Acute Phase Serum Proteins in Diabetic Retinopathy


The serum concentration of various acute phase reactants were studied in patients with non-insulin dependent diabetes mellitus with and without retinopathy and in control subjects. The serum levels of haptoglobin was elevated in diabetics with retinopathy and the levels were highest in those with proliferative diabetic retinopathy. The levels of serum albumin, alpha-1 acid glycoprotein, alpha-1 antitrypsin and caeruloplasmin were not significantly different between the patients with retinopathy and controls. Haptoglobin increases serum viscosity and this could be the mechanism by which it plays a role in pathogenesis of diabetic retinopathy. These preliminary observations need to be confirmed by studies based on larger number of patients. Longitudinal studies on acute phase reactants in various stages of development of diabetic retinopathy would also provide valuable information.

Key Words: Acute phase reactants - Haptoglobin - NIDDM - Diabetic retinopathy - Viscosity.

The term "acute phase reactants" or "acute phase proteins" is used to denote proteins, secreted by the liver, whose levels have been shown to be increased following injury and in both acute and chronic medical disorders. The major proteins of hepatic origin involved in the acute phase reaction are alpha-1 acid glycoprotein (GP), alpha-1 anti-trypsin, caeruloplasmin and haptoglobin.

Levels of acute phase proteins have been found to be elevated in adult diabetics, but normal in childhood diabetes suggesting that the increase could be related to occurrence of microvascular disease rather than to diabetes perse. A recent study by McMillan has shown that the levels of acute phase reactants correlate with the number of microvascular sequelae. However, McMillan's study did not look specifically at diabetic retinopathy but at microvascular complications in general. In this paper, a detailed study is made of the acute phase serum proteins in non-insulin dependent diabetic patients with background and proliferative retinopathy compared to diabetic patients without retinopathy.

MATERIAL AND METHODS

Three groups of diabetic subjects and one control group were studied. The control group comprised 20 healthy non-pregnant adult volunteers who were the spouses and siblings of the patients included in the study. This selection ensured age and sex matching as well as matching for socio-economic and nutritional status which could otherwise affect the results. All control subjects had normal glucose tolerance.

The study was done during a 6 month period from February 1, 1991 to July 30, 1991. The mode of selection of diabetic patients was as follows. From the daily list of patients seen at the diabetic centre, every suitable patient with Non-Insulin Dependent Diabetes Mellitus (NIDDM) (after exclusion criteria) who respectively had no retinopathy, background or proliferative was included in the study. Recruitment was continued until the required numbers of male and female patients were recruited in each group.

All diabetic patients in the study had NIDDM diagnosed according to the WHO study group report criteria. All the diabetic subjects underwent a detailed fundus examination both by direct and indirect ophthalmoscopy and retinopathy, when present, was graded as background or proliferative. Background retinopathy was diagnosed when there were microangiopathy, dot hemorrhages or exudates in the absence of new vessels. Proliferative diabetic retinopathy was diagnosed when new vessels were present on the optic disc or elsewhere on the retina.

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The three diabetic groups were ambulatory NIDDM outpatients without major health problems and included the following:

i) NIDDM without retinopathy (n = 20)

ii) NIDDM with Background Diabetic Retinopathy (BDR) (n = 20)

iii) NIDDM with Proliferative Diabetic Retinopathy (PDR) (n = 20)

The age group of the controls and the three diabetic groups ranged from 25 to 50 years. Table 1 outlines the clinical details of the study groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (n = 20)</th>
<th>Diabetics without DR (n = 20)</th>
<th>Diabetics with BDR (n = 20)</th>
<th>Diabetics with PDR (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male:Female</td>
<td>10:10</td>
<td>10:10</td>
<td>10:10</td>
<td>10:10</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>30±4</td>
<td>32±6</td>
<td>34±7</td>
<td>35±6</td>
</tr>
<tr>
<td>Duration of diabetes (Years)</td>
<td>-</td>
<td>6±4</td>
<td>8±3</td>
<td>10±2</td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>80±6</td>
<td>126±14</td>
<td>138±15</td>
<td>140±16</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.5±0.1</td>
<td>9.2±0.5</td>
<td>9.4±0.6</td>
<td>9.5±0.5</td>
</tr>
</tbody>
</table>

Exclusion criteria were nutritional deficiency (anaemia, skin and hair changes etc.) presence of significant protein excretion (>300 mg/day), serum creatinine elevation (> 1.2 mg/dl) and active inflammatory disease and/or local or systemic infections. Subjects with a history of exogenous hormone administration were also excluded.

Serum was recovered from clotted blood, stored frozen at minus 20°C and thawed at the time of analysis. Single radial immuno diffusion was used to determine the levels of alpha-1 acid GP, alpha 1 antitrypsin, caeruloplasmin and serum albumin. Haptoglobin was measured through its ability to modify haemoglobin's light absorption. Fasting plasma glucose estimations were done by glucose oxidase method using Hitachi 704 autoanalyzer and glycosylated haemoglobin by the method of Eross et al. as modified by Susheela et al.

Statistical analysis was done using analysis of variance (ANOVA) for differences between the groups. Results are expressed as mean ± SD and p<0.05 was considered significant.

RESULTS

Table 2 shows the results of the different acute phase reactants in the four groups studied. The serum haptoglobin levels were not significantly different in the diabetic patients without retinopathy as compared to the control group. In those with background retinopathy (BDR) the serum haptoglobin levels were significantly higher compared to controls and those without retinopathy (p < 0.01). The highest levels of serum haptoglobin were found in those with proliferative retinopathy. The differences between the haptoglobin levels in the PDR group as compared to the controls and the diabetics without retinopathy was highly significant statistically (p < 0.01).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (units)</th>
<th>Alpha-1 acid GP (mg/dl)*</th>
<th>Alpha-1 anti-trypsin* (units)</th>
<th>Caeruloplasmin (units)</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>109±33</td>
<td>72±20</td>
<td>270±58</td>
<td>15.6±1.8</td>
<td>3.9±0.4</td>
</tr>
<tr>
<td>N No DR</td>
<td>127±55</td>
<td>68±21</td>
<td>244±64</td>
<td>16.4±2.8</td>
<td>3.7±0.5</td>
</tr>
<tr>
<td>I ns</td>
<td>148±65</td>
<td>69±16</td>
<td>229±38</td>
<td>15.7±2.7</td>
<td>3.7±0.5</td>
</tr>
<tr>
<td>D p&lt;0.01</td>
<td>181±84</td>
<td>79±18</td>
<td>245±59.2</td>
<td>17.1±3.2</td>
<td>3.8±0.6</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± S.D
NS = Not Significant
NO DR = No retinopathy
BDR = Background Diabetic Retinopathy
PDR = Proliferative Diabetic Retinopathy
* denotes that there were no significant differences between any of the groups studied (p > 0.05).

The serum levels of the other acute phase reactants studied namely alpha-1 acid glycoprotein, alpha-1 antitrypsin, caeruloplasmin and albumin were not significantly different either in the group with background or proliferative diabetic retinopathy as compared to the controls or the diabetic patients without retinopathy.

DISCUSSION

Elevation of one or more acute phase reactants has been commonly found in diabetic subjects with angiopathy. McMillan has shown that haptoglobin, C-reactive protein and alpha-1 acid glycoprotein levels were higher and serum albumin levels lower in patients with microangiopathy. The most striking changes in this study were seen in haptoglobin levels. The present study confirms the elevation of haptoglobin levels and further demonstrates its correlation with the severity of retinopathy. There were however no differences in the other acute phase reactants in our study.
The differences between our study and McMillan's could be explained by the patient selection. McMillan's patients had significant proteinuria (nephropathy). This could explain the lower albumin levels and consequent increase in globulins in their study. In our study we have selected patients without neuropathy and hence did not find any difference in serum albumin levels nor in the alpha-1 and trypsin and acid glycoprotein (GP) concentrations in our diabetic patients.

The relationship between acute phase protein changes and serum viscosity has been elegantly shown by McMillan. The positive correlation with serum viscosity was shown to be strongest for haptoglobin and least so for alpha-1 acid glycoprotein and C-reactive protein. These findings support the conclusion that haptoglobin may exert its effect in diabetic retinopathy by changes in serum viscosity. The haptoglobin shape and the degree of elevation in diabetes, makes it particularly important in increasing serum viscosity. Obviously the findings in our studies are only preliminary observations and need to be confirmed on larger number of patients. Further studies on haptoglobins and serum viscosity in relation to the natural history of diabetic retinopathy would also be of great interest.

Acknowledgements

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REFERENCES