

## Original Article

# Changes in glycosylated proteins in diabetic and non-diabetic patients with and without cardiovascular complications

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The objective of this study was to find the changes in glycoprotein composition in both diabetic and non-diabetic patients with and without cardiovascular complications. The study was carried out in Ziauddin Medical University Karachi, Pakistan. Eighty-three patients and control subjects were selected. Among them twenty-one were diabetic patients without any clinical evidence of chronic diabetic complications, twenty-one were diabetic patients with cardiovascular complications, twenty were non-diabetic patients with cardiovascular complications and twenty-one apparently normal, age, sex and weight matched control subjects were investigated. All these patients were selected on clinical grounds from National Institute of Cardiovascular Disease, Karachi. Fasting plasma glucose was increased in all diabetic patients and correlated significantly with and without cardiovascular complications. Fasting plasma glucose, glycosylated haemoglobin, glycosylated plasma proteins, serum fructosamine, sialic acid, hexosamine and total serum protein and its fractions were increased in diabetic patients with and without cardiovascular complications. Fasting plasma glucose, glycosylated haemoglobin, glycosylated plasma proteins, serum fructosamine, sialic acid and hexosamine were not different in diabetic patients with cardiovascular complications and diabetic patients without chronic complications as compared with control subjects. In conclusion, fasting plasma glucose, glycosylated haemoglobin, glycosylated plasma proteins, serum fructosamine, sialic acid, hexosamine and total serum proteins and its fractions were increased in diabetic patients with and without complications, but these parameters remained within normal limits in non-diabetic patients with cardiovascular complications.

**Key Words:** fasting plasma glucose, glycosylated haemoglobin, glycosylated plasma proteins, serum fructosamine, sialic acid, hexosamine, total serum protein, non-diabetic patients with cardiovascular complications, diabetic patients with cardiovascular complications, Karachi, Pakistan.

## Introduction

Diabetes mellitus, the most common, serious, chronic endocrine disease is characterized by hyperglycaemia, metabolic abnormalities and by long-term complications involving the eyes, kidneys, nerves and blood vessels.<sup>1</sup> It has become a major health problem world wide, reaching epidemic proportions in many developing countries as well as in minority groups in the developed world.<sup>2</sup> Over 12% of the Pakistani population in the age group of 25 years and above suffers from diabetes mellitus and about 10% from impaired glucose tolerance.<sup>3</sup>

The cardiovascular complications are known to be the major cause of morbidity and mortality in diabetics<sup>4</sup> and the risk factors are in common in diabetic and non-diabetic patients. The number and state of plaques of atherosclerosis increase with age, but the rate of progression of the individual plaques, even in the same patient, is very variable. People with more than one risk factor or a combination of risk factors (e.g. smoking, increasing age, a history of congestive heart failure, ST-segment depression, hypertension, genetic factor and diabetes) have the greater risk of developing cardiovascular diseases. Aging has recently been pointed out to influence the association between risk factors and incidence of atherosclerotic diseases.<sup>5</sup> The atherosclerotic lesion in non-diabetics is histologically not different from that of diabetic patients.

Fatty streaks are found throughout aortas of diabetic and non-diabetic patients, although diabetic patients have more coronary artery streaking.<sup>6</sup> Glucose-dependent factors, such as the formation of advanced glycation end products (AGE) that interact with specific receptors and lead to over-expression of a range of cytokines, may play an important role in diabetic vascular complications including atherosclerosis.<sup>7,8</sup> Intracellular hyperglycaemia is the primary initiating event in the formation of both intracellular and extracellular AGEs.<sup>9</sup> AGEs can arise from intracellular auto-oxidation of glucose to glyoxal, decomposition of the Amadori product to 3-deoxyglucosone, and fragmentation of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate to methylglyoxal.<sup>10</sup>

Production of intracellular AGE precursors damages target cells by three general mechanisms. First, intracellular proteins modified by AGEs have altered function. Second, extracellular matrix components modified by AGE precursors interact abnormally with other matrix

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components and with the receptors for matrix proteins (integrins) on cells. Third, plasma proteins modified by AGE precursors bind to AGE receptors on endothelial cells, mesangial cells and macrophages, including receptor-mediated production of reactive oxygen species.<sup>9</sup> Both glycation and AGE formation can alter protein conformation and may impair function in several ways.<sup>11,12</sup>

The objective of the study was to determine the changes in glycosylated protein composition in diabetic and non-diabetic patients with and without cardiovascular complications.

### Subjects and methods

The study included eighty-three patients and control subjects. Among them twenty one were diabetic patients without any clinical evidence of chronic diabetic complications, twenty-one were diabetic patients with cardiovascular complications, twenty were non-diabetic patients with cardiovascular complications and twenty-one apparently normal, age, sex and weight matched control subjects were investigated. Apparently healthy subjects having no history of diabetes and any other major illness like macrovascular disease, cataract, retinopathy, tuberculosis, rheumatoid arthritis, liver diseases, malignancy were selected matched with the patients for age, sex and weight. All these patients were selected on clinical grounds from National Institute of Cardiovascular Diseases, Karachi. The patients were selected who were over fifty years of age. Sex, weight, duration of diabetes, duration of cardiovascular complications in diabetic and non-diabetic patients, type of diabetes and type of treatments received were recorded. The family and medical history were also recorded. Drugs were stopped 48 hours before sample collection (except insulin). Physical examination, including measurement of blood pressure was done.

Individuals were classified as having diabetes mellitus if any of the following criteria were met (American Diabetes Association, 1997). Fasting serum glucose levels of 7.0 mmol/L or more, random glucose levels of 11.1 mmol/L, current use of medications prescribed to treat diabetes (e.g. insulin or drugs) or a positive response to the question "has a doctor ever told you that you had diabetes i.e. (sugar in the blood)"? The patients below the age of fifty years or those with more than one complication were excluded from the study. Only the diabetic and non-diabetic patients having cardiovascular complications like angina, myocardial infarction and hypertension were investigated. Diabetic patients without any complications were also investigated. Macrovascular disease was considered to be present if there was history of myocardial infarction, angina, stroke, intermittent claudication, vascular surgery or amputation for atherosclerotic disease or one or more absent foot pulses on examination.

Blood glucose was determined by glucose oxidase method. The reagents were obtained from glucose enzymatique PAP 7500 kit of bioMerieux. Glycosylated haemoglobin estimated by kit obtained from Bio Systems Reagents and Instruments, Spain. Serum hexoamine was determined by the method of Cessi and Pillego,<sup>13</sup> total serum protein by Biuret Method of Reinhold,<sup>14</sup> sialic acid

by Natelson method<sup>15</sup> and glycosylated proteins by the method of Ma.<sup>16</sup> Serum protein electrophoresis<sup>17</sup> was carried out by Helena Electrophoretic System, using a kit, Titan III Cat. No. 3023 obtained from Helena Laboratories. Serum fructosamine was determined by kit method supplied by Quimica Clinia Aplicada, Spain.

### Statistical analysis of the data

Epi-Info was used for statistical analysis of the data. Epi-Info is a statistical package available from the US center for disease control and prevention. (Health profile of people of Pakistan 1990-1994).

### Statistical analysis by manual method (Bland, 1987)

The statistical significance of the difference between two mean of various parameters between different groups was evaluated by students 't' test. The difference was regarded as highly significant if the *P* value was less than 0.001, statistically significant if the *P* value was less than 0.05 and non-significant if the *P* value was greater than 0.05. The *P* value was found by mean of 't' distribution table and was read against the degree of freedom. To measure the strength of correlation data was analyzed by using Pearson Product-Moment Correlation technique. This method is suitable for studies where both the research variables are linear. The correlation coefficient (*r*) and determination coefficient (*r*<sup>2</sup>) were calculated on computer by using Microsoft Excel.

### Results

Fasting plasma glucose, HbA<sub>1C</sub>, serum fructosamine, glycosylated plasma protein, serum hexosamine, serum sialic acid levels and total serum protein, alpha-1 and alpha-2 globulins were significantly increased in diabetic patients with or without cardiovascular complications, as compared with non-diabetic patients with cardiovascular complications and control subjects (Table 2). These parameters were not significantly different in non-diabetic patients with cardiovascular complications as compared with control subjects (Table 2) except alpha-1 and alpha-2 globulins, which were significantly increased. Total serum protein and serum albumin were significantly increased (*P*<0.05) in diabetic patients with cardiovascular complications as compared with non-diabetic patients with cardiovascular complications (Table 2).

### Discussion

Type 2 diabetes mellitus has emerged as an important condition of older patients in whom, both microvascular and macrovascular complications are a common cause of morbidity and mortality. Hyperglycaemia and insulin resistance, both seem to have an important role in the pathogenesis of cardiovascular complication.<sup>18,19</sup> A connection between impaired glycemic control with protein oxidation was suggested by Odetti *et al.*<sup>20</sup> Glycation cascade also releases free radicals, which become responsible for further oxidative attacks.<sup>20</sup> HbA<sub>1C</sub>, serum fructosamine and glycosylated plasma protein levels were significantly increased in diabetic patients with cardiovascular complications as compared with non-diabetic patients with cardiovascular complications. These observations are similar to those of other

**Table 1.** Age, weight, duration of diabetes, hypertension and lipid profile of control subjects, diabetic patients without complications and diabetic and non-diabetic patients with cardiovascular complications.(The values are expressed as mean  $\pm$  SEM. Units and number of cases are shown in parentheses.)

Parameters	Control subjects (21)	Diabetic patients without complications (21)	Non-diabetic patients with cardiovascular complications (20)	Diabetic patients with cardiovascular complications (21)
Age (years)	53.81 $\pm$ 1.20	54.71 $\pm$ 1.40	59.65 <sup>a</sup> $\pm$ 1.64	57.86 $\pm$ 1.44
Weight (Kg)	64.30 $\pm$ 1.57	64.24 $\pm$ 1.62	68.15 <sup>a</sup> $\pm$ 0.91	68.05 <sup>a</sup> $\pm$ 1.48
Duration of diabetes (years)	-	9.29 $\pm$ 0.50	-	10.48 $\pm$ 0.65
Hypertension (%)	43.5	62.2 <sup>ab</sup>	31.9	62.2 <sup>ab</sup>
Total cholesterol (mmol/L)	6.80 $\pm$ 0.04	6.68 $\pm$ 0.06	7.07 $\pm$ 0.16	7.04 $\pm$ 0.12
HDL cholesterol (mmol/L)	1.50 $\pm$ 0.01	1.23 $\pm$ 0.01	1.32 $\pm$ 0.05	1.08 <sup>ab</sup> $\pm$ 0.02
LDL cholesterol (mmol/L)	4.71 $\pm$ 0.03	4.39 $\pm$ 0.04	4.93 $\pm$ 0.14	4.68 $\pm$ 0.02
Triglycerides (mmol/L)	1.40 $\pm$ 0.02	2.52 $\pm$ 0.03	2.00 $\pm$ 0.15	3.07 <sup>ab</sup> $\pm$ 0.14

<sup>a</sup>Significant as compared with control subjects; <sup>b</sup>Significant as compared with non-diabetic patients with cardiovascular complications**Table 2.** Blood analytes of control subjects, diabetic patients without complications and diabetic and non-diabetic patients with cardiovascular complications (the values are expressed as mean  $\pm$  SEM and coefficients of variation is shown as percentage units and number of cases are shown in parentheses).

Parameters	Control subjects (21)	Diabetic patients without complications (21)	Non-diabetic patients with cardiovascular complications (20)	Diabetic patients with cardiovascular complications (21)
Fasting plasma glucose (mmol/L)	5.04 $\pm$ 0.13 11.7%	7.83 <sup>a</sup> $\pm$ 0.31 18.3%	4.86 $\pm$ 0.22 19.8%	11.81 <sup>ab</sup> $\pm$ 0.19 7.5%
Glycosylated haemoglobin (HbA <sub>1c</sub> %)	4.98 $\pm$ 0.11 9.7%	11.32 <sup>a</sup> $\pm$ 0.18 7.4%	5.11 $\pm$ 0.19 16.6%	11.72 <sup>ab</sup> $\pm$ 0.14 5.4%
Serum fructosamine (mmol/L)	2.25 $\pm$ 0.08 15.8%	3.72 <sup>a</sup> $\pm$ 0.17 20.4%	2.15 $\pm$ 0.05 10.2%	3.67 <sup>ab</sup> $\pm$ 0.18 21.8%
Glycosylated plasma protein (absorbance/g of proteins)	6.20 $\pm$ 0.12 8.7%	7.90 <sup>a</sup> $\pm$ 0.30 15.8%	5.95 $\pm$ 0.13 9.3%	10.21 <sup>ab</sup> $\pm$ 0.04 17.1%
Hexosamine (mg/dl)	67.86 $\pm$ 3.12 20.7%	102.94 <sup>a</sup> $\pm$ 3.63 10.6%	78.52 $\pm$ 3.47 19.1%	118.5 <sup>ab</sup> $\pm$ 3.13 12.1%
Sialic Acid (mg/dl)	35.36 $\pm$ 1.34 17.0%	49.66 <sup>a</sup> $\pm$ 1.78 16.1%	40.15 $\pm$ 1.98 21.7%	52.90 <sup>ab</sup> $\pm$ 1.77 15.3%
Total Serum protein (gm%)	7.32 $\pm$ 0.12 7.1%	7.94 <sup>a</sup> $\pm$ 0.17 9.7%	7.45 $\pm$ 0.16 9.6%	7.99 <sup>ab</sup> $\pm$ 0.11 5.9%
Serum Albumin (gm%)	4.01 $\pm$ 0.10 11.5%	4.03 $\pm$ 0.11 12.1%	3.48 $\pm$ 0.14 18.2%	3.95 <sup>b</sup> $\pm$ 0.12 14.0%
Alpha-1 Globulin (gm%)	0.16 $\pm$ 0.02 41.1%	0.38 <sup>a</sup> $\pm$ 0.06 69.4%	0.29 <sup>a</sup> $\pm$ 0.03 43.3%	0.32 <sup>a</sup> $\pm$ 0.07 69.4%
Alpha-2 Globulin (gm%)	0.77 $\pm$ 0.03 19.0%	0.96 <sup>a</sup> $\pm$ 0.05 22.3%	1.04 <sup>a</sup> $\pm$ 0.06 24.9%	0.93 <sup>a</sup> $\pm$ 0.07 32.2%
Beta Globulin (gm%)	1.00 $\pm$ 0.03 12.8%	0.92 $\pm$ 0.06 27.5%	0.97 $\pm$ 0.04 19.6%	1.00 $\pm$ 0.06 27.7%
Gamma Globulin (gm%)	1.48 $\pm$ 0.07 20.4%	1.67 $\pm$ 0.09 25.5%	1.66 $\pm$ 0.10 27.7%	1.76 <sup>a</sup> $\pm$ 0.09 22.6%

<sup>a</sup>Significant as compared with control subjects; <sup>b</sup>Significant as compared with non-diabetic patients with cardiovascular complications

workers.<sup>21,22</sup> Serum fructosamine and glycosylated plasma protein concentrations have close correlation with HbA<sub>1c</sub> because they reflect glycaemic control with last 2 to 3 weeks and HbA<sub>1c</sub> reflects glycaemic control for the last 4 to 6 weeks.<sup>23</sup> In the present study serum fructosamine

and glycosylated plasma proteins in diabetic patients also had a close correlation with HbA<sub>1c</sub>. The degree of glycosylation of plasma proteins, as an alternative index of control and as a reflection of possible structural alterations of tissue proteins leading to complications was associated with the diabetic state.<sup>16</sup>

Stratton *et al.*,<sup>24</sup> suggested that in patients with type 2 diabetes, the risk of diabetic complications was strongly associated with previous hyperglycaemia. Any reduction in HbA<sub>1C</sub> is likely to reduce the risk of complications, with the lowest risk being in those with HbA<sub>1C</sub> in the normal range (6.0%). Glycosylation occurs mainly at the N-terminal end of the  $\beta$  chain of haemoglobin, which forms part of the binding site for 2,3 diphosphoglycerate. HbA<sub>1C</sub> has less 2,3-DPG attached and a higher affinity for oxygen than HbA. This will result in less oxygen being released to the tissues and causes tissue hypoxia, which may be related to the development of microangiopathy. Walia *et al.*,<sup>21</sup> found that the HbA<sub>1C</sub> was significantly associated with coronary heart disease in type 2 diabetes. In the present study HbA<sub>1C</sub> was 9.67% in diabetic patients and in non-diabetic patients with cardiovascular complications HbA<sub>1C</sub> was 5.11%. It seems that there are different mechanisms for the development of cardiovascular complications in diabetic and non-diabetic patients.

In diabetic patients with cardiovascular diseases serum hexosamine and serum sialic acid levels were significantly increased as compared with non-diabetic patients with cardiovascular complications. These results are similar to those of other workers.<sup>25-29</sup> Hangloo *et al.*,<sup>30</sup> in a study performed on healthy individuals found that age and sex have no influence on sialic acid levels in serum. As sialic acid is incorporated into carbohydrate chains of glycoproteins and glycolipids in serum and tissues, the degree of incorporation of sialic acid has been reported to affect transvascular permeability and accumulation of lipid in the arterial wall. Sialic acid is conjugated with constituents of acute phase reactants, which are highly concentrated on the surface of endothelial cells. One likely hypothesis might be that the relationship between clinical condition and sialic acid concentration is due to the activity of a current inflammatory atherosclerotic process and/or to a direct damage to vascular endothelium causing sialic acid into the circulation.<sup>27</sup> Serum hexosamine levels rise due to its biosynthesis, which is involved in the pathogenesis of insulin resistance in patients with type 2 diabetes mellitus.<sup>31-33</sup> In the present study the values of hexosamine and sialic acid in non-diabetic patients with complications were in normal limits, but the values were increased in diabetic patients with and without complications. Correlation between fasting plasma glucose and HbA<sub>1C</sub> in control subjects was  $r = 0.284$ , between fasting plasma glucose and HbA<sub>1C</sub> in diabetic patients without complications was  $r = 0.478$ , between fasting plasma glucose and HbA<sub>1C</sub> in non-diabetic patients with cardiovascular complications was  $r = 0.064$  and between fasting plasma glucose and HbA<sub>1C</sub> in diabetic patients with cardiovascular complications was  $r = 0.509$ . The uniform increase in fasting plasma glucose, HbA<sub>1C</sub>, serum fructosamine, glycosylated plasma protein, serum hexosamine and serum sialic acid levels in diabetic patients indicates that the process of glycosylation depends upon hyperglycaemia. The parameters do not rise in non-diabetic patients with cardiovascular complications or without complications, which indicate some other underlying mechanism may be responsible for the development of cardiovascular complications.

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