Metal binding acute phase proteins and trace elements in Nigerian children with urinary schistosomiasis.

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Abstract

Fifty-five Nigerian children with urinary schistosomiasis (USS) and 34 apparently healthy children of matched ages and sexes were considered for this study. In these children, metal binding acute phase proteins (transferrin and caeruloplasmin) and trace elements were measured by single radial immunodiffusion and atomic absorption spectrophotometry respectively. The result shows that Cd, Mn, albumin and transferrin were reduced (p<0.01, p>0.20, p<0.01 and p<0.01 respectively) while Fe, Cu and caeruloplasmin were raised (p<0.01, p<0.01 and p<0.01 respectively) in USS subjects when compared with the controls. Low levels of transferrin might be responsible for raised level of plasma Fe, which is in turn modulated by caeruloplasmin. It may be concluded that trace elements and antioxidants are involved in the pathogenesis of USS.

Key words: Schistosomiasis, elements, proteins, antioxidants, children.

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INTRODUCTION

There are various effector mechanisms needed to attack different stages of schistosome infections. Schistosomal worms are damaged at high levels of antibodies and complement factors but low levels of antibody sensitizes neutrophils, macrophages, eosinophils and platelets for antibody-dependent cell-mediated cytotoxicity. Neutrophils and macrophages act by releasing toxic reactive oxygen species and nitric oxide (NO) whereas eosinophils damage the schistosome tegument by the release of major basic proteins (1).

Reactive oxygen species and NO which are parts of immune responses against *Schistosoma haematobium* parasites may also lead to consumption/reduction of antioxidants, or when produced in excess amount may damage surrounding cells and autotoxicity in some cases (2). Prostaglandin E2 (a critical immunomodulator) is produced in the pathway involving the production of NO via L-arginine pathway (3). The most potent of the reactive O₂ species (ROS) are OH⁻, O₂⁻, HO₂⁻, HOCl and H₂O₂, all of which are generated during oxidative burst by phagocytic neutrophils and macrophages. These ROS are also released in relatively high concentrations during inflammation (4).

Inflammation is a common phenomenon in urinary schistosomiasis. However, there are several naturally occurring compounds (antioxidants) that can inactivate/lower these ROS. Antioxidants may be preventive type (such as catalase, transferrin, caeruloplasmin and albumin) or chain breaking type (such as superoxide dismutase, uric acid, vitamin C and bilirubin) (5).

Previous studies showed that during USS, there is inflammation and production of ROS and NO by phagocytes. It is likely therefore that the levels of antioxidants and trace metals may be affected by *Schistosoma haematobium* infection. The present study is designed to assess the levels of preventive antioxidants and trace elements in Nigerians with USS.

MATERIALS AND METHODS

**USS Subjects:** Before the commencement of the study ethical approval was obtained from UCH/UI Ethical Review Committee. A total of 84 Nigerian (aged between 4-15 years) children were involved in the study. Among these are 54 children with USS. *Schistosoma haematobium* infection was identified by location of terminal spined eggs in the urine as previously described (6).

**Controls:** Forty-three *Schistosoma haematobium* free subjects who were age and sex matched with the USS subjects formed the control group. The subjects (USS and controls) with heavy malaria, microfilaria and intestinal helminth infections were excluded from the study. Microfilaria was examined in thick blood films stained with Giemsa while intestinal helminthes eggs were examined in normal saline preparation of faecal samples stained with Dobell iodine (6).

**Determination of acute phase proteins:** The motive behind the study was explained to each subject before sample collection. Five (5) ml of venous blood was withdrawn from each subject. Two ml (2ml) of the blood was put into a plain glass universal bottle for the determination of acute phase proteins, while the remaining 3 ml was put into bottle containing lithium heparin for the determination of trace elements. Serum concentrations of transferrin and caeruloplasmin were estimated using single radial immunodiffusion method (7) while albumin was determined using bromocresol green solution as described by Varley (8). This was based on the principle that peptide linkages in the amino acids which make up a protein are capable of reacting with copper in alkaline solution to produce a violet colour (8).

**Plasma concentrations of trace metals:** This was determined by atomic absorption spectroscopy as described (9). In the vapourised ground state
(unexcited), atom of a trace metal absorb light of the same wavelength as that emitted by the metal in the excited state. The amount of light absorbed is proportional to the trace metal in the solution (9).

Statistical analysis: Statistical analysis was performed by calculating the mean, standard deviation and Students $t$ test.

RESULTS

Table 1 showed that iron and copper were significantly raised ($p<0.01$ in each case) in USS subjects compared with the controls. Both cadmium and manganese were reduced in USS subjects but the difference was significant in the level of cadmium only. Caeruloplasmin was significantly raised ($p<0.01$) in USS while both albumin and transferrin were significantly reduced ($p<0.01$ in each case) in USS subjects compared with the controls.

DISCUSSION

The results of the study showed significantly reduced levels of albumin and transferrin and significantly elevated level of caeruloplasmin in USS subjects compared with the controls. This is a confirmation that inflammation is present in USS subjects and this might have been caused by tissue damage resulting from schistosome eggs, migrating larva and adult worms. Acute phase proteins are synthesized in the liver hence their increase in the plasma may be deduced from increased synthesis (10). This suggests that adequate synthetic function of the liver is maintained in USS subjects.

Decreased level of transferrin detected in USS subjects may be due to its consumption by schistosome larva as well as inflammatory responses during tissue damage by schistosome eggs. Schistosome larva uses transferrin and albumin as growth factors (7). Also transferrin is a negative acute phase reactants whose level decreases with inflammation. Under normal condition most of the iron are bind to transferrin leaving a small concentration of free iron in the plasma. Low level of transferrin in the present study could mean that, there might be excess unbound iron in the plasma. This might explain the significantly raised concentration of iron observed in USS subjects considered for this study.

Table 1: Concentrations of trace elements and metal binding acute phase proteins in urinary schistosomiasis subjects.

<table>
<thead>
<tr>
<th></th>
<th>USS(n=55)</th>
<th>Controls(n=34)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe (mg/dL)</td>
<td>159.9 ± 4.9</td>
<td>156.5 ± 3.5</td>
<td>3.78</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cd (mM/L)</td>
<td>32.1 ± 7.5</td>
<td>36.2 ± 5.4</td>
<td>2.97</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cu (µg/ml)</td>
<td>146.5 ± 26.1</td>
<td>122.1 ± 23.7</td>
<td>4.51</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mn (mg/dL)</td>
<td>4.83 ± 3.3</td>
<td>5.35 ± 2.8</td>
<td>0.79</td>
<td>&gt;0.20</td>
</tr>
<tr>
<td>Albumin(g/dL)</td>
<td>3.37 ± 0.3</td>
<td>3.98 ± 0.41</td>
<td>7.63</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Caeruloplasmin(mg/dL)</td>
<td>35.9 ± 8.1</td>
<td>31.2 ± 5.8</td>
<td>3.36</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Transferrin(mg/dL)</td>
<td>200.1 ± 8.4</td>
<td>206 ± 3.3</td>
<td>4.54</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are in $x \pm s.d$
The relationship between iron level and the effects of iron on the virulence of specific pathogens and host-immune functions is complex. Enhanced growth of infectious agents is a major concern for iron supplementation in iron deficient individuals (10). Direct alterations in immune function are associated with excess iron and alteration in neutrophil defense in haemodialized patients with iron overload have been reported (11). Excessive production of toxic O\textsubscript{2} species in response to high cellular iron contribute to decreased phagocytosis (12). Decreased natural killer cell (NKC) activity in response to multiple transfusions in thalassemia patients has been reported (13). Studies described elsewhere-detected slightly reduced leucocyte ingestion and digestion rates in Nigerian USS subjects (6). The raised iron level may explain these observations.

Caeruloplasmin is reported to be involved in iron metabolism and copper transport (14). Significantly elevated level of caeruloplasmin in USS subjects compared with the controls may be as a result of inflammation and additional need for caeruloplasmin as a result of copper and iron increases. Caeruloplasmin is an O\textsubscript{2} radical scavenger and polymorphonuclear phagocytes produce free O\textsubscript{2} radicals during intracellular killing of ingested particles (15). Excess free O\textsubscript{2} radicals will require corresponding amount of caeruloplasmin (among other O\textsubscript{2} radical scavengers) for its clearance. Therefore, increased caeruloplasmin in the USS subjects may be concluded since it will control the level of excessive concentration of plasma iron and copper in USS subjects. Copper is among the most toxic of the trace elements and was raised in USS subjects. Excessive circulatory copper result in the generation of reactive O\textsubscript{2} radicals and peroxides, thus there is need for its removal from biological systems.

Manganese activates glycosyl transferases that are necessary for polysaccharide and glycoprotein synthesis. Manganese is also involved in cholesterol and protein biosynthesis and alleviation of blood clotting defects (16). Insignificant reduction in manganese levels in USS subjects could be supported by reduced synthesis of protein as indicated by significant reduced level of albumin. This reduction could be explained by raised level of iron since a study showed that individuals with low manganese status may be at risk of excess iron than those with adequate amount (17).

Low concentration of cadmium was detected in USS subjects. High concentration of cadmium is toxic to polymorphonuclear neutrophils (18), decreased phagocytic function of macrophages, increased production of NO, O\textsubscript{2}\textsuperscript{-} and H\textsubscript{2}O\textsubscript{2} but most of these effects are alleviated by addition of selenium (19). The low level of cadmium in USS subjects is not therefore too dangerous.

Albumin was found to be reduced in USS subjects. This result is in line with Hussain et al (20) where hypoalbuminaemia in schistosomiasis was reported. The cause of reduced albumin may be one of the following reasons: protein loss in the urine, being a negative acute reactant and consumption by schistosome larva. Albumin regulates the oncotic pressure of the blood; it binds and transports metal ions, drugs, amino acids and toxic wastes among others. The consequence of albumin deficiency in USS is detrimental since free and toxic forms of these substances may be predominant in the blood.

This study suggests a possible involvement of trace elements and antioxidants in the pathogenesis of USS.

REFERENCES


