PUB: 8/2012

Int J Biol Med Res. 2012; 3(4): 2362-2365

Contents lists available at BioMedSciDirect Publications

International Journal of Biological & Medical Research

Journal homepage: www.biomedscidirect.com

Original Article

Protein carbonyl content as a stable Oxidative stress marker in Type II Diabetes.

Dayanand C. D* Pradeep Kumar Vegi, A. V. M Kutty

1.Associate professor, Department of Biochemistry & Allied Health Sciences
Research Scholar, Department of Biochemistry & Allied Health Sciences.
Dean, Allied Health Sciences, Sri Devaraj Urs Academy of Higher Education and Research, Kolar. Karnataka India PIN-563101

ARTICLE INFO

Keywords:
Protein carbonyl
Insulin
DNPH
Protein marker
Oxidative stress

ABSTRACT

Introduction: Carbonyl groups result from protein oxidation and their level in tissues and plasma is relatively a stable marker of oxidative damage. Proteincarbonyls in cells and tissues have beenobserved during oxidative stress.Increasein oxidative stressduring altered homeostasis of oxidants and antioxidants leads to deleterious effect on cellular components through oxidative damages to proteins, lipids, nucleic acids. Objectives:The present study is to evaluate the levels of protein carbonyls as a stable oxidative stress marker in type 2 diabetes without complications in comparison to the healthy controls. Methods: Three ml ofEDTA bloodwasused for estimations of HbA1c by HPLC, plasma glucose by Glucose oxidase peroxidase, plasma insulin by Chemiluminescence, and plasmaprotein carbonyl by Levine et al using 2,4-dinitrophenylhydrazine.A total of 60 diabetic patients and equal number of healthy individuals as controls were recruited in the study. Results: The Mean ± SD values of total protein carbonyl content was 0.70 ± 0.34nmol/ml, fasting plasma glucose 79.12 ± 11.74mg/dl, HbA1c 5.79±0.66%and plasma insulin 9.58±3.93mcu/ml in control group and the values in the diabetic group respectively were 1.68 ± 0.47nmol/ml, 182.58 ± 102.42 mg/dl, 8.86 ± 2.24%, 10.89 ± 5.37mcu/ml. The levels of protein carbonyl content, fasting blood glucose and Glycated hemoglobin were significantly increased in diabetic group compared to the controls. A positive correlation was observed between Protein carbonyls and glycemic status in controls and type 2 diabetes. Conclusion: Increased oxidative stress, indiabetic group, is doubtlessly induced by hyperglycemia. The present study indicatesthat an increased level of protein carbonyls wasobserved in generalized hyperglycemia. Since protein carbonyl formation was known to later the functional integrity of proteins, it could lead to the development of complicationsin uncontrolled diabetes and the carbonyls could acts as a stable oxidative stress marker in type 2 diabetes.

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficiency in secretion or action of endogenous insulin. Although, the etiology of this disease is not well defined, viral infection, autoimmune disease, and environmental factors have been implicated [1–5]. The most prevalent form of diabetes mellitus is type 2 diabetes mellitus, that onset in adult life due to decreased insulin secretion accelerated by various genetic factors [6, 7]. The underlying metabolic cause of this disease is either impairment in insulin-mediated glucose disposal or defective secretion of insulin by pancreatic β-cells or both. Insulin resistance can also develop from obesity, physical inactivity and genetic susceptibility [8, 9]. Insulin resistance typically accompanied by cardiovascular risk factors such as dyslipidemia, hypertension, and prothrombotic factors [10, 11].Exogenous insulin and other medications can control many aspects of diabetes and its complications.

Reactive oxygen species generally damage all biomolecules due to their catalyticproperties. In vivo, Protein carbonylation is a metal (copper or iron) accelerated modification occurring to protein side chain of many amino acids such as lysine, arginine,

* Corresponding Author : Dayanand C. D*
Associate professor,
Department of Biochemistry & Allied Health Sciences
Sri Devaraj Urs Academy of Higher Education and Research,
Kolar. Karnataka India PIN-563101
Email: cd8905@yahoo.co.in

proline or histidine to produce carbonyls. The hypochlorous acid [HOCL] a major endogenously produced oxidizingspecies released by an enzyme myeloperoxidase, predominantly induce the protein carbonylation [12].The protein carbonyl content (PCC) resulted by oxidation makes the protein resistant to hydrolysis and functional inactivation of proteins in serum or plasma, cellular components, membrane proteins etc. since, protein is major constituents of all forms of the biological system the exact conformation and three dimensional folding are highly connected to the protein functions, the restore of nativity of protein is crucial. Thus, critical evaluation of protein carbonyl content serves asbiomarkers of protein oxidative damage in various conditions like diabetes, ageing, neurodegeneration, smoking etc. [13]

In Diabetes, oxidative stress increased because of persistent hyperglycemia that causes auto oxidation of glucose and the glycation of proteins [14]. The free radicals generation is more apparent and potentiates the pathogenesis in terms of cellular components via destruction, tissue damage and inflammation. The impact of the increased free radicals beyond the homeostasis of oxidants and antioxidants results the oxidative stress that has significant deleterious effect in terms of damage to various cellular and plasma proteins such as antitrypsin, immunoglobulin, stress proteins. The aim of the study was to investigate the status of oxidative stress at generalized increased hyperglycemia in terms of protein carbonyl content (PCC) and predict the importance of protein carbonyl content as a stable oxidative stress marker in type 2 Diabetes mellitus.

Study Area

The oxidative stress is increased in diabetic subjects with hyperglycemia and leads to the formation of protein carbonyls.Glycemic status in terms of plasma glucose, glycosylated hemoglobin and plasma insulin levels were to be assessed in order to find out their relationship between the glycemic status and protein carbonyls in order to predict a possible application by the measurement of protein carbonyls as a stable oxidative stress marker.

2.0 Materials and Methods

Sixty patients with type 2 diabetes mellitus and equal number of normal subjects as volunteersvisited to R. L Jalapa Hospital, Kolar, India were taken into the study after obtaining institutional ethics committee clearance and informed consent from the patients. The exclusion criteria for selection of subjects were similar for both the controls and cases.Alcoholics, smokers, hypertensives, patients suffering from diarrhea/vomiting/diuretics and renal disorders were excluded in the study.

2.1 Procedure

Three ml of fasting venous blood was collected in to EDTA tube, aliquated for measurement of glycosylated hemoglobin (HbA1C) by Bio-Rad HPLC method. The remaining sample is centrifuged at 3500 g at 4°C to obtain the clear plasma which was used for quantification of glucose by glucose oxidase peroxidase method [15]. 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.All values obtained were expressed as Mean ± Standard Error of Mean. Statistical analysis was done to compare the difference in the mean between the controls and the cases, using non-parametric Spearman's rho test. A 'p' value <0.05 was considered as statistically significant. Statistical analysis is performed using SPSS for windows version 11.5.

3. Results:

The Mean ± SD values of total protein carbonyl content was 0.70 ± 0.34nmol/mlas shown in figure 1, fasting plasma glucose 79.12 ± 11.74mg/dl, HbA1c 5.79±0.66%in figure 2 and plasma insulin 9.58±3.93mcu/ml figure 3 in control group and the values in the diabetic group respectively were 1.68 ± 0.47nmol/ml, 182.58 ± 102.42 mg/dl, 8.86 ± 2.24%, 10.89 ± 5.37mcu/ml. The levels of protein carbonyl content, fasting blood glucose and Glycated hemoglobin were significantly increased in diabetic group compared to the controls as shown in table 1& figure 4. A positive correlation was observed between Protein carbonyls and glycemic status in controls and type 2 diabetes. The Fasting blood glucose, HbA1c, protein carbonyl content between control and case group observed (p <001) respectively which is highly statistically significant however insulin found non-significant (p < 0.49).

Table 1 Indicating the mean and ±SD of FBS, HbA1c, Insulin and protein carbonyl content in controls and patients with type II Diabetes Mellitus

	Mean And Standard Deviation			
Groups	FBS (mg/dl)	HbA1c (%)	Insulin (mcu/ml)	Protein carbonyl (nmol/ml)
Controls	79.12±11.74	5.79±0.66	9.58±3.93	0.70±0.34
Type 2 DM	182.58±102.42	8.86±2.24	10.89±5.37	1.68±0.47
P-Value	<0.001**	<0.001**	<0.007^{NS}	<0.001**

P value < 0.005 = statistically significant, 0.001 = highly significant, NS= Non significant**

4. Discussion:

Many studies have demonstrated that the presence of increased oxidative stress in a system where the rate of free radical production is increased and the antioxidant mechanisms are impaired. The oxidative stress-induced free radicals have been implicated in the pathology of IDDM [17-23].Prolonged exposure to hyperglycemia increases oxygen free radicals through auto oxidation of glucose followed by consequent nonenzymatic posttranslational modification between glucose and primary amino groups of proteins in terms of glycation resulting Glycated proteins [24, 25-26].

There are many markers that can be used toindicate the presence of the oxidative stress in Diabetes mellitus. Free radicals are highly reactive and unstable because of their physico- chemical properties and are difficult to measure accurately invivo as well as in biological material such as plasma and other body fluids.

We have evaluated oxidative status by determining the levels of protein carbonyls in patients with type 2 DM and healthy control group. The results of the study indicated that there has an increase in the levels of the protein carbonyls in patients as an index of oxidative stress. It was observed that the levels of protein carbonyl content, fasting blood glucose and Glycated hemoglobin were significantly increased in type 2 diabetes.

In this study, we also focused on plasma glucose, HbA1c and plasmainsulin levels in controls and type 2 diabetic patients. The most significant observation in the present study is the relation between the protein carbonyl content and the glycemia. It can be seen that in controls, protein carbonyl content has a positive correlation with the HbA1c levels and the same was observed in the diabetic group as well where the levels of protein carbonyls, HbA1c were significantly increased than the normal healthy individuals.

Though there was increased in the levels of protein carbonyls and HbA1c in diabetic group, the levels of insulin remained unchanged. Similarly, the levels of HbA1c, plasma glucose in control and diabetic group were found significant according to Odetti and his co-workers [27]. The study of Odetti and his co-workersreports that slight but non- significant result with respect to protein carbonyl content in controls compared with type 2 diabetes. However in the present study the results were consistent and promising as a stable marker for oxidative stress when it is correlated with HbA1c level. The outcome of the study probably might provide usefulness of the measurement of the protein carbonyl as a reliable stable marker of free radical induced oxidative stress and damage.

Summary and Conclusion:

In conclusion, this study demonstrated the prevailing oxidative stress in type 2 diabetes in terms of increased protein carbonyl content which is significantly influenced by generalized glycemic status. Persistent hyperglycemia is a cause for increased production of reactive oxygen species that leads to oxidation of proteins which aremeasured by means of protein carbonyl content. The results of the present study indicate that impaired glycemic status is associated with protein oxidation as a consequence of increased free radical generation.

Since protein carbonyl formation known to alter the functional integrity of proteins, it can lead to development of complications in uncontrolled diabetes. The present study opens up an area of enquiry to study the same extensively into the patients suffering from complications of type 2 diabetes. The studies in this direction are in progress.

Figure 1 Comparison of protein carbonyl content (nmol/ml) levels between control & type 2 diabetes

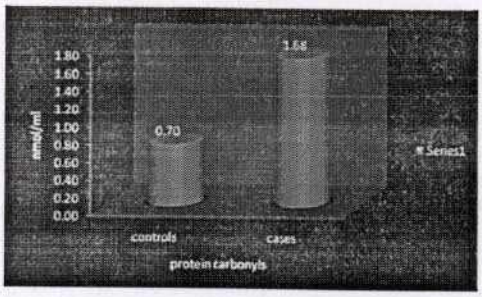


Figure 2 Comparison of Glycated hemoglobin (%) levels between control and type 2 diabetes

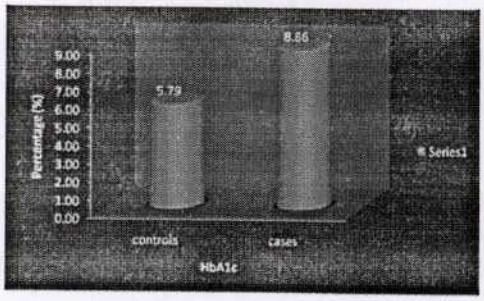


Figure 3 Comparison of plasma insulin (mcu/ml) levels between control and type 2 diabetes

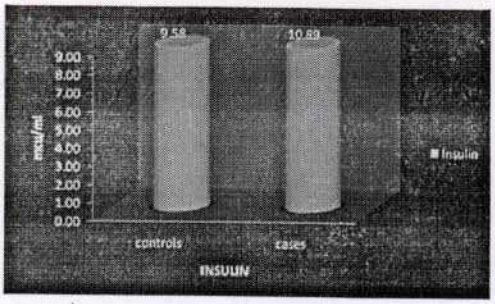
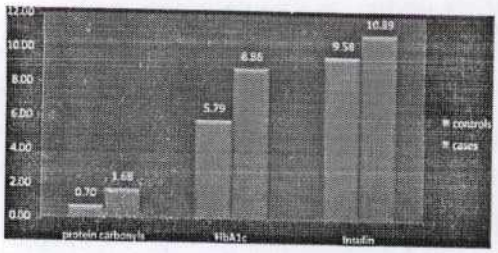


Figure 4 Comparison of Protein carbonyls, HbA1c and plasma insulin levels between control and type 2 diabetes



ACKNOWLEDGEMENT

We would like to thank Dr V.Lakshmaiah Medical Superintendent of RL Jalappa Hospital and Research Centre, and Dr Madhavi Reddy Nutritionist and other authorities of Sri Devaraj Urs Academy of Higher Education and Research for their constant support during carry out of this research.

5.References

[1] Kataoka S, Satoh J, Fujiya H, Toyota T, Suzuki R, Itoh K, Kumagai K. Immunologic aspects of the nonobese diabetic (NOD) mouse. Abnormalities of cellular immunity. Diabetes 1983;32(3):247–253.

[2] Like AA, Rossini AA, Guberski DL, AppelMC,Williams RM. Spontaneous diabetes mellitus: Reversal and prevention in the BB/W rat with antiserum to rat lymphocytes. Science 1979;206(4425):1421–1423.

[3] Paik SG, Blue ML, Fleischer N, Shin S. Diabetes susceptibility of BALB/cBOM mice treated with streptozotocin. Inhibition by lethal irradiation and restoration by splenic lymphocytes. Diabetes 1982;31(9):808–815.

[4] Sandler S, Andersson AK, Barbu A, Hellerstrom C, Holstad M, Karlsson E, Sandberg JO, Strandell E, Saldeen J, Sternesjo J, Tillmar L, Eizirik DL, Flodstrom M, Welsh N. Novel experimental strategies to prevent the development of type 1 diabetes mellitus. Ups J Med Sci 2000;105(2):17–34.

[5] Shewade Y, Tirth S, Bhonde RR. Pancreatic islet-cell viability, functionality and oxidative status remain unaffected at pharmacological concentrations of commonlyused antibiotics in vitro. J Biosci 2001;26(3):349–355.

[6] Pimenta W, Korytkowski M, Mitrakou A, Jenssen T, Yki-Jarvinen H, Evron W, Dailey G, Gerich J. Pancreatic beta-cell dysfunction as the primary genetic lesion in NIDDM: evidence from studies in normal glucose-tolerant individuals with a first-degree NIDDM relative. JAMA. 1995;273:1855–1861.

[7] Humphriss DB, Stewart MW, Berrish TS, Barriocanal LA, Trajano LR, Ashworth LA, Brown MD, Miller M, Avery PJ, Alberti KG, Walker M. Multiple metabolic abnormalities in normal glucose tolerant relatives of NIDDM families. Diabetologia. 1997;40:1185–1190.

[8] Gerick JE. The genetic basis of type 2 diabetes mellitus: impaired insulinsecretion versus impaired insulin sensitivity. Endocr Rev. 1998;19:491–503.

[9] Chisholm DJ, Campbell LV, Kraegen EW. Pathogenesis of the insulinresistance syndrome (syndrome X). ClinExpPharmacol Physiol. 1997;24:782–784.

[10] Hopkins PN, Hunt SC, Wu LL, Williams GH, Williams RR. Hypertension, dyslipidemia, and insulin resistance: links in a chain or spokes on a wheelCurrOpinLipidol. 1996;7:241–253.

[11] Gray RS, Fabsitz RR, Cowan LD, Lee ET, Howard BV, Savage PJ. Risk factor clustering in the insulin resistance syndrome: the Strong Heart Study. Am J Epidemiol. 1998;148:869–878.

[12] Kettle AJ, Winterbourn C.C. Myeloperoxidase- a key regulator of neutrophil oxidant production. Redox Rep. 1997 3:3 15.]

[13] New markers of oxidative damage to macromolecule Eminaolak Institute of Medical Biochemistry, CCS, Belgrade, Serbia JMB 27: 1–16, 2008.

[14] Rajeshwari C, Andallu B Oxidative stress in NIDDM patients: influence of coriander Research Journal of Pharmaceutical, Biological and Chemical Sciences 2011; 2:32.

[15] Barham G, Trinder P, an improved color reagent for the determination of blood glucose by the oxidase system. Analyst 1972;20:470-75.

[16] Levine R. L., Garland D., Oliver C. N., Amici A., Climent I., Lenz A. G., Ahn B. W., Shaltiel S., Stadtman E. R. (1990): Determination of carbonyl content in oxidatively modied proteins. Methods Enzymol. 186, 464|478.

[17] Engler MM, Engler MB, Malloy MJ, Chiu EY, Schloetter MC, Paul SM, Stuehlinger M, Lin KY, Cooke JP, Morrow JD, Ridker PM, Rifai N, Miller E, Witztum JL, Mietus-Snyder M: Antioxidant vitamins C and E improve endothelial function in children with hyperlipidemia: Endothelial Assessment of Risk from Lipids in Youth (EARLY) Trial. Circulation 2003, 108:1059-63.

[18] Ashour M, Salem S, Hassaneen H, EL-Gadban H, Elwan N, Awad A, Basu TK: Antioxidant status and insulin dependent diabetes mellitus (IDDM). J ClinBiochemNutr1999, 26:99-107.

[19] MarraGiampiero, CotroneoPatrizia, Pitocco Dario, Manto Andrea, diLeo Mauro AS, Ruotolo Valeria, Caputo Salvatore, Giardina Bruno, Phirlandalovanni, Santini Stefano A: Early Increase of Oxidative Stress and Reduced Antioxidant Defenses in Patients with Uncomplicated Type 1 Diabetes. Diabetes Care2002, 25:370-375.

[20] Xi LS, Fan HF, ZhiJian G, Ryoji N, Ru ZW, ZhiQiang L, Mei ZZ, et al Advanced oxidation protein products accelerate atherosclerosis through promoting oxidative stress and inflammation. Arterioscler Thromb VascBiol 2006, 26:1156-62.

[21] Ceriello A, Kumar S, Picon L, Esposito K, Giugliano D: Simultaneous control of hyperglycemia and oxidative stress normalizes endothelial function in type I diabetes. Diabetes care 2007, 30:649-54.

[22] Hsu WT, Tsai LY, Lin SK, Hsiao K, Chen BH: Effects of diabetes duration and glycemic control on free radicals in children with type 1 diabetes mellitus. Ann Clin Lab Sci 2006, 36:174-8.

[23] Gleisner A, Martinez L, Pino R, Rojas IG, Martinez A, Asenjo S, Rudolph MI: Oxidative stress markers in plasma and urine of prepubertal patients with type 1 diabetes mellitus. J PediatrEndocrinolMetab2006, 19:995-1000.

[24] Wolff SP, Jiang Z, Hunt J: Protein glycation and oxidative stress in diabetes mellitus and aging. Free Radical Biol Med 1991, 10(5):339-52.

[25] Giampiero M, Patrizia C, Pitocco Dario, Manto Andrea, diLeo Mauro AS, Ruotolo Valeria, Caputo Salvatore, Giardina Bruno, Phirlandalovanni, Santini Stefano A: Early Increase of Oxidative Stress and Reduced Antioxidant Defenses in Patients with Uncomplicated Type 1 Diabetes. Diabetes Care2002, 25:370-75.

[26] Rice-Evans C, Miller NJ: Total antioxidant status in plasma and body fluids. Methods Enzymol234:279-93.

[27] Odetti P, Garibaldi S, Noberasco G, Aragno I, Valentini S, Traverso N, Marinari UM. Levels of carbonyl groups in plasma proteins of type 2 diabetes mellitus subjects. ActaDiabetol. 1999 Dec;36(4):179-83.