Am. J. Trop. Med. Hyg., 47(3), 1992, pp. 291–297

CONSEQUENCES OF *SCHISTOSOMA HAEMATOBIUM* INFECTION ON THE IRON STATUS OF SCHOOLCHILDREN IN NIGER

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Abstract. The relationship between iron status and degree of infection by *Schistosoma haematobium* was studied in 174 schoolchildren from Niger in an area endemic for urinary schistosomiasis. Iron deficiency was defined by a combination of three reliable indicators: a low serum ferritin level combined with a low transferrin saturation, a high erythrocyte protoporphyrin level, or both. Hematuria and proteinuria were found in 76.4% and 79.9% of the children, respectively, while 95.4% excreted eggs (geometric mean egg count of 31.5 eggs per 10 ml of urine). Anemia was observed in 59.7% of the subjects. The prevalence of iron deficiency was 47.1%. Anemia was associated with iron deficiency in 57.7% of the cases. The hemoglobin level and transferrin saturation decreased significantly when the degree of hematuria increased, while prevalence of anemia and prevalence of iron deficiency increased significantly. The hemoglobin level and the hematocrit were negatively correlated with egg count, while prevalence of anemia increased with increasing egg count. This inverse relationship between degree of infection by *S. haematobium* and iron status shows a deleterious consequence of urinary schistosomiasis on nutrition and hematopoietic status, which should be considered in the design of nutrition intervention programs.

Anemia constitutes a major public health problem throughout the world, but is of particular importance in developing countries, especially in Africa. The prevalence of anemia in Africa has been estimated to be 59% in children, 57% in schoolchildren, and 50% in pregnant women.[1] In a tropical context, the causes of anemia are multiple and often combined: deficiency of hematopoietic factors (iron, folates), genetic disorders (sickle cell disease, thalassemia, etc.), and parasitic infections. Although the individual role of these various causes is difficult to assess, iron deficiency is by far considered the most important etiologic factor.[2] However, evaluation of iron status is difficult because many confounding factors (chronic inflammatory syndromes, protein malnutrition, infections) interfere with a determination of the significance of various indicators, such as hemoglobin and hematocrit levels, and hematuria. Therefore, it is essential to use a combination of indicators in this evaluation. Although the relationship between parasitic infections and the hematopoietic status has long been debated, few studies have used sufficient laboratory measurements to provide a clear description of this relationship. So far, only hookworm infections have been demonstrated to be a major determinant of iron-deficient anemia.[3]

The consequences of urinary schistosomiasis on iron status and on anemia have not yet been clearly established. Estimations of daily blood losses due to hematuria, the most frequent symptom of an infection by *Schistosoma haematobium*, vary greatly but they are important enough to potentially affect the iron balance. However, few studies showed a relationship between intensity of infection and hemoglobin level,[4–7] and none between intensity of infection and iron status, although the degree of hematuria is positively correlated with the intensity of infection.[8] The major goal of the present study was to determine the relationship of degree of hematuria and intensity of infection with the hemoglobin level and iron status in schoolchildren living in an area endemic for urinary Schistosomiasis, using a combination of reliable indicators.

MATERIALS AND METHODS

Study population

The study population consisted of 174 schoolchildren from the primary school of Daekena, a village located 100 km west of Niamey, the capital of the Republic of Niger, on the banks of the Niger river. This village is surrounded by rice paddies that provide the main source of revenue. The population is composed of the Djerma and the Bella ethnic groups. The diet consists mainly of millet, rice, and vegetable sauce, to which fish is sometimes added. The climate is Sahelian: a long dry season from September to June, followed by a short rainy season from June to August. The average rainfall is 400 mm a year. The estimated prevalence of urinary schistosomiasis in this endemic area is 69%.[9] No child had been previously treated specifically for *S. haematobium* infection.

Experimental design

This study was authorized by the Niger Ministries of Health and Education. A meeting was held with the local authorities and the parents of the study population, at which all methods and goals were explained. Informed consent was obtained in all cases. All schoolchildren were interviewed, examined, and screened for hematuria, proteinuria, and egg output. All infected children received at different intervals a single dose of praziquantel (40 mg/kg) under the supervision of the research team.

Parasitology

Schistosoma haematobium infection was measured by a quantitative urine filtration technique.[10] Immediately after micturition between 10 AM and 2 PM, 10 ml of urine was filtered through a 2-mm diameter paper filter, and a Lugol coloration test was performed. Eggs were then counted and expressed as the number per 10 ml of urine. Chemical reagent strips (Neotstix 3®; Miles Laboratories, Puteaux, France) were used to screen for urinary blood (results were recorded as negative, + = 25 erythrocytes/μl, ++ = 80 erythrocytes/μl, and +++ = > 200 erythrocytes/μl) and urinary proteins (results were recorded as negative, + = 30–100 mg/dl, ++ = 100–300 mg/dl, and +++ = 300–1,000 mg/dl).

Hematology

Blood samples were collected by venipuncture, stored at 4°C, and transported to the central laboratory in Niamey. The hemoglobin concentration was determined using a spectrophotometer, and the erythrocyte protoporphyrin level was determined using an automatic hematofluorometer (Model 5; Helena Corp., St. Leu La Foret, France). The hematocrit was determined by microcentrifugation. Electrophoresis of hemoglobin on cellulose acetate (Helena Corp.) was also performed. Serum fractions were collected by centrifugation, frozen at −20°C, and shipped on dry ice by air to France where they were assayed for serum iron by a colorimetric method using ferrozine as a chromogen,[11] and for transferrin by an automated immunoturbidimetric assay. From this analysis, the total iron binding capacity was derived. Serum ferritin was determined by an enzyme-linked immunosorbent assay that was standardized using the international reference (NIBSC, London, UK).

Definitions

Anemia was defined according to the World Health Organization reference values; for the age group in our study, this was a hemoglobin level < 12 g/100 ml.[12] Iron deficiency was defined as a low serum ferritin level ($\leq$ 12 μg/l) combined with a low transferrin saturation coefficient (< 16%), a high erythrocyte protoporphyrin level (> 3 μg/g of hemoglobin), or both.[13] Iron deficiency associated with an inflammatory syndrome was suggested by a moderate serum ferritin level (13–50 μg/l) associated with a low transferrin saturation and a high erythrocyte protoporphyrin saturation.[14] Infection by *S. haematobium* was considered if egg output was positive.

Data analysis

Statistical analysis was performed with SPSS (Chicago, IL) PC+ version 2.0 software using the Student's *t*-test, analysis of variance, Pearson's correlation coefficient (and residual anal-

TABLE 1

Relationship between hematuria and Schistosoma haematobium *egg output in urine samples of the study population*

	Hematuria*				
	0	+	++	+++	P†
No. of subjects	41	41	20	72	
Egg output, geometric mean (eggs/10 ml)	7.76	18.54	27.93	83.65	10^{-4}‡
% of infected subjects	14.6	95.12	100.0	100.0	10^{-4}§

* + = 25 erythrocytes/μl; ++ = 80 erythrocytes/μl; +++ = >200 erythrocytes/μl.
† Comparisons between all four groups.
‡ By analysis of variance.
§ By chi-square test.

ysis), and the chi-square test. A log transformation of ferritin and egg count was done to ensure a log normal distribution.

RESULTS

All 174 schoolchildren (58% boys and 42% girls) in the study were screened. Their mean ± SD age was 10.64 ± 2.2 years. Hematuria and proteinuria were observed in 76.4% and 79.9% of the children, respectively. No differences in these indicators were observed between boys and girls. In 41.4% of the hematuric children, hematuria was severe (+++); it was macroscopic in 36% of the cases.

The overall prevalence of infection was very high (95.4%). The geometric mean egg count was 31.5 eggs per 10 ml of urine. Intensity of infection was significantly related to the degree of hematuria and proteinuria ($F = 20.18$ and $F = 21.4$, respectively; $P < 10^{-4}$) (Table 1). Anemia was observed in 59.7% of the children, and five (2.9%) had severe anemia (hemoglobin < 8 g/dl). Seventy-five (43.1%) children had stigmata of iron deficiency associated with an inflammatory syndrome. Iron deficiency without evidence of inflammatory syndrome was found in seven (4%) children. Thus, the overall prevalence of iron deficiency in our sample was 47.1%. Anemia was associated with iron deficiency in 60 (57.7%) of the 104 anemic children.

The results of the analysis of iron indicators are listed in Table 2. Hemoglobin levels and transferrin saturation coefficients decreased significantly when the degree of hematuria increased ($F = 4.02$, $P = 0.008$ and $F = 3.04$, $P = 0.02$, respectively), while prevalence of anemia and prevalence of iron deficiency increased significantly ($\chi^2 = 11.12$, $P = 0.02$) (Figures 1 and 2). The odds ratio of anemia when hematuria is present is 1.47. Hematocrit and erythrocyte protoporphyrin are associated with the intensity of proteinuria ($F = 2.98$, $P = 0.03$ and $F = 22.4$, $P = 10^{-4}$, respectively). Prevalences of anemia and iron deficiency are also associated with proteinuria ($\chi^2 = 14.15$, $P = 0.003$ and $\chi^2 = 11.44$, $P = 0.02$, respectively). This may be explained by the fact that an increased egg count is associated with increased hematuria and proteinuria. Hemoglobin levels and hematocrit are negatively correlated with egg count ($r = -0.18$ and $r = -0.189$, $P = 0.001$, respectively) (Figure 3), while the prevalence of anemia increases with higher egg counts ($\chi^2 = 10.2$, $P = 0.01$).

Hemoglobin typing showed that 80.5% of the children had type AA, 15.5% had type AS, and 4% had type AC. No homozygote sickle cell hemoglobin was observed, and no relationship was found with any of the indicators of hematopoietic status.

DISCUSSION

The prevalence of anemia in schoolchildren living in an area of Niger that is endemic for *S. haematobium* is very high, but is comparable to

TABLE 2

Iron parameters in the study population

	Mean ± SD	% of abnormal value
Hemoglobin (g/10 ml)	11.7 ± 1.5	59.7
Erythrocyte protoporphyrins (μg/g of hemoglobin)	3.5 ± 5.07	39.1
Serum ferritin (μg/l)	2.4 ± 30.6	73.5
Transferrin saturation (%)	19.9 ± 6.9	32.7

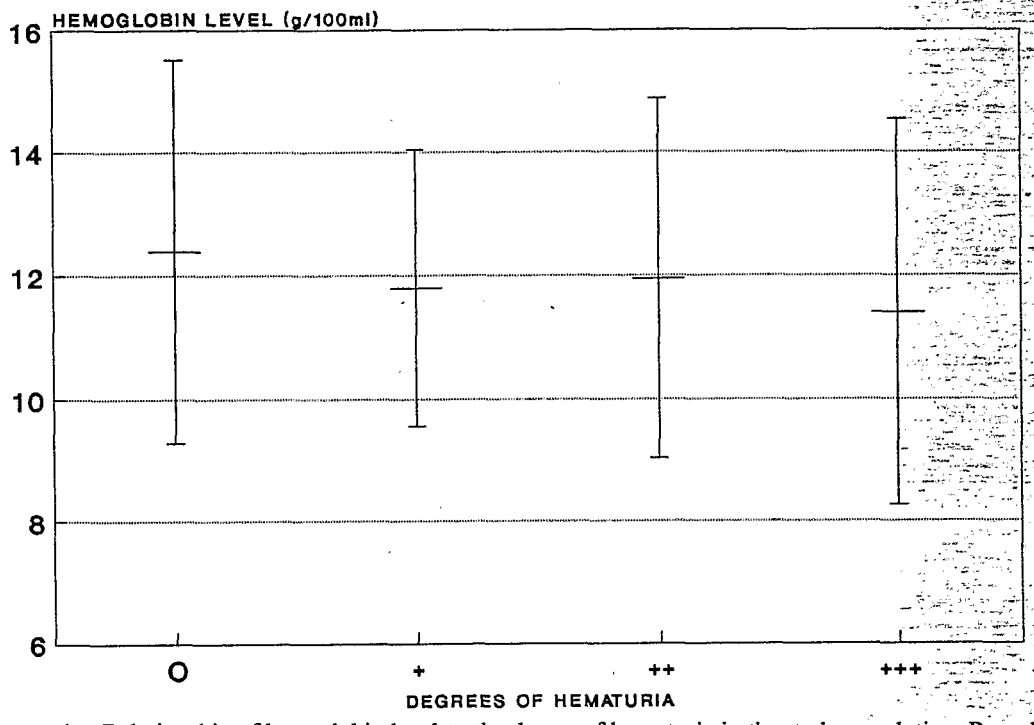

FIGURE 1. Relationship of hemoglobin level to the degree of hematuria in the study population. Bars show the mean ± SD.

that reported by other studies carried out in West Africa, where other parasitic infections are common.[15, 16] In our study, 95.4% of the children were infected with *S. haematobium:* 44.3% had a severe infection[17] and 41.3% had severe hematuria (+++). Such heavy infections induce significant losses of blood and iron. Estimations of urinary blood losses due to *S. haematobium* infections range from 0.5 to 126 ml per day.[18–20] This corresponds to the loss of 0.6–37.3 mg of iron. In the severe cases, this is 40 times the normal physiologic loss in an adult male (65 kg) and is estimated to be 0.9 mg/day.[21]

Stephenson and others[22] found that iron losses in Kenyan schoolchildren ranged from 0.149 mg/day in the control group to 0.652 mg/day in the group heavily infected by *S. haematobium,* which is comparable to menstrual blood losses in women. Although this chronic blood and iron loss can theoretically lead to iron deficiency anemia, especially in people whose diet is poor in biologically available iron, the relationship between urinary schistosomiasis and anemia is controversial.[23] Furthermore, the role of iron deficiency in anemia of urinary schistosomiasis is unknown. The prevalence of anemia in our study population is proportional to the degree of hematuria. The presence of hematuria increased the risk of anemia by 47%, and hemoglobin and hematocrit levels were negatively correlated with the intensity of infection. This relationship has been found in other cross-sectional studies[4–7] and in two longitudinal studies.[24, 25] When such a relationship has been found, sample sizes were small, intensity of infection was not measured, or moderate and severe anemia were uncommon.[26, 27]

A relationship between hematuria and hemoglobin level has not yet been reported. However, hematuria and egg count are strongly correlated, as was demonstrated by Mott and others.[8] The mechanism involved in anemia when urinary schistosomiasis is present is not clear, despite the significant losses of urinary iron. In the only reported study concerning the relationship between iron status and urinary schistosomiasis, Bretagne and others[7] found a significant difference in the prevalence and intensity of anemia between a population infected with *S. haematobium* and a comparable but uninfected population, but they did not find any difference in the iron status. However, they did not use a com-

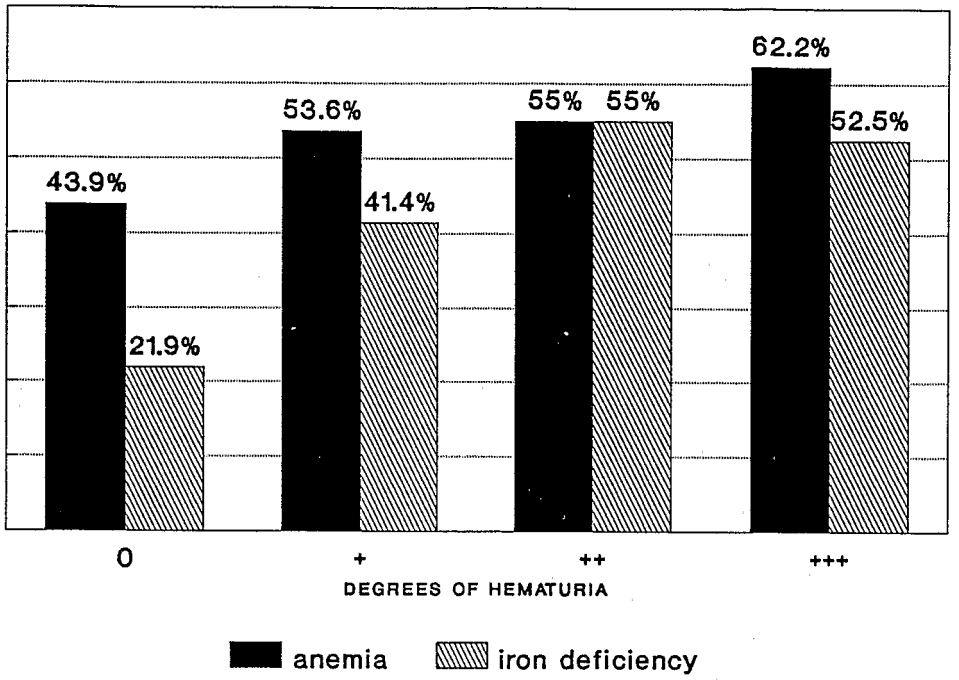

FIGURE 2. Relationship of the prevalence of anemia and iron deficiency to the degree of hematuria in the study population.

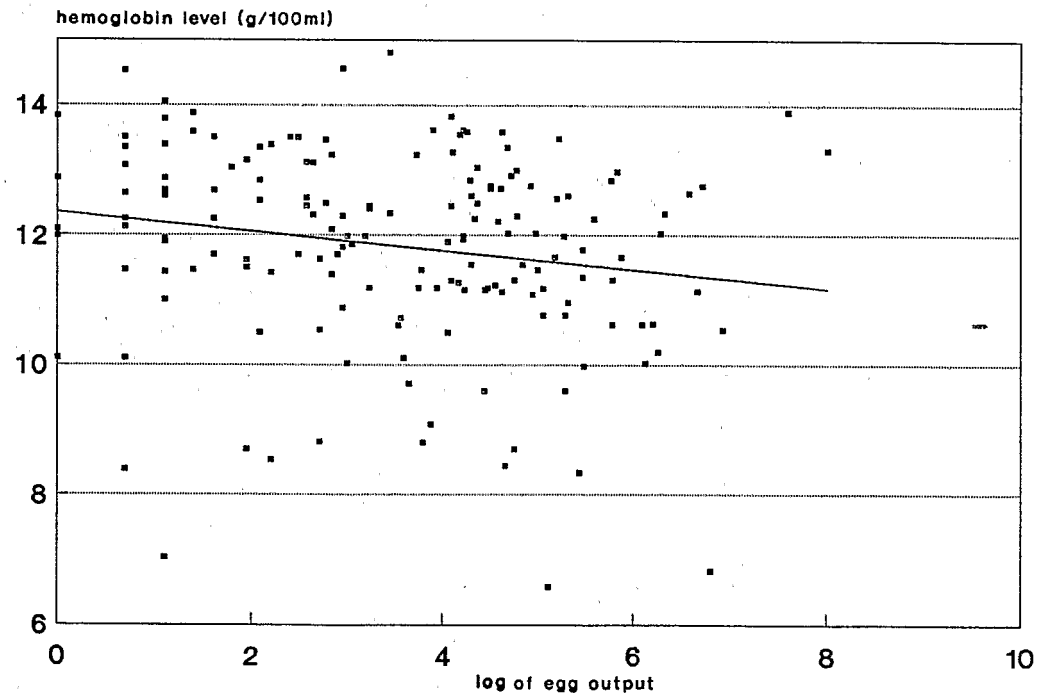

FIGURE 3. Linear regression analysis of the hemoglobin level as a function of *Schistosoma haematobium* urinary egg output in the study population. Each point represents a study subject. log 2 = 7.4 eggs/10 ml; log 4 = 54.6 eggs/10 ml; log 6 = 403.4 eggs/10 ml; log 8 = 2,980.9 eggs/10 ml.

bination of iron indicators that could correctly identify iron deficiency and therefore ensure a correct classification of iron status. In addition, hematuria was not taken into account in their study.

The diagnosis of the source of iron deficiency is very difficult, especially in a tropical context, where numerous confounding factors interfere with the measurements and modify the significance of each test. This is especially true when studying infection and parasitism. In our study area, which is characterized by a very dry climate, intestinal helminthic infections are uncommon, despite poor sanitation. *Schistosoma mansoni* has not been found[28] because *Biomphalaria pfeifferi,* its snail host in West Africa, is not present in this area.[29] For the same climatic reason, hookworm, *Ascaris lumbricoides,* and *Trichuris trichiuria* have an extremely low prevalence.[28] In addition, at the end of the dry season when this study was carried out, the prevalence of malaria was less than 10%.[7] Heterozygote sickle cell hemoglobin was found in 15.5% of the children. However, no relationship was found with anemia or with indicators of iron status as previously observed by Hercberg and others.[16]

A combination of four indicators of iron status is necessary to improve the accuracy of the diagnosis of iron deficiency.[30] Such a combination was used in our study. Anemia was associated with iron deficiency in more than half of the cases. This proportion was also found in areas where *S. haematobium* is not endemic.[16] The prevalence of iron deficiency increased significantly with increased hematuria. The lack of a relationship between each single indicator of iron status and the indicators of infection by *S. haematobium* may be explained by the possible effect of an inflammatory syndrome on the iron parameters. Although this has never been reported, urologic lesions of this type of active schistosomal infection found in our study population could be responsible for such an inflammatory syndrome, thus masking an iron deficiency. However, our study suggests that a relationship exists between iron status, anemia, and urinary schistosomiasis. Such a relationship shows the deleterious consequences of an infection by *S. haematobium* on nutrition and on the hematopoietic status. Moreover, this demonstrates the necessity to take into account these consequences and relationships when designing intervention programs on nutrition.

Acknowledgments: We thank Adamou Kaka and Soumaila Mamadou (Laboratory of Biochemistry, Faculte des Sciences de la Sante), and Ali Sidiki (Centre de Recherche sur les Meningites et Schistosomoses), for active participation in the different phases of specimen collections and laboratory work. We also thank Jim Hyde and his group (Department of Community Health, Tufts University, Boston, MA) for financial and technical support.

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