GLYCOSYLATED PROTEINS IN DIABETIC NEPHROPATHY

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Summary

This study was carried out on 55 diabetic patients, 20 of whom had diabetic nephropathy, and 10 controls. Glycosylated haemoglobin, glycosylated serum protein, glucoprotein, serum protein electrophoresis, blood urea, serum creatinine and β2-microglobulin were measured.

A significant increase of glucoprotein was observed in patients with diabetic nephropathy. No correlation was found between glycosylated serum protein and glycosylated haemoglobin and duration of diabetes. Glycosylated serum protein showed a positive correlation with β2-microglobulin, indicating a link between renal involvement and the rise in glycosylated serum protein. Whether there is a pathogenic relation between glycosylated serum protein and the development of nephropathy awaits further evidence.

Introduction

Renal disease is a major cause of morbidity in people with type I diabetes mellitus. About half of the patients with insulin dependent diabetes mellitus develop renal failure by a mean of 20 years after the apparent onset of the disease. The pathogenesis of diabetic glomerulopathy, which is characterised by increased thickness of the capillary basement membranes and accumulation of proteinaceous material in the mesangium is still speculative [1]. This proteinaceous material was previously described as glycoproteins and on other occasions as immunoglobulins with various complement components and fibrin. Currently it is assumed to be glycosylated proteins, especially albumin.

Tischler [1] concluded that beside the metabolic effect of hyperglycaemia, there are additional factors, such as hypertension, growth hormone, platelet aggregation and abnormalities in the coagulation and fibrinolytic systems. Other factors include abnormalities in metabolism of proteoglycans and glycosaminoglycan and genetic factors.
In this study we investigated the possible pathogenic role of glycosylated proteins in diabetic nephropathy.

Materials and methods

This study included 55 patients with insulin dependent diabetes mellitus, 10 of which were without clinically evident complications (6 females and 4 males) with a mean age of 44.9±14.1 years. Twenty patients had diabetic nephropathy (13 females and 7 males) with a mean age of 51.2±9.2 years. Fifteen patients had ischaemic heart disease (10 males and 5 females) with a mean age of 52.5±10.8 years. The remaining 10 patients had diabetic retinopathy (6 males and 4 females) with a mean age of 54.4±7.4 years.

Ten healthy persons with no history of family predisposition to diabetes, of matching age and sex were chosen as normal controls (5 males and 5 females with a mean age of 40.8±16.8 years).

Our patients were classified according to the main clinical presentation as most of the cases had combined complications.

For the classification of our material all cases were subjected to full clinical assessment, fundoscopic examination, and the following investigations: electrocardiogram; blood sugar, both fasting and two hours post-prandial; full urine analysis including quantitative urinary proteins per 24 hours; urine culture and bacterial count (cases with upper urinary tract infection were excluded); blood picture; serum creatinine and blood urea; serum proteins (total and albumin): diabetics with recent febrile illness or serious overt infections were excluded to avoid any change in plasma proteins secondary to increased acute phase of protein production; serum glucoproteins electrophoresis; glycosylated haemoglobin; glycosylated serum proteins; urinary tract plain X-ray and intravenous pyelogram or sonogram (cases with any suspicion of obstructive uropathy were excluded); renal biopsy for cases with proteinuria more than 1g/24 hours, and serum β2-microglobulin as an index of glomerular filtration rate (cases with any possible tubular injury as infection, obstruction or receiving nephrotoxic drugs were excluded).

Results

Blood sugar, serum creatinine and blood urea are shown in Table I.

β2-microglobulin (β2Mug/L) In the controls the mean was 1656.2±863.8 (mean ± SD), in uncomplicated diabetic patients 1693.3±789.7 (NS), patients with diabetic nephropathy 9824.2±1250.7 (p<0.01), those with diabetic retinopathy 3731.8±243.4 (p<0.05) and those with ischaemic heart disease 3244.5±132 (p<0.05).

Plasma proteins: (a) total serum proteins (mg%) Total serum proteins of uncomplicated diabetic patients were 7.3±0.5 which is insignificantly different from control values (7.6±0.6). The mean values in the complicated subgroups were 6.7±0.8 for diabetic nephropathy, 6.9±0.6 for diabetic retinopathy and 6.5±0.5 for ischaemic heart disease.
<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>Duration of diabetes</th>
<th>Fasting glucose</th>
<th>Two hours post-prandial</th>
<th>Blood urea</th>
<th>Serum creatinine</th>
<th>$\beta_2$ M</th>
<th>Proteinuria</th>
<th>GSP mmol HMF/mg prot.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>40.8</td>
<td>–</td>
<td>79.9</td>
<td>125.5</td>
<td>30.8</td>
<td>0.8</td>
<td>16562</td>
<td>–</td>
<td>7.9</td>
</tr>
<tr>
<td>n=10</td>
<td>±16.8</td>
<td>±21.8</td>
<td>±15.5</td>
<td>±10.3</td>
<td>±0.2</td>
<td>±863.6</td>
<td>±1693.3</td>
<td>±0.6</td>
<td>±0.04</td>
</tr>
<tr>
<td>Uncomplicated diabetics</td>
<td>44.9</td>
<td>3.2</td>
<td>170.5**</td>
<td>195.5*</td>
<td>35</td>
<td>0.8</td>
<td>1693.3</td>
<td>11.4**</td>
<td>0.133</td>
</tr>
<tr>
<td>n=10</td>
<td>±14.1</td>
<td>±3.1</td>
<td>±94.1</td>
<td>±96.5</td>
<td>±4.8</td>
<td>±0.3</td>
<td>±789.7</td>
<td>±1.5</td>
<td>±0.146</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>51.2</td>
<td>9.4</td>
<td>263.6**</td>
<td>317.4**</td>
<td>139.3</td>
<td>5.7</td>
<td>9824.5**</td>
<td>4.2</td>
<td>12.9**</td>
</tr>
<tr>
<td>n=20</td>
<td>±9.2</td>
<td>±7.9</td>
<td>±105</td>
<td>±125.2</td>
<td>±34.1</td>
<td>±3.9</td>
<td>±1250.7</td>
<td>±1.9</td>
<td>±2.5</td>
</tr>
<tr>
<td>Diabetic retinopathy</td>
<td>54.4</td>
<td>7.7</td>
<td>234.1**</td>
<td>321**</td>
<td>43</td>
<td>1.9</td>
<td>3731.8</td>
<td>0.9</td>
<td>13.6**</td>
</tr>
<tr>
<td>n=10</td>
<td>±7.4</td>
<td>±4.5</td>
<td>±132</td>
<td>±134.2</td>
<td>±18</td>
<td>±0.2</td>
<td>±243.4</td>
<td>±2.2</td>
<td>±0.17</td>
</tr>
<tr>
<td>Ischaemic heart disease</td>
<td>52.5</td>
<td>12.7</td>
<td>283.8**</td>
<td>384.7**</td>
<td>41.9</td>
<td>1.2</td>
<td>3244.5**</td>
<td>0.3</td>
<td>14.4**</td>
</tr>
<tr>
<td>n=15</td>
<td>±10.8</td>
<td>±9.8</td>
<td>±93.1</td>
<td>±114.3</td>
<td>±16</td>
<td>±0.1</td>
<td>±132.0</td>
<td>±0.01</td>
<td>±2.2</td>
</tr>
</tbody>
</table>

** p<0.01  
* p<0.05  
$\beta_2$ M = $\beta_2$-microglobulin  
GHB = Glycosylated haemoglobin  
GSP = Glycosylated serum protein  
HMF = 5-hydroxy methylfurfural
(b) **serum albumin (mg%)** in uncomplicated diabetic patients was 4.1±0.7 which is insignificantly different from controls (4.5±0.3). In complicated subgroups it was 3.3±0.7 in diabetic nephropathy, 3.6±0.7 in diabetic retinopathy and 4.1±0.8 in ischaemic heart disease, a significant reduction in both diabetic nephropathy and diabetic retinopathy groups.

**Glucoproteins (GP%)** alpha₁, alpha₂ and beta-glucoproteins in uncomplicated diabetic patients was 11.46±1.42, 35.62±3.6 and 24.28±4.51 respectively. The corresponding control figures were 12.76±2.05, 36.56±2.75 and 24.19±4.45 respectively. The mean value of alpha₁ glucoproteins was 13.0±2.7 in diabetic nephropathy, 9.8±4.2 in diabetic retinopathy and 9.6±3.4 for ischaemic heart disease. The corresponding figures for alpha₂ glucoproteins were 42.03±5.9, 36.6±4.7 and 38.5±6.2 respectively. A highly significant increase was found in the renal group compared to controls (p<0.01) and to uncomplicated diabetic patients (p<0.05). While beta glutoprotein was 22.9±4.9, 23.6±3.2 and 23.8±4.1 respectively. Beta-glucoproteins showed insignificant differences between all groups.

**Glycosylated haemoglobin (GHB %)** in uncomplicated diabetic patients was 11.36±1.5 which is significantly higher than the control value (7.89±0.6) (p<0.01). In diabetic nephropathy it was 12.95±2.5, in diabetic retinopathy 13.6±2.2 and 14.4±2.2 for ischaemic heart disease. Glycosylated haemoglobin was significantly high in all complicated subgroups compared to controls (p<0.01), while compared to uncomplicated diabetic patients significantly higher values were found in both ischaemic heart disease (p<0.01) and diabetic retinopathy (p<0.05). No correlation could be found between glycosylated haemoglobin and age, sex or duration of diabetes.

**Glycosylated serum proteins (GSP, mmol HMF/mg protein)** in uncomplicated diabetics it was 0.133±0.14 which was insignificantly higher than controls (0.036±0.04). The mean value in diabetic nephropathy was 0.59±0.23, in diabetic retinopathy was 0.29±0.17 and 0.35±0.24 in ischaemic heart disease.

Comparing these later subgroups to uncomplicated diabetic patients and comparing the different complicated subgroups to each other, diabetic nephropathy was the only group that showed significantly higher values throughout. No correlation was found between glycosylated serum proteins and fasting blood sugar. No correlation could be found between glycosylated serum proteins and glycosylated haemoglobin, total serum protein, serum albumin, serum creatinine or blood urea. A positive correlation was found between glycosylated serum proteins and β₂-microglobulin in the nephropathy group (r=0.8637, p<0.001).

**Discussion**

In our study, a highly significant increase in alpha₂-glucoproteins in the nephropathy group was found whether compared with the control group or the uncomplicated diabetic patients. This agrees with the studies of Levin et al [2], who
reported abnormal glucoproteins in the glomerular basement membrane resulting in diabetic glomerulosclerosis, and later in 1982 Tischer [1] described diffuse accumulation of glucoproteins in intercapillary glomerulosclerosis.

The percentile value of beta-glucoproteins did not show any statistically significant difference between controls and diabetic patients, whether complicated or uncomplicated. A significant increase of glycosylated haemoglobin was found in all diabetic groups (complicated and uncomplicated) compared to controls, while a significant rise was found only in the retinopathy and the coronary groups on comparing the complicated with the uncomplicated diabetic patients. The relatively low glycosylated haemoglobin in the renal cases compared to other diabetic groups could be explained by the shortened life span of erythrocytes in renal failure [3] and frequent blood transfusions in these cases.

A positive correlation could be found between glycosylated haemoglobin and fasting blood sugar in all diabetic groups. This is in agreement with the findings of Graf et al [4]. Glycosylated haemoglobin was not related to age, sex or duration of diabetes. This disagrees with Goldstein et al [5], who found a significant relationship between glycosylated haemoglobin and the duration of diabetes.

In our study, glycosylated serum protein values were similar to the figures found by Kinney et al [6], but contrary to the same author, we found no correlation between fasting blood sugar and glycosylated serum protein. A significant increase in glycosylated serum protein was found in all complicated groups while an insignificant rise was found in uncomplicated diabetic patients. Patients with diabetic nephropathy were the only group that showed a significant rise in glycosylated serum proteins on comparing different complicated subgroups to each other.

These data are suggestive of a possible pathogenic role of glycosylated serum proteins in the pathogenesis of diabetic renal disease. This is in agreement with the experimental study of Cohn [7], who demonstrated glycosylation of rat glomerular basement membrane after incubation with glucose. Also, it has been found that incorporation of glycosylated serum proteins in renal basement membrane may explain the pathological changes seen in diabetic nephropathy.

No correlation could be found between glycosylated serum proteins and other variables, such as glycosylated haemoglobin, total serum proteins, serum albumin, serum creatinine or blood urea. There was also no correlation between glycosylated serum proteins and age, sex or the duration of the disease. This is in agreement with Kinney et al [6].

A statistically significant correlation could be found between glycosylated serum proteins and B2-microglobulins as it is a sensitive index of renal function in diabetic patients [8]. The accumulating evidence points to changes in the plasma proteins of diabetic patients playing a role in accelerating the progression rate of diabetic microangiopathy. A disturbance in the average molecular shape of plasma proteins due to raised glycosylated serum proteins directly increases both plasma viscosity and erythrocyte aggregation leading to impairment of the microcirculation [9]. These findings can explain the positive correlation between glycosylated serum proteins and B2-microglobulins, especially in the late second junctional and early third phase of diabetes.
Therefore we can conclude that elevated glycosylated serum protein, together with other factors, have a pathogenic role in the evolution of diabetic renal disease.

References

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3 Horton BF, Huisman THJ. *Br J Haematol* 1965; 11: 196
5 Goldstein DE et al. *Diabetes* 1982; 31 (suppl 3): 70
9 McMillan DE. *Diabetes* 1976; 25 (suppl 2): 858

Open Discussion

MIGONE (Chairman) May I ask you if you related the microproteinuria to the increased $\beta_2$-microglobulin you have measured in the serum?

RAMZY Yes, the serum $\beta_2$-microglobulin is an index of glomerular filtration rate.

MIGONE Was there a parallel increase in both serum and urine?

RAMZY No, the increase was between the serum $\beta_2$ and the glycosylated serum proteins.

MIGONE You did not find any increase of microproteinuria in general without looking for the $\beta_2$-microglobulin – I mean the microproteinuria, the so-called tubular proteinuria?

RAMZY No, in some cases there is increased $\beta_2$-microglobulin but I have excluded them because I tried to concentrate on the glomerulus. That is why if we have increased $\beta_2$ it is suggestive of tubular injury of unknown cause. That is why these cases had been excluded because they may have infection or something else.

MIGONE In any case you are dealing with $\beta_2$-microproteinuria?

RAMZY Yes.