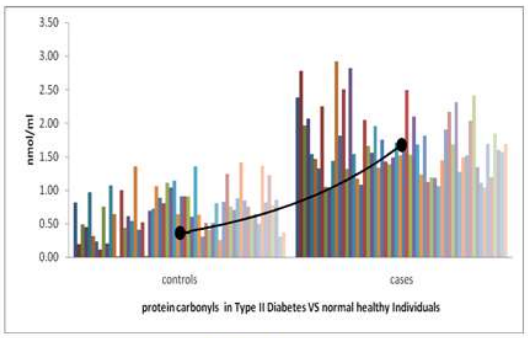

Figure 1:

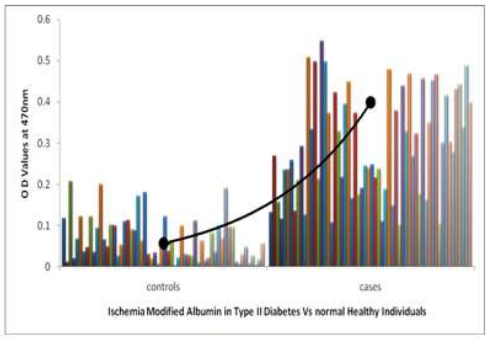

Figure 2:

Figure 1: The series of variations with protein damage through protein carbonyls in type II diabetes Vs. Control; **Figure 2:** The series of variations with generation of hypoxic risk in Hyperglycemia condition via IMA in type II diabetes when compared to the Controls.

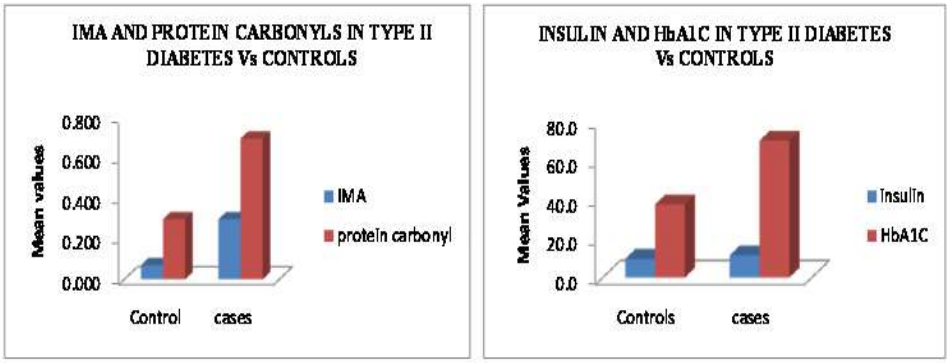

Figure 3: The Mean variations of IMA, Protein Carbonyls,HbA1c and Insulin in type II diabetes when compared with controls.

DISCUSSION

Diabetes mellitus is a chronic, systemic, metabolic disease defined by hyperglycemia and associated with metabolic derangements of carbohydrate, protein and lipid. Hyperglycemia through few mechanisms results oxidative stress represents the increased generation of free radicals in a system. Free radicals are highly reactive and unstable due to their physico- chemical properties and therefore, their accurate measurement found to be difficult in vivo as well as in biological samples such as serum/plasma and other body fluids. In recent years, reports available on theoxidative stress-induced by free radicals that have been implicated in the pathology of IDDM[19, 20-24]. The present study prophesies the relation of oxidative stress and the level of protein oxidation as protein carbonyls in type II diabetes patients. Prolongedexposure to hyperglycemia also increased oxygen free radicals through auto oxidation ofglucose [25-27] and consequent nonenzymaticposttranslational modification of proteins resulting from chemical reaction between glucose and primary amino groups of proteins – glycation. During acute ischemic condition, the metal binding capacity of albumin at the N terminal position of albumin for transition metals such as copper, nickel, and cobalt, is reduced due to amino terminal dithyrosine 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 of response to ischemia. Increased IMA levels have been reported in a variety of clinical conditions which have an ischemic element in their patho physiology [28,29]. Significantly increased IMA levels have been reported in Diabetic Nephropathy[30, 31]. Chief factor involved in modifying the metal binding domains of albumin molecule is the generation of reactive oxygen species due to ischemia reperfusion injury as seen in Diabetes nephropathy[32]. Plasma IMA levels are reported to correlate with parameters of oxidative stress like advanced oxidation protein products [AOPP] and thiol groups [33]. 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 by the same authors, non-significant increase in IMA levels was observed in patients with type 2 diabetes which were lacking of any observation on micro and macro angiopathic complications [34]. But 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 shows the relationship between increased risk of oxidative stress measured in terms of a marker IMA and also extent of protein oxidation by protein carbonyl occurred by overproduction of reactive oxygen species [ROS] and Reactive nitrogen species [RNS] as free radicals in type II diabetics. To ourknowledge, reporting the association between oxidative risk as IMA and protein carbonyl content levels as stable marker of protein damage in type II diabetes is the newfangled characteristic.

CONCLUSION

The inference drawn from the present study is that although, the availability of conventional biomarkers is essential for detection of type II diabetes. Several studies reported that conventional biomarkers might not be 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 ischemia modified albumin as ischemic risk indicator. Further studies are necessary to authenticate the screening of these parameters in type II diabetes can be treated as beneficial in early understanding of long term type II 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 type II diabetes mellitus.

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