Plasma Protein Patterns in Sudanese Patients with Recurrent Malaria Using Protein Electrophoresis

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Abstract: Malaria is a parasitic disease in tropical and subtropical climates. It causes a number of life-threatening complications including cerebral malaria, pulmonary edema, organ failure (kidneys, liver, or spleen), hypoglycemia and anemia. The study was conducted on 30 adult’s patients with recurrent falciparum malaria and 41 adults healthy individuals at New Halfa (Sudan) during the period from 2008 to 2010. The aim of the study was to evaluate the effect of recurrent falciparum malaria on the plasma protein patterns. Serum protein was analysed by electrophoresis and photometric method. The mean values in g/dL ± SD of the different patterns in the patients group are as follows; Total protein: (7.34 g/dL ± 0.59), Albumin: (3.32 g/dL ± 0.48), α-1: (0.55 g/dL ± 0.27), α-2: (1.45 g/dL ± 0.50), β-1: (1.02 g/dL ± 0.32), β-2: (0.70 g/dL ± 0.32), and γ globulins: (0.78 g/dL ± 0.53). Only nine sera of the patients group were found to show β-2 fraction (mean 0.70 g/dL SD ± 0.32). The mean values in g/dL± SD of the different patterns in the control group are as follows; Total proteins: (7.29 g/dl ± 0.61), albumin: (4.01 g/dl ± 0.54). α-1: (0.27 g/dl ± 0.12), α-2: (1.26 g/dl ± 0.46), β-1: (0.94 g/dl ± 1.02), β-2: (0.57 g/dl ± 0.18), and γ globulins: (0.84 g/dl ± 0.18). The study revealed that there was a significant difference in the albumin and α-1 levels between the patients and the control (P value = 0.000). In conclusion, falciparum malaria group showed lower albumin higher α-1 levels when compared to the control.

Keywords: electrophoresis, Recurrent Malaria, New Halfa

INTRODUCTION:
Malaria is found in tropical and subtropical climates where the parasites can live[1]. A number of life-threatening complications can be caused by this disease. These complications include cerebral malaria [1], pulmonary edema [2], organ failure (kidneys, liver, or spleen) [3], hypoglycemia and anemia [2].

Nearly all proteins are synthesized in the liver. Synthesis and maintenance of proteins in circulation is assessed by the measurement of the total serum protein concentration. Hepatic failure is a cause of decreased serum protein [4]. Serum total protein is not a sensitive measure of hepatic failure because proteins differ in their biological half-lives [5]. Hypo proteinemia results from deficient synthesis due to hepatic failure and malnutrition, or from protein loss due to kidney involvement [3].

Hyper proteinemia is caused by protein concentration due to dehydration and decreased blood volume and by overproduction of immunoglobulins due to infections and neoplasms [3, 5].

The synthesis of acute-phase reactants proteins (CRP, ferritin, ceruloplasmin, haptoglobin, and α-1 antitrypsin) increases rapidly in response to inflammation [6, 7]. Changes in the concentrations of these proteins can be detected by serum protein electrophoresis [8].
Components of Serum Protein Electrophoresis:

**Albumin:**
Albumin comprises from 55.8% to 65% of the total human serum protein concentration. Synthesized in the liver [9] at a capacity of 120 mg/kg daily the synthetic rate can be doubled when serum albumin falls [10].

**The Globulins:**

a) **Albumin – α 1- Interzone:**
The predominant protein in this zone is α 1–lipoprotein (high density lipoprotein). A decrease can be caused by renal and liver diseases, and severe inflammation. Increased in severe alcoholism, in young people during puberty and in women during pregnancy [11].

b) **α 1 –Zone:**
AFP, α 1– glycoprotein, thyroid-binding globulin, and transporting are the constituents of α1– protein fraction. A decrease in this band is seen in nephrotic syndrome and liver cirrhosis. Free light chains may bind to and retard the α1- band and thus lead to a decreased α 1- zone, whereas acute-phase reactants may increase the α 1– protein band [12].

c) **α 1 – 2– Interzone:**
This zone is composed of tow bands; α 1–antichymotrypsin and vitamin D binding protein. The zone increase in early inflammation due to an increase in α 1 –antichymotrypsin [13].

d) **α 2 –Zone:**
This zone is composed of Ceruloplasmin, α 2 – macroglobulin, and haptoglobin. Haptoglobin decrease the intensity of the zone in hemolytic anemia (haptoglobin binds with free hemoglobin and removed by phagocytes) and increases the intensity during inflammation [14]. Higher levels are shown in children and elderly people. The zone may be raised in liver cirrhosis, diabetes mellitus, and malignant tumors [15].

e) **α 2 – Interzone:**
High levels of pre- α 2- lipoprotein are found in Frederickson type II hyper-cholesterolemia and in nephritic syndrome (large size) [14].

f) **β –Fraction:**
This band is separated by higher resolution techniques into α β 1 and a β 2 –band. Transferrin comprises the β 1- band, increase in iron-deficiency anemia due to elevated levels of free transferrin, pregnancy and estrogen therapy. Complement protein 3 (C3) and β– lipoprotein form the β 2– band. IgA, IgM, and sometimes IgG also can be identified in the β2- fraction [13].

g) **γ- Fraction:**
Most of the γ –band is made up by the various immunoglobulin of the classes (IgG, IgA, IgM, IgD and IgE), but they can also be found in the β – γ – and β –regions, and may occasionally extend into the α2- globulin area. The γ- zone is decreased in agamma globulinemia and hypo-gammaglobulinemia syndromes. It is increased in malignant lymphoma, Waldenström’s macroglobulinemia, Hodgkin’s disease, chronic lymphocytic leukemia, chronic infections, liver cirrhosis, amyloidosis, and rheumatological, granulomatous and connective tissue disorders [16].

**Rationale:**
- Electrophoresis is important in evaluating the overall pattern of change in one single test.
- Relatively little is known about the procedure and interpretation of results of electrophoresis in Sudan.
- Training in electrophoresis will indeed extend our experience in this field, allowing most institutions to carry on the same procedure.
- Performing electrophoresis locally will decrease the cost of investigation compared to the high cost when doing it outside the Sudan.

**General objective:**
To study the serum protein patterns in Sudanese adults with endemic diseases (recurrent malaria) using electrophoresis.

**Specific objectives:**
To
1. Introduce the protein electrophoresis technique in Sudan. This will significantly improve the chance of resolving a complex mixture of proteins into individual constituents, and so improve diagnosis.
2. Provide new information (our own data) on the plasma protein patterns in Sudan, and to compare the results with those obtained from some countries.
3. Determine the impact of some diseases (mainly endemic) on the plasma protein concentration and fractions.
4. Evaluate the performance of the test regarding diagnostic and prognostic parameters in plasma protein.

**MATERIALS AND METHODS:**

**Study Design:**
Cross- sectional, case control study.

**Study Area:**
This study had been conducted in New Halfa, Sudan.
Study Duration:
From July 2008 up to 2010

The Study Groups:
A. Patients group (recurrent malaria):
Sample size is 30 adults with recurrent malaria attending New Halfa Hospital. Selection of patients was based on the direct microscopy on the blood film stained with Geimsa stain and ICT test for malaria antigen.

B. The control group (Healthy individuals):
Sample size is 41 healthy individuals. Individuals with diseases, or taking drugs known to affect serum protein levels or electrophoretic patterns were excluded. Among drugs known to affect serum protein levels or electrophoretic patterns are; Phenytoin, procaainamide, oral contraceptives, methadone, therapeutic gamma globulin, aspirin, bicarbonates, chlorpromazine, corticosteroids, and neomycin.

A. Laboratory analysis:
Five milliliters of blood samples were collected from each individual, sera were separated and analysed immediately for:

B. Serum protein electrophoresis (SPE):
Serum protein electrophoresis (SPE) for the participants and reference sera was undertaken using the Biotec Fischer Protein kit on the Filippo system (Biotec Fischer W. Germany).

C. Serum Total Protein:
Serum total protein concentrations were measured photo metrically using the Biuret reaction, on BA Semi- Auto chemistry analyzer. Results were compared with the published reference range.

Statistical Analysis:
Statistical analysis was performed by the statistical software SPSS 16. The data were presented as mean ± SD, and the range. The means of the patients and the control protein patterns were compared using ANOVA test. P ≤ 0.05 was considered statistically significant

RESULTS:
A. Patients:
The mean values ± SD of the different patterns in this group are as follows; total proteins (7.34 g/dL ± 0.59), albumin (3.32 g/dL ± 0.48), α1 (0.55 g/dL ± 0.27), α2 (1.45 g/dL ± 0.50), β1 (1.02 g/dL ± 0.32), β2 (0.70 g/dL ± 0.32), and γ globulins (0.78 g/dL ± 0.53).

Only nine sera of this group were found to show β-2 fraction (mean 0.70, SD ± 0.32).

Table-1: Mean values, range and SD of the different protein patterns of the patients group

<table>
<thead>
<tr>
<th>Patterns</th>
<th>No</th>
<th>Minimum (g/dl)</th>
<th>Maximum (g/dl)</th>
<th>Mean (g/dl)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>30</td>
<td>6.0</td>
<td>8.3</td>
<td>7.34</td>
<td>0.59</td>
</tr>
<tr>
<td>Albumin</td>
<td>30</td>
<td>2.6</td>
<td>4.7</td>
<td>3.32</td>
<td>0.48</td>
</tr>
<tr>
<td>α1</td>
<td>30</td>
<td>0.2</td>
<td>1.0</td>
<td>0.55</td>
<td>0.27</td>
</tr>
<tr>
<td>α2</td>
<td>30</td>
<td>0.3</td>
<td>2.7</td>
<td>1.45</td>
<td>0.50</td>
</tr>
<tr>
<td>β1</td>
<td>30</td>
<td>0.5</td>
<td>1.6</td>
<td>1.02</td>
<td>0.32</td>
</tr>
<tr>
<td>β2</td>
<td>9</td>
<td>0.2</td>
<td>1.1</td>
<td>0.70</td>
<td>0.32</td>
</tr>
<tr>
<td>γ–globulin</td>
<td>30</td>
<td>0.2</td>
<td>1.9</td>
<td>0.78</td>
<td>0.52</td>
</tr>
</tbody>
</table>

B. CONTROL:
The mean values in g/dL ± SD of the different patterns in this group are as follows; total proteins (7.29 g/dL ± 0.61), albumin (4.01 g/dL ± 0.54), α1 (0.42 g/dL ± 1.06), α2 (1.26 g/dL ± 0.46), β-1 (0.94 g/dL ± 1.02), β-2 (0.57 g/dL ± 0.18), and γ globulins (0.84 g/dL ± 0.18).

Table-2 Mean values, range and SD of the different protein patterns of the control group

<table>
<thead>
<tr>
<th>Protein fractions</th>
<th>Number</th>
<th>Minimum (g/dl)</th>
<th>Maximum (g/dl)</th>
<th>Mean (g/dl)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>41</td>
<td>5.9</td>
<td>8.3</td>
<td>7.29</td>
<td>0.61</td>
</tr>
<tr>
<td>Albumin</td>
<td>41</td>
<td>3.1</td>
<td>5.2</td>
<td>4.01</td>
<td>0.54</td>
</tr>
<tr>
<td>α1</td>
<td>41</td>
<td>0.1</td>
<td>0.7</td>
<td>0.27</td>
<td>0.12</td>
</tr>
<tr>
<td>α2</td>
<td>41</td>
<td>0.4</td>
<td>2.1</td>
<td>1.26</td>
<td>0.46</td>
</tr>
<tr>
<td>β1</td>
<td>41</td>
<td>0.2</td>
<td>1.6</td>
<td>0.94</td>
<td>1.02</td>
</tr>
<tr>
<td>β2</td>
<td>7</td>
<td>0.3</td>
<td>0.8</td>
<td>0.57</td>
<td>0.18</td>
</tr>
<tr>
<td>γ</td>
<td>41</td>
<td>0.2</td>
<td>1.8</td>
<td>0.84</td>
<td>0.48</td>
</tr>
</tbody>
</table>
C. Comparison between patients and control groups:

The study revealed that there was a significant difference in the albumin and alpha-1 levels between the patients and the control. (Table-3)

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP</td>
<td>Between Groups</td>
<td>.037</td>
<td>1</td>
<td>.037</td>
<td>.103</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>24.986</td>
<td>69</td>
<td>.362</td>
<td></td>
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<td>ALB</td>
<td>Between Groups</td>
<td>8.221</td>
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<td>8.221</td>
<td>30.832</td>
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<tr>
<td></td>
<td>Within Groups</td>
<td>18.398</td>
<td>69</td>
<td>.267</td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>Between Groups</td>
<td>1.375</td>
<td>1</td>
<td>1.375</td>
<td>35.881</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>2.644</td>
<td>69</td>
<td>.038</td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>Between Groups</td>
<td>.619</td>
<td>1</td>
<td>.619</td>
<td>2.677</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>15.953</td>
<td>69</td>
<td>.231</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>Between Groups</td>
<td>.107</td>
<td>1</td>
<td>.107</td>
<td>.165</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>44.568</td>
<td>69</td>
<td>.646</td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>Between Groups</td>
<td>.065</td>
<td>1</td>
<td>.065</td>
<td>.916</td>
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<tr>
<td></td>
<td>Within Groups</td>
<td>.994</td>
<td>14</td>
<td>.071</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>Between Groups</td>
<td>.059</td>
<td>1</td>
<td>.059</td>
<td>.233</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>17.321</td>
<td>69</td>
<td>.251</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION:

The decrease in serum albumin observed in the patient group in this study may be due to both the role of human serum albumin in the intraerythrocytic development of parasites [17, 18] and also due to the release of cytokines TNF-α and IFN-γ during the pro-inflammatory response against the asexual blood stages of human malaria [19].

α-1 acid glycoprotein (AGP) is the principal component of α-1 zone in protein electrophoresis. It is an acute phase protein [20]. The increase of α-1 zone of the patient group of this study (0.55 g/dL± 0.27) compared to the control group (0.27 g/dL± 0.12), (P. value 0.000) may be due to the acid glycoprotein produced by acute phase reaction (immediate response) of the disease. This is in agreement with previous studies which concluded that when in human an acute phase response is induced by P. falciparum-infected RBCs, serum levels of α1-acid glycoprotein (AGP) peak to inhibit the growth of P. falciparum [21].

CONCLUSION:

In recurrent malaria, the reduction in serum albumin may be due to the role of human serum albumin in the intraerythrocytic development of malaria parasites and the cytokines TNF-α and IFN-γ released during the proinflammatory response against the asexual blood stages of human malaria. The significant increase in serum globulins in malaria group is due to the increase in α-1 zone caused by the increase in the acute phase reaction (immediate response) of the disease.

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