DOI: 10.7860/NJLM/2021/45501:2470

Original Article

Biochemistry Section

Correlation of Glycated Haemoglobin with Protein Carbonyl Content as Biomarkers of Oxidative Stress in Type 2 Diabetes Mellitus

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ABSTRACT

Introduction: Diabetes is chronic disease which is characterised by defect in insulin secretion and insulin action leading to hyperglycaemia.

Aim: To detect protein carbonyl as oxidative stress marker in Type 2 Diabetes Mellitus (T2DM).

Materials and Methods: The present cross-sectional study was conducted on 100 subjects (aged 40 to 70 years) out of which 50 were T2DM and 50 were normal individuals. Serum was tested for Fasting Blood Sugar (FBS) and Glycated Haemoglobin (HbA1c) and Protein Carbonyl Content (PCC). Serum PCC was measured by spectrophotometric DNPH (dinitrophenylhydrazone) method of Reznick AZ and Packer L.

Results: The protein carbonyl content, Fasting blood sugar and HbA1c were observed as positively correlated in type 2 diabetes mellitus as the levels of PCC, FBS and HbA1c were raised in T2DM as compared to controls.

Conclusion: Increased HbA1c may be associated with generation of oxygen free radicals in the form of protein carbonyls and decreased antioxidants in T2DM.

Keywords: Dinitrophenylhydrazone, Fasting blood sugar, Hyperglycaemia

INTRODUCTION

The prevalence of diabetes globally in 2019 was assessed to be 9.25%,which may rise to 10.15% by 2030. The prevalence in urban population may be higher i.e 10.75% and in rural it may be 7.2% [1]. American Diabetes Association (ADA) has recommended that all people with diabetes aim for a target haemoglobin HbA1c level below 6.5%. For FBS reading 100 mg/dL is normal. A level from 100 to 125 mg/dL is considered as prediabetes. If your FBS is 126 mg/dL or higher on two separate tests, you have diabetes. Insufficiency in either secretion or action of endogenous insulin leads to a metabolic disorder called as T2DM, characterised by hyperglycaemia. Viral infection, environmental factors, autoimmune disease have been implicated in the etiology of diabetes mellitus but, it is not well defined [2]. Reactive oxygen species are generated due to hyperglycaemia which in turn causes damage cells leading to many complications in diabetes [3]. Oxidative stress leads to molecular and cellular damage which lead the way to circulatory dysfunction and hence development of T2DM [4]. When proteins are exposed to reactive oxygen species, it causes damage to protein structure and hence, it leads to inhibition of enzyme activity, alteration in aggregation and proteolysis, less or more uptake by cells and alteration in immunogenicity [5,6]. So, the protein activity is decreased by glycation and this can indicate the oxidative stress [7].

The most commonly used marker and most common indicator for protein oxidation is PCC. Protein carbonyl accumulation is done in many pathological conditions [6]. Aminoacids such as lysine, arginine, proline or histidine have side chains of protein which are modified by protein carbonylation to produce carbonyls which is a metal (copper or iron). Myeloperoxidase enzyme releases hypochlorous acid (HOCL), which is a major endogenously produced oxidising species induce protein carbonylation [7]. Protein becomes resistant to hydrolysis by oxidation, resulted by protein carbonyl content. In protein oxidative damage in diseases like diabetes and conditions like ageing, neurodegeneration and smoking, the protein carbonyl content serves as biomarkers [8]. Recently, India had more diabetics than any other country in the world according to the International Diabetes Foundation. The aim of the study was to investigate and correlate the status of oxidative stress as generalised increased hyperglycaemia in terms of PCC and anticipate the importance of PCC as a stable oxidative stress marker in T2DM.

MATERIALS AND METHODS

The present study was a hospital based cross-sectional study in which 50 patients with T2DM and equal number of normal subjects as volunteers visited to Muzaffarnagar Medical College and Hospital, Uttar Pradesh. Ethics Committee clearance was obtained from Muzaffarnagar Medical College (MMC/PO/2020/65) and informed consent from the patients. This study was done in the Department of Biochemistry, Muzaffarnagar Medical College and Hospital, Muzaffarnagar, Uttar Pradesh, India, from October 2019 to March 2020. Alcoholics, hypertensives, smokers and patients with renal disorders or on diuretics and patients who were vomiting were in **exclusion criteria** for both controls and cases.

Sample collection and analysis: 10 mL of blood was taken with all aseptic precautions after overnight fasting of controls and of T2DM patients. Sample was distributed into three parts marking them as 1,2 and 3.

Fasting blood samples of patients and control were collected in plain vial and sodium fluoride vial. Clear serum was seperated by centrifugation for 5 min at 2500 rpm.

First part of sample i.e. 5 mL of blood was tested for total proteins by biuret method [9] and Protein carbonyl assay by spectrophotometric DNPH method of Reznick AZ and Packer L [10].

Second part of sample i.e. 2 mL of blood with anticoagulant (EDTA) was used for estimation of glycated haemoglobin.

Third part of sample contains 3 mL of blood, with no anticoagulant after two hours of meals, which was used for estimation of Post Prandial Blood Sugar (PPBS).

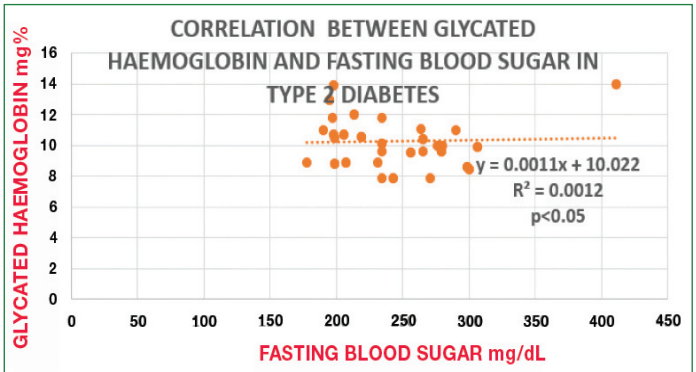
Sample analysis: FBS, PPBS and HbA1c were measured with the help of automated biochemistry analyser (Beckman and coulter Au 480).

STATISTICAL ANALYSIS

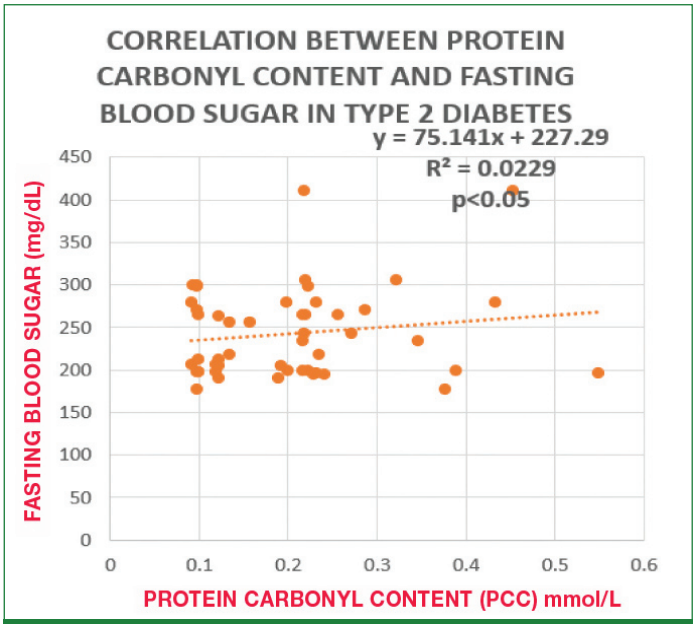
Statistical analysis was done by Graph Pad Quick Cals t-test calculator'. To assess the significance of difference between the groups, Student's t-test was used. Mean±Standard Deviation (SD) was used to present the results. The p-value <0.05 was considered significant.

RESULTS

Protein carbonyls and Glycaemic status showed a positive correlation in type 2 diabetes. Significant increase in levels of protein carbonyl content, HbA1c and fasting blood glucose was seen in diabetic group compared to the controls [Table/Fig-1-4].

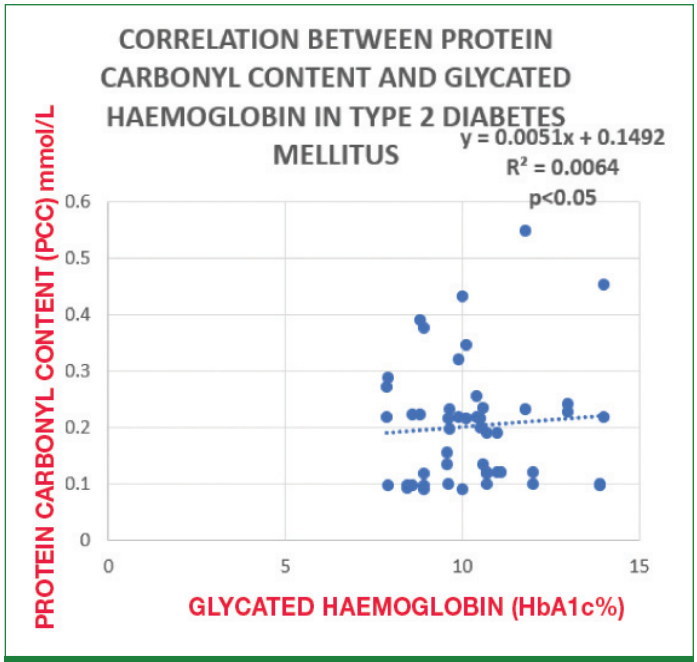


[Table/Fig-1]: Correlation between glycated haemoglobin (HbA1c%) and Fasting Blood Sugar (FBS) in T2DM.
R^2= correlation coefficient of two data sets depicting the trendline between glycated haemoglobin and Fasting Blood Sugar (FBS) and y is the axis showing the trendline for the positive correlation.



[Table/Fig-2]: Correlation between Protein Carbonyl Content (PCC) and Fasting Blood Sugar (FBS) in T2DM.
R^2= correlation coefficient of two data sets depicting the trendline between Protein carbonyl content (PCC) and Fasting blood sugar (FBS) and y is the axis showing the trendline for the positive correlation

Increase in fasting blood glucose was observed as comparison to normal healthy group, which was statistically significant (p<0.0001). Out of 100 subjects, 50 subjects were diabetic and 50 were normal healthy subjects. In diabetic patients, the mean levels of FBS, PCC and HbA1c increased significantly (<0.0001) when compared to normal healthy individuals. Among diabetic patients, HbA1c positively correlated with PCC (y=0.0051x+0.1492 R^2 =0.0064 [Table/Fig-3], FBS positively correlated with PCC (y=75.141x+227.29 R^2=0.0229 [Table/Fig-2] and HbA1c haemoglobin positively correlated with FBS fasting blood sugar (y=0.0011x+10.022 R^2=0.0012 [Table/Fig-3]. R^2 is the correlation



[Table/Fig-3]: Correlation between Protein Carbonyl Content (PCC) and glycated haemoglobin (HbA1c%) in T2DM.
R^2=correlation coefficient of two data sets depicting the trendline . between glycated haemoglobin and Protein carbonyl content and y is the axis showing the trendline for the positive correlation

Parameters	Diabetics (50) Mean±SD	Non-diabetics (50) Mean±SD	p-value
FBS (mg\dL)	156.38±19.13	93.50±8.64	<0.0001
Protein carbonyl content (mmol/L)	0.094±0.14	0.029±0.011	<0.0001
HbA1c (%)	7.56±0.94	4.85±0.47	<0.0001

[Table/Fig-4]: Shows mean and standard deviation of variants in diabetic and non diabetic patients.
Student's t-test used; p<0.001: highly significant; FBS: Fasting blood sugar; HbA1c: glycated haemoglobin

coefficient and y is the axis showing the trendline for the positive correlation. The Mean±SD values of total PCC, fasting plasma glucose and HbA1c for diabetes and for non-diabetes is shown in [Table/Fig-4].

DISCUSSION

T2DM is chronic metabolic disorder characterised by hyperglycaemia and is defined by alteration in metabolism of carbohydrates, protein and lipids. Oxidative stress in a T2DM is either due to increase in the rate of free radical production or damage in the antioxidant mechanisms [11].

Hyperglycaemia when it is high raises oxygen free radicals where glucose is auto oxidated leading to non-enzymatic post-translational modification between protein amino groups and glucose resulting in glycated proteins. In plasma and other body fluids, free radicals are difficult to measure as they are highly reactive and unstable [12]. Many markers can be used to detect the presence of the oxidative stress in DM. Due their physico-chemical properties, free radicals are highly reactive and unstable and are difficult to measure accurately invivo as well as in biological material such as plasma and other body fluids. Oxidative stress causes several notable modifications in protein and since it has specific biological functions, consequences resulted from their modifications are accumulation of proteins, endure damage by glycation and oxidation, loss of protein solubility and function is impaired [13].

Protein carbonyl is generated by modifications of proteins by oxidative stress either by α-amidation pathway or by oxidation of glutamyl side chains, which 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 give rise to carbonyl derivatives [14].

In present study, oxidative status was assessed by determining the levels of protein carbonyls in patients with T2DM 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 PCC, fasting blood glucose and HbA1c were significantly increased in T2DM patients.

In this study, it was also concentrated on plasma glucose and HbA1c levels in controls and T2DM patients. In the present study, the relation between the PCC and the glycaemia was the most significant observation. A positive correlation was seen in controls and Type 2 Diabetics with PCC levels, glycated haemoglobin where the levels were significantly increased than the normal healthy individuals. Odetti P et al., found significant levels of HbA1c and plasma glucose in control and diabetic group [15]. Results were encouraging in the present study as a stable marker for oxidative stress, when it was correlated with HbA1c level. To conclude, the study will be useful to measure PCC as a stable marker of oxidative stress by free radicals damage.

Limitation(s)

A study of a larger population size would have been better to verify the results results of present study.

CONCLUSION(S)

In conclusion, this study demonstrated the correlation between the prevalent oxidative stress in T2DM in terms of increased PCC which is notably influenced by generalised glycaemic status. Reactive oxygen species are increased by persistent hyperglycaemia that leads to oxidation of proteins which are measured by means of PCC. The results of the present study may indicate that impaired glycaemic status is associated with protein oxidation as a consequence of increased free radical generation. Since, protein carbonyl formation is known to change 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 T2DM. The studies in this direction are making headway.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Jul 26, 2020
- Manual Googling: Oct 26, 2020
- iThenticate Software: Jan 29, 2021 (24%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jul 22, 2020**
Date of Peer Review: **Sep 03, 2020**
Date of Acceptance: **Nov 10, 2020**
Date of Publishing: **Apr 01, 2021**