















Original Research

# Haptoglobin Phenotypes in School-age Children Infected with Schistosoma haematobium: A case-control study

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## Abstract

**Background:** Acute phase proteins (APPs), including haptoglobin (Hp), are a large and varied group of plasma proteins that can be used as biomarkers for disease diagnosis/detection/severity. **Objective:** The main objective was to assess the levels of haptoglobin (Hp) in serum and detect Hp phenotypes using polyacrylamide gel electrophoresis in 100 school-aged children infected with Schistosoma haematobium compared with 60 healthy control. **Methods:** We conducted a case-control study on 160 schoolchildren (ages 9-15 years) recruited from Tayba Eltejanja village, Sinar state, Sudan. Unrelated children with Schistosoma haematobium (case group 100) and unrelated healthy children (control group 60) were included, while those with both Schistosoma types were excluded. The enrolled subjects were evaluated for the levels of Hp and its phenotypes as early markers for disease severity. ELISA quantified biochemical analysis for the serum Hp level. Hp phenotypes were determined, and their frequency was compared between cases and controls. **Results:** The Hp 2-1 was the highest frequency among cases and controls 72/143 (50.3%), followed by Hp 2-2 (28%), while Hp 1-1 phenotype was 22%. The Hp 2-1 and Hp 2-2 frequency did not differ significantly between cases and controls, considering the Hp 1-1 as the reference group. Multiple comparisons were executed between Hp phenotypes; the differences between these groups were not statistically different. The disease severity was set according to the egg count (Group I: moderate infection  $\leq 35/10$  ml, Group II: severe infection  $\geq 36/10$  ml), the Hp 2-2 was four times more frequent in cases with severe infection considering Hp 1-1 as the reference phenotype (OR=3.85, 95% CI: 1.044-14.24). Confirming the result, Hp 2-2 was significantly associated with disease severity than Hp 1-1 and Hp 2-1 (OR= 3.77 95% CI: 1.39-10.20). **Conclusion:** There was evident that the egg count increased in subjects with Hp 2-2 indicating severe infection.

**Keywords:** schistosoma haematobium; haptoglobin; school-age children; haptoglobin phenotypes; haptoglobin 2-2

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## INTRODUCTION

Schistosomiasis is caused by infection with blood flukes of the genus *Schistosoma*. It has been estimated that *Schistosomiasis* infects more than 230 to 250 million people per year and is the cause of 280,000 deaths annually.<sup>27</sup> It is a typical tropical and sub-tropic blood infection.<sup>20</sup> Nevertheless, it is one of the neglected tropical diseases. The African region is the most affected, with 42 countries endemic for the infection.<sup>2</sup> Urinary schistosomiasis was reported in 53 countries across Africa and the Middle East, where it is responsible for most *Schistosoma*-associated pathology.<sup>26</sup> In Sudan, the risk for *Schistosoma haematobium* is widespread in the different regions, and school-age children were reported to be at a higher risk for *Schistosoma haematobium* infection (Ahmed et al., 2009). It is the only blood fluke that infects the urinary tract, causing urinary schistosomiasis. Left untreated can result in bladder fibrosis or cancer.<sup>34</sup>

Haptoglobin (Hp) is a positive acute-phase protein (APP) that forms 1% of the total plasma mass. It is synthesized mainly by the hepatocytes in the liver. Cytogenetic Location: 16q22.2.<sup>31</sup> Hp is the major hemoglobin-binding protein and an APP, the expression of which increases during inflammation.<sup>36</sup> The primary biological function of Hp is to bind free hemoglobin released from erythrocytes, stabilize it (i.e., prevent the dissociation of the heme group), and hence prevent its oxidative damage. The haptoglobin-hemoglobin complex formation speed its removal through the reticuloendothelial system, mainly in the spleen.<sup>35</sup> On the other hand, free hemoglobin (Hb) can damage renal tissues, so any reduction in Hp concentration or Hb binding ability may result in increased renal damage. Free Hb may also be a source of iron for pathogenic bacteria, and a decrease in Hp concentration or Hb binding ability may aggravate bacterial infection.<sup>12</sup>

Humans are polymorphic for Hp, with three significant phenotypes: Hp 1-1, Hp 2-2, and the heterozygous Hp 2-1.<sup>3</sup> Hp phenotyping is generally based on electrophoretic separation

of the different subtypes according to their molecular size in an appropriate gel medium.<sup>32</sup> The Hp 1-1 protein migrates as a single fast band, while the Hp 2-2 protein shows a series of slow bands. The Hp 2-1 protein displays a series of Hp 2-2 slow bands and a weak Hpn1-1 band.<sup>11</sup>

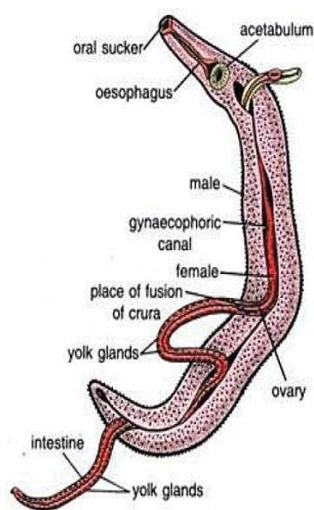
Hp is an integral part of the inflammatory acute phase, a response where its synthesis is stimulated by interleukin (IL-6). The resulting increase in Hp levels can result in feedback dampening the severity of the initial acute phase reaction.<sup>4</sup> Downregulation may also relate to the binding of the Hp-Hb complex by macrophage CD163. This leads to the secretion of the inflammatory cytokine IL-10 and the breakdown products of heme, which also have potent anti-inflammatory activity.<sup>23</sup> The release of IL-10 induces CD163 synthesis and haem oxygenase-1 via an autocrine mechanism. Thus, there is a coordinated regulation of Hb uptake and breakdown.<sup>30</sup> There are differences in the binding of Hp types by the CD163 receptor. The Hp (2-2)-Hb complex has a 10-fold higher functional affinity for CD163 than the Hp (1-1)-Hb complex, probably due to the clustering effect of several binding sites in the multimeric ligand complex. This may increase the efficiency of the macrophage in clearing the Hp (2-2) Hb complexes from the plasma when compared with that of the Hp (1-1) Hb complex binding to CD163.<sup>14</sup> However, studies in cultured CHO cells transfected with CD163 have demonstrated more rapid uptake of the Hp (1-1)-Hb complex than the Hp (2-2)-Hb complex.<sup>5</sup> Both high and low plasma Hp levels are associated with different clinical conditions. Studies have shown that functional differences between these phenotypes have essential consequences in several pathological disorders (e.g., cardiovascular disease, autoimmune disorders, infectious disease), making phenotype determination of potential use in the clinical field.<sup>38</sup> In addition, Hp 2-2 is over-expressed among subjects with advanced tuberculosis.<sup>17</sup> In subjects with a human immune virus (HIV), Hp 2-2 is associated with a higher mortality rate and a worse prognosis for subjects with other Hp phenotypes. People with Hp 2-2 have a more robust antibody

## Schistosoma haematobium

**Background** Acute phase proteins (APPs), including haptoglobin (Hp), are used as biomarkers for disease diagnosis/detection/severity.

**Objective** Assess the levels of haptoglobin (Hp) in serum and detect Hp phenotypes in 100 school-aged children infected with *Schistosoma haematobium* compared with 60 healthy control.

**Methods** A case-control study on 160 schoolchildren (ages 9-15 years).



**Results** The Hp 2-1 was the highest frequency among cases and controls 72/143 (50.3%), followed by Hp 2-2 (28%), while Hp 1-1 phenotype was 22%. Hp 2-2 was significantly associated with disease severity than Hp 1-1 and Hp 2-1 (OR= 3.77 95% CI: 1.39-10.20).

**Conclusion** There was evident that the egg count increased in subjects with Hp 2-2 indicating severe infection with *Schistosoma*.

**Keywords** *Schistosoma haematobium*; haptoglobin; school-age children; haptoglobin phenotypes; haptoglobin 2-2

response to typhus and tetanus vaccination.<sup>18</sup>

### Rationale

In Sudan, a study has revealed that Hp1-1 percentage frequency was significantly higher in infected individuals than in healthy control individuals. Hence, it was concluded that the Hp1-1 phenotype may increase the susceptibility to Schistosoma parasites infection in central Sudan among agricultural laborers.<sup>39</sup> Hence, we wanted to investigate if the same association exists among school children.

### Objective

The main objective of this study was to measure the levels of haptoglobin (Hp) in serum and obtain Hp phenotypes using polyacrylamide gel electrophoresis in 100 school-age children infected with *S. haematobium* compared with 60 healthy control ages ranging between 9-15years.

### Ethical

The study ethics committee approved the faculty of Medicine, University of Gezira-Wad Madani, Sudan, from the Ministry of Health in Sinnar State-Sudan; and the Administration of General Education, Sinnar State-Sudan.

### METHODS

We have conducted a case-control study on 160 schoolchildren (ages 9-15 years) recruited from Tayba Eltejanian village, Sinar state, Sudan. We have included unrelated children diagnosed with Schistosoma haematobium (case group) and unrelated healthy children (control group). We have **excluded** school children infected with *Schistosoma mansoni* and had a co-infection. 100One hundred of them were infected with *Schistosoma haematobium* representing the case group, and 60 healthy schoolchildren as a control group. We ensured that both groups were matched as closely as possible (e.g., age, gender) to avoid the effects of a confounding variable. Informed consent was obtained from each participant's guardian. The consented and recruited subjects were evaluated for the levels of Hp and its phenotypes as early markers for disease severity and tissue damage. The infected schoolchildren with Schistosoma haematobium infection received a dose of 40 mg/kg praziquantel (PZQ). This dose entails the treatment outcome regarding egg reduction rate (ERR), calculated as the difference of the arithmetic means egg counts (preferred over geometric means) between pre- and post-treatments.

### Data, sample collection, preparation, and storage

Data for this study was obtained from questionnaires and lab analyses for urine and blood samples. **Collection and examination of urine** 10 mL urine samples were collected from the study participants during school day activities during midday noontime to maximize the number of eggs in the urine around midday 10:00 am to 2:00 pm. The urine sample was filtered using a 15-mm polycarbonate filtered r, and a drop of urine was placed for microscopy. The Schistosoma eggs detected and egg count considered a determinant for the severity of infection.

**Blood samples collection and storage:** three ml venous blood samples were collected from each infected child and controls. The blood was centrifuged at 3000 rpm for 5 minutes; separate serum was stored at -20 c until used to estimate serum levels of Hp and Hp phenotypes.

**Biochemical analysis of the Hp level** was quantified by ELISA (Enzyme-Linked Immunosorbent Assay) kit (code 108856). An Huma read the optical density of serum samples at 450 nm. **Hp phenotypes analysis** Hp phenotypes were separated according to the method described (Santoro, Boccazzi, et al. 1982).

### Statistical analysis

Statistical analysis was carried out using the statistical package for social sciences (SPSS version 26). Data were expressed as mean  $\pm$  standard error of the mean (SEM). The Hp phenotypes frequency of diseased and controls were compared using a logistic regression test. Means of the studied parameter according to the Hp phenotypes were compared by ANOVA test in cases, and-value < 0.05 was taken as significant.

### RESULTS

#### The distribution and analysis of HP phenotypes in cases and controls

Frequencies of Hp phenotypes were calculated and presented in [Table 1]. The phenotyping was successful in 143 samples out of 160 (89.3%). The Hp 2-1 was the highest frequency among cases and controls 72/143 (50%) of the total study subjects. They were followed by Hp 2-2 (28%), while Hp 1-1 phenotype was 22%. The logistic regression analysis was conducted on the Hp phenotypes in cases and controls. Table 2 showed that Hp 2-1 and Hp 2-2 frequency did not differ between cases

Table 1. The distribution of Hp phenotypes in cases and controls

Hp phenotypes	Cases (n=89) F (%)	Controls (n=54) F (%)	Total F (%)
1.1	16 (18.0)	15 (28.0)	31 (21.7)
2.1	45 (51.0)	27 (50.0)	72 (50.3)
2.2	28 (31.0)	12 (22.0)	40 (28.0)
Total	89 (100.0)	54 (100.0)	143 (100.0)

Hp: haptoglobin; n: population; F: frequency; %: percent

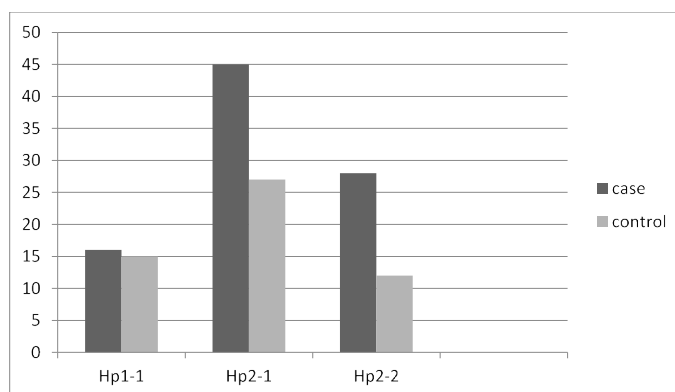


Figure 1. Distribution of HP phenotypes in cases and controls



and controls, considering the Hp 1-1 as the reference group. Multiple comparisons were executed between Hp phenotypes 1-1 versus 2-1 and 2-2, 2-2 versus 2-1 and 1-1, and 2-1 versus 1-1 and 2-2: the differences between these groups were not statistically different.

### Hp phenotypes related to disease severity

(group I with egg count  $\leq$  35/10 mL, and group II with egg count  $\geq$  36/10 mL)

The disease severity was set according to the egg count (Group I: moderate infection  $\leq$  35/10 ml, Group II: severe infection  $\geq$  36/10 ml); after conducting several classifications, we found that the Hp 2-2 was four times more frequent in patients with severe infection considering Hp 1-1 as the reference phenotype (OR=3.85, 95% CI: 1.044-14.24). The result confirmed that Hp 2-2 was significantly associated with disease severity than Hp

1-1 and Hp 2-1 (OR= 3.77 95% CI: 1.39-10.20). Subjects with Hp 1-1 and Hp 2-1 were less likely to have severe infections [Table 3].

### DISCUSSION

In this study, the Hp phenotypes analysis was successful in 143 samples. Hp 2-1 was the highest frequency among cases and controls, followed by Hp2-2, while Hp 1-1 was the lowest frequency in cases and controls. However, the differences in the frequencies of the three phenotypes were insignificant between cases and controls. Hp1-1 is a better antioxidant and binds more strongly with free hemoglobin than Hp2-2. Because the  $\alpha$ 1-chain which is found in Hp1.1, is small than the  $\alpha$ 2-chain found in Hp2.2 and Hp2.1, the increased antioxidant function of Hp1-1 is thought to confer protection from angiopathies;

Table 2. The analysis of Hp phenotypes in cases and controls

Hp phenotypes	Cases (n=89) F	Controls (n=54) F	OR (CI: 95%)	F value
1-1	16	15	Reference	
2-1	45	27	1.562(0.667-3.659)	0.289
2-2	28	12	2.187 (0.824-5.808)	0.304
<b>Hp phenotypes 1-1 versus 2-1 and 2-2</b>				
1-1	16	15	Reference	
2-1 and 2-2	73	39	1.75 (0.785-3.923)	0.171
<b>Hp phenotypes 2-2 versus 2-1 and 1-1</b>				
1-1 and 2-1	61	42	Reference	
2-2	28	12	1.607 (0.735-3.512)	0.235
<b>Hp phenotypes 2-1 versus 1-1 and 2-2</b>				
1-1 and 2-2	44	27	Reference	
2-1	45	27	1.023 (0.520-2.01)	0.948

Hp: haptoglobin; n: population; F: frequency; %: percent

Table 3. The Hp phenotypes related to disease severity (group I with egg count  $\leq$  35/10 mL, and group II with egg count  $\geq$  36/10 mL)

Hp phenotypes	Group II (n=48) F	Group I (n=41) F	OR (CI 95%)	P-value
1-1	7	9	Reference	
2-1	20	25	1.029 (.326 - 3.247)	0.962
2-2	21	7	3.85 (1.044 - 14.24)	0.043*
<b>Hp phenotypes 2-1 and 2-2 versus 1-1</b>				
1-1	7	9	Reference	0.370
2-1 and 2-2	41	32	1.64 (0.554 - 4.902)	
<b>Hp phenotypes 2-2 versus 2-1 and 1-1</b>				
1-1 and 2-1	27	34	Reference	0.009*
2-2	21	7	3.77 (1.39 - 10.20)	
<b>Hp phenotypes 2-1 versus 1-1 and 2-2</b>				
1-1 and 2-2	28	16	Ref.	0.071
2-1	20	25	0.457 (0.195 - 1.07)	

CI: confidence interval; F: frequency; Hp: haptoglobin; n: population; OR: odds ratio

\*: significance < 0.05

%: percent





however, Hp2-2 is believed to be a significant risk factor in several oxidative stress-related disease states.<sup>24</sup> In comparing Hp phenotypes in cases, the egg count significantly increased in the Hp 2-2 phenotype. It has been reported that Hp2-2 may provide less protection against hemoglobin iron-driven peroxidation.<sup>10</sup> In line with the researcher's findings, Indian patients suffered from *P. falciparum* malaria with complications piloted that the Hp2-2 phenotype was associated with susceptibility to severe *P. falciparum* malaria.<sup>37</sup> Moreover, in a study from Mali, the Hp2-2 phenotype was associated with a higher susceptibility to *Plasmodium falciparum* infection.<sup>29</sup> The incidence of febrile malaria and other childhood illnesses about the Hp genotype in a prospective cohort of 312 Kenyan children scrutinized, the Hp 2-2 genotype was associated with a 30% reduction in clinical malarial episodes.<sup>6</sup> A study from Sudan also studied the association between the Hp phenotypes and falciparum malaria complications, suggesting the role of Hp phenotype I-I in the modulation of the pathophysiology of the disease (Elagib, Kder t al. 998). The Hp1-1 phenotype was more frequent in people infected with *S. mansoni*, *Schistosoma haematobium*, and coinfection group irrespective of the type of infection, suggesting that individuals with Hp1-1 phenotype may be at higher risk of *Schistosoma* parasites infection compared to those carrying Hp2-1 or Hp2-2.<sup>13</sup> It seems this discrepancy is because there was no consideration of the type of schistosomiasis, whereas, in our study, *Schistosoma mansoni* and co-infection were excluded. Egg counts continue to be the gold standard for *Schistosoma haematobium* infection, and infection intensity is thought to reflect the disease's severity.<sup>16</sup>

In this study, comparing three Hp phenotypes, the egg counts were significantly increased in Hp 2-2 than in Hp 2-1 or Hp 1-1 infected children. The relation between eggs count and disease severity was demonstrated in a study that found the immunopathological consequence of *Schistosoma* infections mainly due to the parasite eggs trapped in the host tissue, which releases antigens that stimulate granulomatous responses followed by fibrotic reaction.<sup>28</sup> Likewise, Bichler and his colleagues reported that the disease is caused by the presence of the eggs rather than the worms themselves.<sup>7</sup> In our study, the egg counts (>35/10 ml) were applied as an indicator for the severity of *S. haematobium* infection; we found that children with Hp 2-2 were four times more likely to have severe schistosomiasis than Hp 1-1. Numerous studies found associations between Hp 2-2 and diseases. A study in Ghana established an increased risk of complications for individuals with the Hp 2-2 phenotype. The odds ratio of having difficulties in subjects with diabetes with a Hp 2-2 phenotype was 18.3 times greater than other Hp phenotypes, which was justified by the poor antioxidant activity of the Hp 2-2 phenotype compared to the different phenotypes.<sup>1</sup> American trypanosomiasis was studied in 92 cases and 197 controls; Hp2-2 was more prevalent in patients than in the healthy controls.<sup>9</sup> Furthermore, a study investigated the frequency of polymorphisms in the gene encoding Hp of patients with chronic Chagas disease; it was suggested that the Hp1-1 genotype confers protection due to

the rapid metabolism of the Hp1-1-Hb complex and its anti-inflammatory activity while the presence of Hp2-2 genotype increases susceptibility towards the chronic condition of the disease.<sup>25</sup> A study reported that patients with Hp2-2 have a significantly higher risk for cardiovascular, neurological, and infectious complications.<sup>21</sup> In addition, the Severity of Chronic Obstructive Pulmonary Disease was linked to Hp concentrations and phenotype, especially Hp2-2.<sup>19</sup> The strong association between Hp 2-2 and disease complications might be due to many causes, as reported by the authors. The differences in Hb binding capacities of the Hp phenotypes, shape, and size of the Hp phenotypes, and an increase in redox-active iron in the plasma of humans with the Hp 2-2. In addition to the "clearance" of Hp-Hb complex in the blood circulation after intravascular hemolysis by CD163, which is less effective in Hp 2-2 individuals than other HP phenotypes.<sup>22,5,8,18</sup>

### Strength and weakness

One of the strengths of our study was the use of matching with certain limits to avoid over-matching. Moreover, we applied measures of selection of appropriate controls and matching of both groups to minimize the confounding factors. We ensured that the controls were proper and that both groups were matched as closely as possible (e.g., age, gender) to avoid the effects of a confounding variable. One of the weaknesses of our study may be relevant to the sample size and the one-center study setting.

### The limitations of the study

The quality of the data collected retrospectively may be affected by the recall bias of risk factors.

## CONCLUSION

The Hp levels were significantly higher in diseased cases than in controls, which might indicate the hemolysis and tissue damage associated with schistosomiasis. There was no relation between Hp phenotypes and susceptibility to *Schistosoma haematobium* infection, while egg count was increased in patients with Hp 2-2 phenotype.

## ABBREVIATIONS

- APPs: Acute phase proteins
- CD163: is a scavenger receptor for haptoglobin-hemoglobin complexes
- ELISA: Enzyme-Linked Immunosorbent Assay
- Hb: hemoglobin
- Hp: Haptoglobin
- HIV: human immune virus
- IL: interleukin
- rpm: rotation per minute
- SPSS: statistical package for social sciences



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