Serum glycoproteins in diabetic and non-diabetic patients with and without cataract

ANJUMAN GUL1*, M. ATAUR RAHMAN2, NESSAR AHMED3*

1Department of Biochemistry, Ziauddin University, Shah rah-e- Ghalib, Clifton, Karachi-75600, Pakistan
2International Centre for Chemical and Biological Sciences, HEJ Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan
3School of Biology, Chemistry and Health Science, Manchester Metropolitan University, Manchester M1 5GD, UK

*Corresponding authors: A. Gul – anjummurtazagul@yahoo.com, N. Ahmed – N.Ahmed@mmu.ac.uk

This study describes the changes in serum glycoproteins from type 2 diabetic and non-diabetic patients with and without cataract. A total of 85 subjects were selected for the study and divided into four groups. The first group consisted of 21 healthy subjects, the second group consisted of 21 diabetic patients with no chronic complications, the third group consisted of 20 diabetic patients with cataract, and the fourth group had 23 non-diabetic patients with age related cataract. The patients with and without cataract were selected on clinical grounds from the Ziauddin University and Jinnah Postgraduate Medical Centre in Karachi, Pakistan. As expected diabetic patients with and without cataract had significantly higher levels of fasting plasma glucose, glycated haemoglobin, glycated plasma proteins and serum fructosamine. In addition to these parameters, the levels of hexosamine, sialic acid and serum total protein were higher in diabetic compared to non-diabetic subjects with age related cataract and healthy subjects. Analysis of the protein fractions showed that alpha-1-globulins and alpha-2-globulins were higher in diabetic patients without complications compared to non-diabetic subjects with age related cataract and healthy subjects. Serum alpha-1-globulin, alpha-2-globulin, beta globulins and gamma globulins were all significantly higher in diabetic patients with cataract compared to healthy subjects but not serum albumin. In conclusion, the levels of beta globulins and gamma globulins were significantly higher in diabetic patients with cataract and non-diabetic age related patients with cataract compared to healthy subjects. Thus, mechanisms other than hyperglycaemia are responsible for the development of cataract in these patients.

Keywords: glycoprotein, diabetes mellitus, cataract, fructosamine, sialic acid, hexosamine.

1. Introduction

Diabetes mellitus is a common endocrine disorder characterized by hyperglycemia, metabolic abnormalities and long-term complications afflicting the eyes, kidneys, nerves
and blood vessels [1]. Worldwide projections suggest that more than 220 million people will have diabetes by the year 2010 and the majority of these will suffer from type 2 diabetes mellitus [2]. In Pakistan, diabetes mellitus is a major health problem affecting more than 16% of people over the age of 25 years in some areas with a further 10% suffering from impaired glucose tolerance [3].

Cataract is one of the major causes of impaired vision and blindness worldwide and often referred to as senile cataract to indicate that it is more common in advanced age. It is also a serious consequence of long-term diabetes and according to the WHO, affects about half of the 45 million blind people worldwide [4]. Senile cataract is characterized by opacification of the eye lens affecting mainly the nuclear, cortical, and posterior subcapsular regions. Pathological studies of cataractous lenses have revealed that cataracts are composed of protein aggregates that precipitate in the lens of the eye. The insoluble aggregates obstruct the passage of light through the lens preventing it from reaching the photoreceptors in the retina [5]. The lens crystallins may be divided into \( \alpha \), \( \beta \) and \( \gamma \)-crystallins. Diabetic cataract occurs much earlier than senile cataract and causes opacification of the lens and eventual loss of vision. It has been suggested that increased non-enzymatic glycosylation (glycation) of lens crystallins may cause conformational changes resulting in exposure of thiol groups to oxidation and cross-link formation [6–8]. Furthermore, the lens crystallins have virtually no turnover and are ideal candidates for accumulation of glycation-derived cross-links. Thus, increased cross-linking of lens crystallins may cause aggregation producing the high molecular weight material responsible for opacification. Increased glycation of serum proteins could cause an increase in the serum of circulating advanced glycation endproducts (AGEs). These AGEs are responsible for glycation-induced cross-linking of structural proteins and believed to underlie the pathogenesis of diabetic complications [9]. Previous studies have also shown an increase in enzymatically glycosylated proteins such as alpha-2 glycoprotein fractions which were increased in diabetic patients [10].

The aim of this study was to investigate changes in serum glycoproteins (both enzymatic and non-enzymatic) in diabetic and non-diabetic patients with and without cataract.

2. Materials and methods

Patients over 50 years of age were selected on clinical grounds from Ziauddin University Hospital, Karachi and Jinnah Postgraduate Medical Centre, Karachi. The study included a total of 85 subjects which were divided into four groups. Group one consisted of 21 apparently healthy subjects who had no history of diabetes, cataract or any other major illness, like macrovascular disease, retinopathy, tuberculosis, rheumatoid arthritis, liver disease or malignancy. Group two consisted of 21 type 2 diabetic patients without any clinical evidence of chronic diabetic complications, whereas group three had 20 type 2 diabetic patients with cataract. Finally, group four
Serum glycoproteins in diabetic and non-diabetic patients...

consisted of 23 non-diabetic patients suffering from age related cataract. The age, sex, weight, duration of diabetes and treatment received were recorded. Drugs were stopped 48 hours prior to any sample collection. Physical examination including measurement of blood pressure and any family medical history was recorded. Individuals were classified as having diabetes mellitus if they had a fasting plasma glucose concentration ≥ 7.0 or random plasma glucose level ≥ 11.1 mmol/L according to established criteria [11]. Patients with a history of blurred vision or double vision and spots were examined with a slit lamp to determine the type of cataract. The local ethical committee approved the protocol and blood samples were collected from subjects after completing a consent form for each patient and explaining the nature of the study.

Blood glucose was determined by the glucose oxidase method [12]. The reagents were obtained from glucose enzymatic PAP 7500 kit of bioMerieux. For the estimation of glycated haemoglobin, haemolysate was prepared by treating blood with a detergent in a buffered medium and removal of the labile fraction. Haemoglobins are retained by a cationic exchange resin. Haemoglobin A\textsubscript{1C} is specially eluted after washing away the haemoglobin A\textsubscript{1A} and haemoglobin A\textsubscript{1B} fractions and is quantified by direct photometric reading at a wavelength of 415 nm. The kit was obtained from Bio Systems Reagents and Instruments, Spain and based on an established procedure [13]. Fructosamine was detected by the nitro-blue tetrazolium reaction. The kit was obtained from Quimica Clinia Aplicada, Spain. Serum hexoamine was determined by Cessi and Pillego’s method [14], total serum protein by the Biuret method of Reinhold [15], sialic acid by Natelson method [16], and glycosylated proteins by the method of Ma et al. [17]. Glycated plasma proteins were hydrolysed with oxalic acid to release 5-hydroxymethyl furfural which was detected by reaction with thiobarbituric acid. This method gives overall estimation of ketoamine linkages. Serum protein electrophoresis [18] was carried out by Helena Electrophoretic System, using a kit method (Titan III Cat. No. 3023 obtained from Helena Laboratories).

3. Statistical analysis

Epi-Info was used for statistical analysis of the data. Epi-Info is a statistical package available from the US Centre for Disease Control and Prevention. The statistical significance of the difference between two means of various parameters between different groups was evaluated by Student’s 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.

4. Results

The mean age of non-diabetic and diabetic patients with cataract was significantly higher ($P < 0.05$) as compared with control subjects (the Table). Fasting plasma
Table. The age, weight and concentration of blood analytes in non-diabetic and diabetic patients with and without cataract. (The values are expressed as mean ± SEM. The units and number of cases are shown in parentheses.)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control subjects (21)</th>
<th>Diabetic patients without any complications (21)</th>
<th>Non-diabetic patients with cataract (20)</th>
<th>Diabetic patients with cataract (23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53.81±1.20</td>
<td>54.71±1.40</td>
<td>59.83±1.69a</td>
<td>57.50±1.58</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>10/11</td>
<td>10/11</td>
<td>10/13</td>
<td>10/10</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.30±1.57</td>
<td>64.24±1.62</td>
<td>65.22±1.45</td>
<td>67.78±1.55a</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>—</td>
<td>9.29±0.50</td>
<td>65.34±1.45</td>
<td>7.68±1.00</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>5.04±0.13</td>
<td>7.83±0.32a</td>
<td>5.34±0.18</td>
<td>9.32±0.34ab</td>
</tr>
<tr>
<td>% Glycosylated haemoglobin (HbA1c)</td>
<td>4.98±0.11</td>
<td>9.30±0.37a</td>
<td>5.04±0.09</td>
<td>8.80±0.34ab</td>
</tr>
<tr>
<td>Serum fructosamine (mmol/L)</td>
<td>2.25±0.08</td>
<td>3.72±0.17a</td>
<td>1.98±0.07b</td>
<td>3.05±0.21ab</td>
</tr>
<tr>
<td>Glycosylated plasma protein (absorbance/g)</td>
<td>6.20±0.12</td>
<td>7.90±0.30a</td>
<td>5.87±0.12</td>
<td>8.90±0.34ab</td>
</tr>
<tr>
<td>Hexosamine (mg/dL)</td>
<td>67.86±3.12</td>
<td>102.94±3.63a</td>
<td>77.52±3.31</td>
<td>118.80±3.43ab</td>
</tr>
<tr>
<td>Sialic acid (mg/dL)</td>
<td>35.36±1.34</td>
<td>49.66±1.78</td>
<td>39.22±1.38</td>
<td>50.49±1.76ab</td>
</tr>
<tr>
<td>Total serum protein (gm%)</td>
<td>7.32±0.12</td>
<td>7.94±0.17a</td>
<td>6.94±0.20</td>
<td>7.79±0.12ab</td>
</tr>
<tr>
<td>Serum albumin (gm%)</td>
<td>4.01±0.10</td>
<td>4.03±0.11</td>
<td>3.01±0.09</td>
<td>3.57±0.12b</td>
</tr>
<tr>
<td>Alpha-1 globulin (gm%)</td>
<td>0.16±0.02</td>
<td>0.38±0.06a</td>
<td>0.17±0.02</td>
<td>0.36±0.09ab</td>
</tr>
<tr>
<td>Alpha-2 globulin (gm%)</td>
<td>0.77±0.03</td>
<td>0.96±0.05a</td>
<td>0.87±0.05</td>
<td>1.54±0.56a</td>
</tr>
<tr>
<td>Beta globulin (gm%)</td>
<td>1.00±0.03</td>
<td>0.92±0.06</td>
<td>1.08±0.07a</td>
<td>1.14±0.07a</td>
</tr>
<tr>
<td>Gamma globulin (gm%)</td>
<td>1.48±0.07</td>
<td>1.67±0.09</td>
<td>1.89±0.12a</td>
<td>2.01±0.11a</td>
</tr>
</tbody>
</table>

a – significant as compared with control subjects; b – significant as compared with non-diabetic patients with cataract.
glucose, HbA$_{1C}$, serum fructosamine, glycosylated plasma protein, serum hexosamine and serum sialic acid levels were significantly higher ($P < 0.05$) in all diabetic patients with or without cataract as compared with control subjects (the Table). These parameters did not change ($P > 0.05$) in non-diabetic patients with age related cataract as compared with control subjects (the Table). However, they were higher in diabetic patients when compared to non-diabetic patients with age related cataract ($P < 0.001$; the Table). Total serum protein, alpha-1- and alpha-2 globulins were significantly higher ($P < 0.05$) in diabetic patients with or without cataract as compared with control subjects (the Table). Beta globulin and gamma globulin were significantly higher ($P < 0.05$) in non-diabetic and diabetic patients with cataract as compared with control subjects (the Table).

The correlation between fasting plasma glucose and HbA$_{1C}$ in control subjects was $r = 0.284$, between fasting plasma glucose and HbA$_{1C}$ in diabetic patients without cataract $r = 0.478$, between fasting plasma glucose and HbA$_{1C}$ in non-diabetic patients with age related cataract $r = 0.267$ and between fasting plasma glucose and HbA$_{1C}$ in diabetic patients with cataract $r = 0.467$.

5. Discussion

Diabetes mellitus and its complications constitute an important health problem in both developing and developed countries. Cataract remains the commonest cause of blindness worldwide. In the present study, patients were selected with cataract and the possibility of other complications was excluded by the absence of any sign and symptoms on physical examination. Changes in protein concentration and increased enzymatic glycation of various proteins in diabetic patients have been correlated with hyperglycemia, which in turn causes early functional alterations in different tissues. In the present study, non-diabetic patients with age related cataract have normal values except beta and gamma globulin which are increased in non-diabetic patients with age related cataract. Glycaemic related analytes increased in all diabetic patients with and without cataract and the levels did not change in non-diabetic patients with age related cataract and control subjects (the Table). The presence of cataract in non-diabetic patients did not affect the glycaemic related analytes, and increase in diabetic patients without complications, reflect that the diabetes was uncontrolled. They may be developing changes at the subclinical level which later on present a complication.

The correlation between fasting plasma glucose and HbA$_{1C}$ in control subjects was $r = 0.284$, between fasting plasma glucose and HbA$_{1C}$ in diabetic patients without cataract $r = 0.478$, between fasting plasma glucose and HbA$_{1C}$ in non-diabetic patients with age related cataract $r = 0.267$, and between fasting plasma glucose and HbA$_{1C}$ in diabetic patients with cataract $r = 0.467$. Our results show that fasting plasma glucose is directly proportional to HbA$_{1C}$ level. The correlation among HbA$_{1C}$, blood glucose concentrations and late complications has been established over the last 30 years [19–21]. Serum fructosamine and glycated plasma protein concentrations have close correlation with HbA$_{1C}$ because they reflect glycaemic control within the last 2
to 3 weeks and HbA_1C reflects glycaemic control for the last 4 to 6 weeks [22, 23]. In the present study, serum fructosamine and glycated plasma proteins in diabetic patients also have a close correlation with HbA_1C. The degree of glycation of plasma proteins, as an alternative index of control and as reflection of possible structural alterations of tissue proteins leading to complications was associated with the diabetic state [22]. STRATTON et al. [23] suggested that in patients with type 2 diabetes, the risk of diabetic complications was strongly associated with previous hyperglycemia. Any reduction in HbA_1C is likely to reduce the risk of complications, with the lowest risk being in those with HbA_1C in the normal range. In the present study, the coefficient of variation of HbA_1C was higher in diabetic patients with cataract as compared with non-diabetic patients with age related cataract. It seems that there could be different mechanisms for the development of cataract in diabetic and non-diabetic patients. Serum hexosamine and sialic acid levels were significantly increased in all diabetic patients and were non-significant in non-diabetic patients with age related cataract as compared with control subjects (the Table). Other workers have made similar observations [24, 25]. HANGLOO et al. [26] found that age and sex had no influence on serum sialic acid levels. 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 surface of endothelial cells [26]. One likely hypothesis might be that the relationship between clinical condition and sialic acid concentration is due to the activity of a current inflammatory atheroslerotic process and/or to a direct damage to vascular endothium causing sialic acid into the circulation [27]. Serum hexosamine levels rise due to hexosamine biosynthetic pathway, which is involved in the pathogenesis of insulin resistance in patients with type 2 diabetes mellitus [24, 28, 29]. In the present study, the values of hexosamine and sialic acid in non-diabetic patients with age related cataract were in normal limits, but the values were increased in diabetic patients with cataract and without complications.

The uniform increase in fasting plasma glucose, glycosylated haemoglobin (HbA_1C), serum fructosamine, glycosylated plasma protein, serum hexosamine and serum sialic acid levels in diabetic patients indicates that the process of glycosylation depends upon hyperglycemia. The parameters do not rise in non-diabetic patients, and hence some other underlying mechanism may be responsible for the development of complications in these patients. However, at present we cannot decide whether or not there is relevant dysfunction of the β- and γ-globulin and what role any such dysfunction would play in the development of cataract. A limitation of this study was the low number of patients and if the study is carried out on a larger sample of patients with other factors, which is necessary for definite conclusion.

Acknowledgement – We are grateful to Dr. Arif Hussain, in charge of Clinical Laboratory, Ziauddin University Hospital, Pakistan for his cooperation and assistance with the laboratory work.
Serum glycoproteins in diabetic and non-diabetic patients ...

References


Received November 5, 2007
in revised form January 12, 2008