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# NONENZYMATIC GLYCATION OF PLASMA PROTEINS IN SMOKERS

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**Abstract :** Glycation is known to play a key role in complications of many pathophysiological processes. The present study was carried out to assess whether there are abnormalities of nonenzymatic glycation of proteins in smokers. Fourteen current smokers and 10 healthy nonsmokers were enrolled for the present study. Fasting plasma glucose, insulin, fructosamine and total plasma glycated proteins were evaluated. A significant rise in the mean levels of fructosamine and total plasma glycated proteins were found in smokers when compared with controls. Significant difference in insulin values was observed between these two groups. When Pearson's correlation analysis was performed, no significant correlation was found between fasting plasma glucose with either fructosamine or total plasma glycated protein levels. These data suggest an increased glycation of proteins in smokers independent of glucose concentration.

**Key words :** smoking fructosamine
total plasma glycated protein insulin

## INTRODUCTION

Nonenzymatic glycation of both circulating and structural proteins is a process of particular physiopathological relevance for the development and progression of many pathological conditions like diabetes, chronic renal failure and atherosclerosis (1–3). Non-enzymatic glycation is a common posttranslational modification of proteins in which reducing sugars bind covalently to the free amino groups (4).

This non enzymatic modification of proteins alters not only the structure, but also the biological properties of protein. This can lead on to a variety of chemical entities and induce structural changes in enzymes starting from conformational alterations, progressing to thiol oxidation, aggregation, formation of disulphide and other covalent cross-links, and inactivation of enzymes (5, 6).

The estimation of fructosamine provides an accurate index of the mean concentration

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of blood glucose during the preceding one to three weeks, complimenting more traditional measure of glucose control (7). The two classical factors known to incite the glycation of proteins *in vivo* are glucose concentration and half life of the protein (8). But evidences have accumulated indicating the existence of other non-classical factors in non-diabetic pathological conditions (9–12).

We have previously demonstrated an increased fructosamine levels in non-diabetic renal failure, nephrotic syndrome, asthma and chronic obstructive pulmonary disease patients (13–16). We have also reported an elevated level of glycated hemoglobin in nondiabetic asthma, renal failure and hyperthyroid patients (15, 17, 18).

Recent reports have indicated that tobacco smoke is a source of toxic reactive glycation products (19–21). Nilsson et al have reported an increased level of glycated hemoglobin in non-diabetic smokers (22), yet to our knowledge no previous reports exist on the levels of glycated plasma protein levels in non-diabetic smokers. As glycation can cause deleterious effect on biological processes, it was of interest to investigate the levels of glycated plasma protein in smokers.

## METHODS AND MATERIALS

Fourteen men aged between 30 and 45 (mean±SD : 37.7 ± 2.1) years without a history of any disease or abnormal biochemical tests were selected for this study. All this subjects have smoked for more than 10 years (> 10 cigarettes smoked/day). The control group consisting of 10 healthy age (38.9 ± 4.3 years) and sex matched volunteers comprising the staff of our institute were enrolled for the study. Written informed consent was obtained from all the participating subjects.

Blood sample (10 ml) was collected in EDTA bottles by venipuncture. The sample was centrifuged (2500 × g 10 minutes at 4°C). The plasma obtained was used for the estimation of glucose, insulin, fructosamine and total glycated protein. Plasma glucose was estimated by glucose oxidase method in 550 express plus auto analyzer (Ciba Corning Diagnostics, Oberlin, Ohio, Canada) using commercial kits from Accurex (Thane, India). Fasting plasma insulin levels were determined by radioimmunoassay kits from BARC (Mumbai, India). The total plasma glycated protein was estimated by a method based upon measurement of 5 hydroxymethylfurfural (HMF) released during mild acid hydrolysis of glycated protein (23). Plasma fructosamine was determined by p-indonitrotetrozolium violet assay with RAICHEM kit (Hemagen diagnostics, San Diego, CA) adapted to the 550 Express Plus analyzer. Homeostasis model assessment (HOMA-IR) was calculated using the formula: HOMA-IR = fasting insulin (µU/ml) X fasting glucose (mmol/L)/22.5.

### Statistical analysis

All variables are shown as mean ± SD. The data between control and test groups were compared using unpaired 2-tailed Student's t test. Correlation was determined by Pearson's correlation coefficient. The level of significance used was p less than 0.05.

## RESULTS

The levels of fasting plasma glucose,

TABLE I: Levels of fasting glucose, insulin and fructosamine levels in healthy smokers (n=14) and nonsmokers (n=10).

| *Smokers* | *Fasting plasma glucose (mg/dl)* | *Fasting Insulin (μU/ml)* | *HOMA-IR* | *Fructosamine (mmol/l)* | *TPGP[1] (nmol/mg/protein)* |
|---|---|---|---|---|---|
| 1 | 98 | 10.37 | 2.51 | 3.69 | 3.2 |
| 2 | 102 | 20.15 | 5.07 | 5.60 | 4.2 |
| 3 | 98 | 89.92 | 21.74 | 3.54 | 3.2 |
| 4 | 92 | 62.74 | 14.24 | 2.85 | 2.2 |
| 5 | 95 | 52.44 | 12.29 | 2.26 | 3.4 |
| 6 | 97 | 13.24 | 3.17 | 4.34 | 3.4 |
| 7 | 94 | 67.97 | 15.76 | 3.48 | 3.2 |
| 8 | 93 | 27.94 | 6.41 | 1.50 | 0.9 |
| 9 | 94 | 33.19 | 7.7 | 2.49 | 2.7 |
| 10 | 96 | 46.52 | 11.02 | 1.70 | 1.8 |
| 11 | 98 | 11.39 | 2.75 | 3.40 | 3.2 |
| 12 | 93 | 18.30 | 4.33 | 2.80 | 2.9 |
| 13 | 89 | 10.49 | 2.3 | 1.75 | 2.5 |
| 14 | 102 | 16.49 | 4.15 | 1.77 | 2.8 |
| Mean±SD | 96.0±3.6* | 34.4±25.5* | 8.1±6.0* | 2.9±1.2* | 2.8±0.8* |
| *Nonsmokers* | | | | | |
| 1 | 87 | 25.16 | 5.40 | 1.72 | 1.2 |
| 2 | 91 | 12.65 | 2.84 | 1.32 | 1.65 |
| 3 | 89 | 14.31 | 3.14 | 1.54 | 1.58 |
| 4 | 81 | 20.15 | 4.03 | 2.18 | 1.43 |
| 5 | 86 | 12.94 | 2.75 | 2.85 | 1.24 |
| 6 | 95 | 21.65 | 5.07 | 1.89 | 1.58 |
| 7 | 92 | 11.60 | 2.63 | 2.25 | 1.65 |
| 8 | 88 | 21.33 | 4.63 | 1.78 | 1.25 |
| 9 | 87 | 10.08 | 2.16 | 1.93 | 1.25 |
| 10 | 80 | 20.99 | 4.14 | 1.84 | 1.58 |
| Mean±SD | 87.6±4.6 | 17.1±5.3 | 3.7±1.1 | 1.9±0.4 | 1.4±0.2 |

[1]TPGP = Total plasma glycated protein; *P<0.05 compared with nonsmokers.

insulin, fructosamine and total plasma glycated protein of smokers and healthy control group are shown in Table I. The results showed a mild, but statistically significant increase in the fasting plasma glucose levels in smokers when compared to control. The fructosamine levels were significantly higher in the smoker group as compared to controls. Similarly the levels of total plasma glycated protein were significantly increased in the smokers when compared with healthy controls. Significant difference in the level of fasting insulin was obtained between smokers and nonsmokers. In addition, there was also a significant difference in insulin sensitivity between the two groups as measured by HOMA-IR. Univariate analysis showed no significant correlation between plasma fasting glucose with either fructosamine ($r = 0.51$, $P = 0.06$) or total plasma glycated protein ($r = 0.54$, $P = 0.05$). Similarly, no significant correlation was observed between HOMA-IR with either fructosamine ($r = –0.02$, $P = 0.95$) or total

plasma glycated protein ($r = -0.04$, $P = 0.89$).

## DISCUSSION

The term nonenzymatic glycation of proteins refers to a wide variety of spontaneous reactions between reducing sugars and protein-bound amines. This process, described by Maillard (24) in 1912 which was of considerable interest to food chemist first, has now attained special prominence biologically. The Maillard reaction begins with the reaction of the carbonyl groups (aldehyde or ketone) of the reducing sugar to form a reversible Schiff's base with amino groups of biomolecules. Schiff's base can undergo an intramolecular rearrangement to form the Amadori products (25); this can undergo a series of further rearrangements, dehydration and condensation to form irreversible end products which may be fluorescent and yellow brown in color, some can form stable intermolecular and intramolecular cross – links (26, 27). There is an increasing body of evidence indicating that glycation products are major factors in the development of complications entangled with diabetes, chronic renal failure and atherosclerosis (1–3).

Even though, tobacco has been found to be a source of toxic reactive glycation products (21), to our knowledge, no study to date has attempted to clarify whether the levels of plasma glycated proteins are altered in smokers. In our study, the fructosamine levels were found to be higher in smokers when compared to controls. Fructosamine is a ketoamine formed by spontaneous nonenzymatic condensation of glucose and proteins. Its measurement provides a valuable retrospective index of the mean prevailing glucose level during a time interval ranging from 7 to 21 days (7). Glycated albumin is the single major contributor to the fructosamine concentration (7). We have also found an increased glycated plasma protein levels when thiobarbituric acid method was used.

In the present study, even though the level of fasting plasma glucose was elevated in smokers, the levels were within the normal fasting glucose range. The plasma insulin was found to be higher among smokers. Among the smokers insulin sensitivity was found to be compromised as determined by HOMA-IR. The published evidence of an association between smoking and insulin resistance is inconsistent (28–30). Wareham et al have found no causal relationship between smoking and insulin resistance (28). Evidence of a link between cigarette smoking and insulin resistance has also been reported (29, 30). Hyperinsulinemia has been identified as a risk factor for hypertension and obesity as well as lipid abnormalities in adults (31). Prospective studies have also demonstrated that high plasma concentrations of insulin predict the development of coronary heart disease, independent of other risk factors (31). Insulin resistance syndrome may put these participants at risk for developing coronary heart disease.

In the present study when Pearson's correlation analysis was performed, we found no significant relationship between plasma fasting glucose with either fructosamine or with total glycaled plasma protein. Similarly there was no significant relationship between HOMA-IR with either fructosamine or total glycated plasma protein. These data

indicate that there is an aberrant glycation of plasma protein independent of glucose homeostasis in smokers. Increased glycation of hemoglobin and plasma proteins have been observed in few nondiabetic clinical situations (32–34).

The mechanism(s) underlying enhanced glycated protein in smokers is difficult to entangle from the present study. Increased $HbA_{1C}$ levels have been previously reported in nondiabetic smokers (22). A close association between oxidative stress and glycation has been reported previously (35). We have also reported in our previous study that lipid peroxides *per se* can enhance glycation of proteins (36). So it can be hypothesized that the oxidative milieu generally associated with smoking would be a reason for the enhanced glycation of proteins in smokers.

Evidence has also been published demonstrating the *in vitro* ability of nicotine to promote glycation of amyloid P-protein (20). Consonant with this study, Dickerson et al has proposed that nicotine *per se* can increase glycation of bovine serum albumin *in vitro*. Thus it can also be possible that compounds present in smoke can *per se* enhance the glycation of proteins in smokers.

In conclusion, the results from the present study provide evidence of an increased glycated protein levels in smokers. Given the detrimental role of glycation in pathological conditions, strategies to avert glycation of protein in smokers would be of great help in controlling the menace associated with smoking.

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