SERUM PROTEIN AND LIPIDS IN ADOLESCENT SICKLE CELL PATIENTS

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Summary

The somatosexual growth retardation seen in sickle cell anaemia (SCA) has multifactorial aetiology.

Since these factors can alter serum protein patterns and lipid concentrations in ways which may affect human growth rate, serum proteins and lipids have been compared in SCA patients and normals. There were no differences in the total serum protein concentrations and electrophoretic patterns in the two groups. Among the lipids, only triglycerides were significantly elevated ($p < 0.001$) in the SCA patients ($0.8 \pm 0.1 \text{mmol/l}; n = 62$; compared with $0.5 \text{mmol/l}$ in the normals; $n = 39$).

The poor nitrogen absorption and utilisation in SCA subjects does not appear to affect their serum protein levels. The high triglyceride level may be due to increased fat mobilization for energy production in sickle cell anaemia.

Key Words: Sickle Cell Anaemia, growth, nutrition.

Introduction

In recent studies aimed at finding explanations for the somatosexual growth retardation seen in sickle cell anaemia, it has been found that some of these patients show:

(a) decreased intestinal absorption of dietary protein and sugars$^{1-4}$;
(b) poor nitrogen utilisation$^{1,2,5}$;
(c) subnormal levels of circulating testosterone and human growth hormone$^{6,7}$, and
(d) higher basal metabolic rate without increased levels of the thyroid hormones$^{8}$.

Even though the absorption of proteins and sugars is impaired, there appears to be normal absorption of fat$^{9}$. The metabolic consequences of these problems are only speculative at the present time.

It is not unlikely that the increased susceptibility of sickle cell patients to infections might be due to the combined effect of poor nutrition, un-
satisfactory living conditions, and the well-known tissue hypoxia that underlies the sickling phenomenon. The ideal solution to this problem seems a long way off so we must pursue attainable short- and medium-term goals. These will depend on a more thorough understanding of the rather abnormal physiology and biochemistry of sickle cell anaemia.

To this end, serum proteins and lipids have been determined as part of the general evaluation of the nutritional status of sickle cell anaemia patients. Deficiencies in dietary intake of proteins and fats can alter serum protein patterns and lipid and lipoprotein concentrations in various ways which will ultimately affect the rate of growth of the individual.

Methods

Subjects made up of 62 sickle cell anaemia patients (HbSS) and 39 normals (HbAA) who served as controls, were investigated. All the subjects were adolescent male Ghanaians (aged 14–16 years). Their haemoglobin status was confirmed by electrophoresis on cellulose acetate strips. They were all healthy and had no oedema, hepatomegaly, gastrointestinal symptoms, clinical evidence of infections, or any stigmata of malnutrition. A tinge of jaundice was however found in some of the sickle cell patients; however, they were all in a “steady state” and had been “crisis-free” for at least 3 months prior to the investigations.

Blood was obtained from an antecubital vein and serum proteins and lipids were determined. Total serum protein and albumin were determined by the biuret method and bromocresol green binding assay respectively. Serum protein was fractionated by cellulose acetate electrophoresis.

Total cholesterol was determined by the method of Abell and Levy. This involved precipitation of lipoprotein complexes by ethanolic potassium hydroxide to liberate cholesterol and extraction of the cholesterol into petroleum ether, a portion of which was evaporated. Cholesterol was then measured after the Liebermann-Burchard reaction had been carried out on the residue. Since haemolysis liberates cholesterol from erythrocytes, haemolysed serum specimens were rejected to eliminate false positive elevations.

Triglycerides were measured in terms of their glycerol content after preliminary separation from phospholipids by adsorption to a zeolite mixture. After removing phospholipids, glycerol was liberated from the triglycerides by saponification with ethanolic potassium hydroxide followed by acidification. The glycerol yield was oxidized to formaldehyde by metaperiodate and the formaldehyde liberated was determined spectrophotometrically by reaction with chromotropic acid. The zeolite mixture contained Lloyd’s reagent which removes bilirubin and other serum chromogens as well as phospholipids.

Phospholipids were estimated in terms of lipid phosphorus. Isopropanol extraction of serum was performed initially to recover the phospholipid fraction which was subsequently dried. A wet acid digestion step with sulphuric acid and hydrogen peroxide was carried out to destroy all organic materials and to oxidise the phosphorus in the residue to inorganic phosphate which was subsequently determined by Gomori’s method. Since lipid phosphorus represents about 1/25th of the weight of the phospholipid molecule, a factor of 25 was used to convert the concentration of lipid phosphorus to phospholipid.

Non-esterified fatty acids were determined by the titrimetric method of Trout, et al. Serum was extracted with an isopropanol - heptane mixture containing sulphuric acid, and the extract was washed with dilute sulphuric acid (0.05%) to remove lactic acid and an acetone insoluble material that interferes. The fatty acids were titrated with sodium hydroxide and thymolphthalein as indicator, using a stream of nitrogen for mixing.

The results were analysed using Student’s ‘t’ test.

Results

Serum protein concentrations in SCA patients were compared with serum protein concentrations in the normal controls, and the results are presented in table 1.
There was no statistically significant difference in the serum protein concentrations determined in the two groups of subjects. Also, there were no qualitative or quantitative differences in the electrophoretic patterns.

The serum lipids results are found in Table 2.

(a) Total Cholesterol

There was no statistically significant difference between the mean levels of total cholesterol in both the sickle cell patients and the normal controls. These levels contrast with the slightly higher values of 3.9 – 6.5 mmol/l quoted in the literature for normal subjects in developed countries\textsuperscript{18}.

(b) Triglycerides

The level of triglycerides found in the sickle cell patients and in the normal controls fall within the published normal range of 0.11-2.09 mmol/l for the developed countries\textsuperscript{18}. However, our results clearly show that sickle cell patients have a mean triglyceride level that is almost double that found in the normal controls. This difference is statistically significant (p<0.001).

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TOTAL PROTEINS</th>
<th>ALBUMIN</th>
<th>GLOBULINS</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Controls</td>
<td>Mean</td>
<td>71.4</td>
<td>34.2</td>
</tr>
<tr>
<td>(n = 39)</td>
<td>SD</td>
<td>5.8</td>
<td>5.1</td>
</tr>
<tr>
<td>Sicklers</td>
<td>Mean</td>
<td>73.9</td>
<td>37.3</td>
</tr>
<tr>
<td>(n = 62)</td>
<td>SD</td>
<td>8.2</td>
<td>7.7</td>
</tr>
</tbody>
</table>

Controls = HbAA  
Sicklers = HbSS

<table>
<thead>
<tr>
<th>GROUP</th>
<th>CONTROLS</th>
<th>SICKLERS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 39</td>
<td>n = 62</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/l)</td>
<td>2.9 ± 0.9</td>
<td>3.1 ± 1.1</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.5 ± 0.1</td>
<td>0.8 ± 0.1*</td>
</tr>
<tr>
<td>Phospholipids (mmol/l)</td>
<td>50.5 ± 13.2</td>
<td>50.7 ± 19.4</td>
</tr>
<tr>
<td>Non-esterified fatty acids (mmol/l)</td>
<td>1.2 ± 0.3</td>
<td>1.3 ± 0.4</td>
</tr>
</tbody>
</table>

Controls = HbAA  
Sicklers = HbSS

*\(p<0.001\) compared with controls
It seems, therefore, that the poor nitrogen absorption and utilisation found in sickle cell anaemia does not compromise serum protein synthesis, either qualitatively or quantitatively. Thus even though protein absorption is impaired in the sickle cell patient, there is probably enough protein absorbed to guarantee the synthesis of serum proteins.

The most common disturbances in lipid metabolism involve the deposition and mobilization of triglycerides in fat depots, leading to obesity when deposition exceeds mobilization, or to cachexia when mobilization exceeds deposition. Sickle cell patients tend to be skinny and have very little subcutaneous fat deposition. This might be due to excessive fat mobilization for the production of energy in the face of poor absorption of sugars. The excessive fat mobilization could also be due to the increased BMR and the frequent intercurrent infections found in sickle cell anaemia. Thus, increased fat mobilization for the supply of energy may be the cause of the relatively higher than normal levels of triglycerides found in the sickle cell patients.

(c) Phospholipids

The mean phospholipid level in the sickle cell patients was similar to that found in the normals, and these once again, were the lower limit of the normal range of 1.9-4.9 mmol/l quoted for individuals in advanced societies. This is not surprising because serum phospholipid bears a close relationship to serum cholesterol.

(d) Non-esterified Fatty Acids (NEFA)

The mean NEFA value in our sickle cell patients is not significantly different from the mean value for the normal controls. These values are however above the normal range of the normal range of 300-480 μmol/l quoted in the literature.

Discussion

The major fraction of total serum proteins is synthesized by the liver cells. Thus, analysis of the various serum proteins may provide helpful insight into the nature and extent of liver disease. Many non-hepatic factors also affect the metabolism of these proteins and decreased synthesis of albumin, for example, can result from an inadequate amino acid supply, as in starvation, protein – calorie malnutrition, or malabsorption syndromes.

Serum proteins in sickle cell anaemia were analysed because of suspicion that the decreased intestinal absorption of dietary protein and the poor nitrogen utilization that have been found in these patients may affect the synthesis of the serum proteins. Additionally, it was thought that the frequent inter-current infections seen in sickle cell anaemia may cause quantitative changes in the serum protein electrophoretic pattern. These suspicions have not been borne out by the evidence available in our results.

The results of serum protein analysis show that there is no significant difference in the serum proteins of the sickle cell patients compared with the normal controls. Indeed, the total serum protein concentrations in the adolescent sickle cell patients were similar to levels found in healthy adult Ghanaians. And, in a study of pre-school Nigerian children with sickle cell trait (HbAS), da Rocha Afodu found that these children had serum protein levels which were similar to levels found in normal (HbAA) children.
Acknowledgement

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References