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Morbidity assessment in urinary schistosomiasis infection through ultrasonography and measurement of eosinophil cationic protein (ECP) in urine

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Summary In a *Schistosoma haematobium*-endemic village in western Madagascar we evaluated ultrasonography and Eosinophil Cationic Protein (ECP) in urine as means to detect the associated urinary tract pathology. 192 individuals were matched according to age and sex, and grouped into infected persons with bladder and, if present, kidney pathology ($n = 96$); infected persons without pathology ($n = 48$) and noninfected persons without pathology ($n = 48$). The median urinary egg count was significantly higher in individuals with ultrasonographically detectable urinary tract pathology (115 eggs/10 ml urine) than in infected persons without (45 eggs/10 ml of urine). At 136 ng/ml, the median ECP level was significantly higher in the 144 infected individuals than in the 48 noninfected persons (0.35 ng/ml). Egg excretion correlated positively with ECP level. The median ECP level was significantly higher in the group with ultrasonographically detectable urinary tract pathology than in the group without (183 ng/ml *vs*. 67 ng/ml). The results suggest that minor degrees of pathology, particularly at an early stage of infection with *S. haematobium*, might be overlooked by ultrasonography despite the presence of marked inflammation, as indicated by markedly increased urinary ECP levels in infected individuals without ultrasonographically detectable urinary tract pathology. ECP may therefore provide important information on the evolution of *S. haematobium*-associated urinary tract morbidity.

keywords *Schistosoma haematobium*, morbidity, ultrasonography, eosinophil cationic protein, Madagascar

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Introduction

The severity of *Schistosoma haematobium* infection and risk of complications depend in general on the intensity and duration of infection. Prevalence is a relatively poor indicator of morbidity (Wiest 1996) and intensity of infection as assessed parasitologically alone is not necessarily an optimal parameter to identify those at risk of severe morbidity (Smith & Christine 1986). Ultrasonography has been shown to be useful in assessing urinary tract pathology due to *S. haematobium* infection, particularly in large-scale epidemiological surveys (Doehring *et al*. 1985; Hatz *et al*. 1992; Strickland & Abdel-Wahab 1993). Unfortunately, only gross pathology can be visualized by ultrasonography and bladder lesions of less significance cannot be detected by this technique (Burki *et al*. 1986). Development of pathology in the urinary tract commences early in life. However, the course of the disease and the prognostic features of infection in untreated individuals are unpredictable at the early stage of infection. It would therefore be useful if ultrasonography could be supplemented with other methods to obtain a more detailed understanding of development of urinary schistosomiasis-associated

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morbidity, especially if one could detect the extent of egg-induced granulomatous inflammation in the bladder wall during the early stages of *S. haematobium* infection.

The eosinophil leucocyte is one of the principal cells involved in the human immune response to *S. haematobium* infection (Butterworth *et al*. 1975), and eosinophiluria has been demonstrated in patients with urinary schistosomiasis (Eltoum *et al*. 1989). Eosinophil granules contain several highly cationic proteins, including the eosinophil cationic protein (ECP), which are released after activation and stimulation of the cells (Olsson *et al*. 1977). ECP can be detected by an enzyme-linked immunosorbent assay (Reimert *et al*. 1991), and the urine ECP level is significantly higher in *S. haematobium*-infected children than in noninfected controls (Reimert *et al*. 1993). The ECP level is also elevated in serum from patients infected with *Schistosoma mansoni* (Tischendorf *et al*. 1996).

The main objective of this study was to elucidate the characteristics of ECP as a marker of morbidity in *S. haematobium* infection by comparing ECP levels with other parameters such as egg counts and ultrasonographical findings.

Materials and methods

The study was conducted in the village of Betalatala in western Madagascar, where only *S. haematobium* transmission occurs (Coulanges 1978). The region has a subhumid tropical climate with an annual precipitation of 1500 mm. The major agricultural activities are rice, corn and tobacco cultivation. Of 641 registered village inhabitants, >5 years old, 574 (89.5%) were examined for *S. haematobium* infection in a baseline survey which revealed a prevalence of 75.9% and a geometric mean egg count of positive individuals of 36 eggs/10 ml of urine (Serieye *et al*. 1996).

A subgroup of 192 individuals (33.4%) was selected from these 574 persons and stratified into a 6–19 years and a ≥20 years age group to compare outcomes in two age groups with an expected difference in intensity of *S. haematobium* infection. The three study groups, matched for age and sex and formed according to infection status and results of the ultrasonographical examination of the urinary tract, comprised infected persons with bladder and, if present, kidney pathology ($n = 96$); infected persons without pathology ($n = 48$) and healthy controls ($n = 48$).

To measure prevalence and intensity of *S. haematobium* infection, *S. haematobium* egg excretion was determined by filtration of urine through a Nuclepore® membrane (Peters *et al*. 1976). Two urine samples were collected on two days between 0900 h and 1300 h. The individual egg count (eggs/10 ml) was expressed as the mean of the two counts. Haematuria was semiquantitatively assessed by using urine reagent strips (Nephur 7-test® Boehringer Mannheim, Germany).

An ultrasonographical examination of the urinary tract was performed. The ultrasonographer was unaware of the person's status regarding *S. haematobium* infection. A Microimager 2000® ultrasonograph (Ausonics Ltd, Sydney, Australia) was used with a 3.5 Mhz sectorial transducer, a 9 inch (c. 23 cm) additional monitor and a printer. Approximately 500 ml of water were given to each individual half an hour before the examination. This was performed in the supine position and only when the bladder was completely full. Pathological changes in the urinary tract were recorded according to the Cairo Working Group (1992) classification with minor modifications: fissures of the kidneys and calcifications in the bladder were not taken into account. Bladder abnormalities were recorded as follows: change in the shape of the bladder, thickened regular wall (>5 mm), irregular wall (focal or diffuse) and one or more localized areas of hypertrophy. Abnormalities in the kidneys were recorded as mild (calyceal dilatation with normal cortex), moderate (more marked dilatation with normal cortex) or severe (marked dilatation with cortical loss). All individuals presenting signs of renal congestion underwent a second examination about 30 min after emptying the bladder. The grading of persisting congestion was definitively recorded following the re-examination. Ultrasonographically detected bladder and kidney pathology were classified as mild, moderate or severe according to a staging system (Hatz *et al*. 1998).

Ten-ml specimens from the first urine sample were kept at + 4 °C for a maximum of one week followed by storage at − 20 °C. One volume of well suspended urine was mixed with one volume of extraction buffer (1% *N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide, CTAB, in 0.15 M NaCl). After one cycle of freeze-thawing the sample was mixed on a vortex mixer and centrifuged for 10 min at 3000 g. The supernatant containing the extracted proteins was siphoned off and used for ECP determinations. ECP in urinary extracts was measured by an ELISA as described in Reimert *et al*. (1991). The method is a polyclonal sandwich-type ELISA using a biotin-avidin-peroxidase amplification step and measures ECP in the range of 15–1000 pg/ml. ECP purified from extracts of human blood eosinophils was used as standard. Before measurement, standards and urine extracts were diluted in sample buffer (0.1% Tween 20, 0.1%).

The study was approved by the Ethics Committee at the Pasteur Institute and by the Ministry of Health in Madagascar.

The χ^2-test for comparison of relative frequencies, Wilcoxon's signed rank test for comparison of egg counts and ECP levels between the study groups and Spearman's rank correlation coefficient (ρ) test to assess if egg counts and ECP levels were correlated.

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Table 1 Median egg count (eggs/10 ml urine), distribution of egg count categories and median ECP content (ng/ml) in urine from *Schistosoma haematobium* infected individuals according to the result of ultrasonographical examination

	Pathology categories						
	Without pathology ($n = 48$)	With pathology * ($n = 96$)	*P*	Mild† pathology ($n = 65$)	Moderate‡ pathology ($n = 24$)	Severe¶ ($n = 7$)	Total ($n = 144$)
Median egg count/10 ml	45	115	<0.05	103	115	180	101
Egg count classes (*n*)							
1–49 eggs/10 ml	27 (56.2%)	28 (29.2%)	<0.01	22 (33.8%)	5 (20.8%)	1 (14.3%)	55 (38.2%)
50–399 eggs/10 ml	21 (43.8%)	57 (59.4%)	NS	38 (58.5%)	15 (62.5%)	4 (57.1%)	78 (54.2%)
400-eggs/10 ml	0	11 (11.5%)	<0.02	5 (7.7%)	4 (16.7%)	2 (28.6%)	11 (7.6%)
Median ECP content(ng/ml) in each egg count category:							
1–49 eggs/10 ml	12	35	NS	50	10	28	
50–399 eggs/10 ml	346	266	NS	284	97	544	298
400-eggs/10 ml	628	403	733	397	628		
Total	67	183	<0.001	181	116	384	136

NS, Non significant at p≥0.05; Staging of urinary tract pathology by ultrasonography according to Hatz and coll. † Mild pathology: focal thickening (≥5 mm) and/or focal irregularity of the bladder wall. ‡ Moderate pathology: diffuse thickening (≥5 mm) and/or diffuse irregularity and/or mass/pseudopolyp of the bladder wall and/or mild hydronephrosis without compressed parenchyma. ¶ Severe pathology: diffuse thickening (≥5 mm) and/or more than one mass/pseudopolyp of the bladder wall and/or moderate/major hydronephrosis with compressed parenchyma.

Results

The median urinary egg count was significantly higher in the 96 individuals with ultrasonographically detectable urinary tract pathology than in the 48 infected individuals without (115 *vs*. 45 eggs/10 ml of urine; $P \leq 0.05$). Cases with heavy infection (≥400 eggs/10 ml) were only observed in the group of individuals with ultrasound-detectable pathology (Table 1). The median urinary egg count was significantly higher in the 6–19 years age group than in the ≥20 group (148 *vs*. 65 eggs/10 ml urine; $P \leq 0.05$) (Table 2). The intensity of infection in females in both age groups, irrespectively of pathology status, exceeded that in males.

Of the 96 individuals with ultrasonographically detectable urinary tract abnormalities, 88 (91.7%) had bladder lesions only and 8 (8.3%) had renal abnormalities in addition to

Table 2 Median egg count (eggs/10 ml urine) and median ECP content (ng/ml) in urine from *Schistosoma haematobium* infected individuals with and without ultrasonographically detectable urinary tract pathology, according to age and sex

	Median egg count, eggs/10 ml urine (range)					Median ECP, ng/ml (range)					
Age/Sex	+ Pathology		− Pathology		*P*	+ Pathology		− Pathology			Controls
Age											
6–19 years (all)	148*					172*					
20-years(all)	65*					91*					
P	<0.05						< 0.05				
6–19 years											
Males	98	(6–1074)†	37	(1–355)‡	NS	193.0	(2.9–1430)†	36.4	(<0.3–1366)‡	<0.3	(<0.3–5.7)‡
Females	212	(5–690)†	224	(2–327)‡	NS	161.0	(2.9–2256)†	552.0	(<0.3–3014)‡	0.84	(<0.3–38.4)‡
P	NS		<0.05			NS	<0.02		NS		
20-years											
Males	47	(1–633)†	20	(1–292)‡	NS	163.0	(1.6–1632)†	12.6	(<0.3–91)‡	0.4	(<0.3–6.4)‡
Females	93	(2–684)†	46	(9–272)‡	NS	193.0	(3.2–4330)†	67.8	(1.1–1166)‡	0.4	(<0.3–51.4)‡
P	NS		NS		NS	<0.05		NS			
Total	115	(1–1074)¶	45	(1–355)§	<0.05	183	(1.6–4330)¶	67	(<0.3–1366)§	0.3	(<0.3–51.4)‡

NS, Non significant at $P \leq 0.05$. * $n = 72$; † $n = 24$; ‡ $n = 12$; ¶ $n = 96$; § $n = 48$.

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bladder pathology. Sixty-five (67.7%) were classified as having mild pathology, 24 (25%) as having moderate pathology and 7 as having severe pathology (7.3%) (Table 1). Median egg counts increased, although not significantly, with severity of pathology.

ECP levels in urine varied markedly within each study group (Table 2). Regardless of the result of the ultrasonography examination, infected individuals presented a significantly higher median ECP level than noninfected individuals (136 ng/ml *vs*. 0.3 ng/ml of urine, $P \leq 0.0001$) (Tables 1 and 2). Six (12.5%) of the 48 individuals in the control group had ECP levels > 5 ng/ml. Looking more specifically at intensity of infection, the median ECP level was significantly lower in individuals with light infections than in individuals with moderate infections, with figures being 28 ng/ml and 298 ng/ml of urine, respectively ($P \leq 0.02$) (Table 1). The median ECP level increased further, although not significantly, to 628 ng/ml of urine in heavily infected individuals ($\geq$400 eggs/10 ml).

The median ECP level in urine was significantly higher in the group with ultrasonographically detectable urinary tract pathology than in the group without (183 ng/ml *vs*. 67 ng/ml, $P \leq 0.001$) (Table 1). Thus the pattern was the same as observed for intensity of infection with a higher median urinary egg count in the former group. In the group with severe pathology, the median ECP value was significantly higher than in the two groups with mild and moderate pathology ($P \leq 0.01$) (Table 1).

ECP levels were comparable in males and females of both age groups in the group with ultrasonographically detectable urinary tract pathology, although intensity of infection in general was higher in females than in males. By contrast, in the group without ultrasonographically detectable pathology, the median ECP level in females was significantly higher than in males in both age groups ($P \leq 0.02$ and $P \leq 0.05$, respectively) (Table 2).

Figure 1 shows ECP levels and corresponding egg counts. Egg excretion was positively correlated to the ECP level in the same urine sample (*rho* = 0.435, $P \leq 0.0001$). The correlation coefficient (*rho*) increased further to 0.563 when the ECP level in the first urine sample was compared to the mean egg count from the first and the second urine samples. Looking specifically at the two groups of infected individuals with and without ultrasonographically detectable urinary tract pathology, the *rho* value increased when the ECP level was related to the mean egg count of two urine samples rather than to the egg count in the same urine sample (0.404 *vs*. 0.290 in the group with pathology; 0.718 *vs*. 0.528 in the group without).

Haematuria as detected by reagent strip was associated with egg excretion in urine ($P < 0.0001$), and it was equally observed in the group with ultrasonographically detectable urinary tract pathology and in the group without, with frequencies of 88.5% and 83.3%, respectively. The median ECP level was significantly higher in individuals with haematuria than in those without (172 ng/ml *vs*. 14 ng/ml, $P < 0.0001$).

Discussion

This study has not only confirmed previous observations that the ECP level in urine is increased in children with *S. haematobium* infection (Reimert *et al*. 1993), but likewise in infected adults. These findings may, after all, be expected in any patient, regardless of age, with urinary schistosomiasis infection due to a uniform pathogenesis with egg sequestration in the bladder tissue followed by granulomatous inflammation containing ECP-secretoric eosinophils (Eltoum

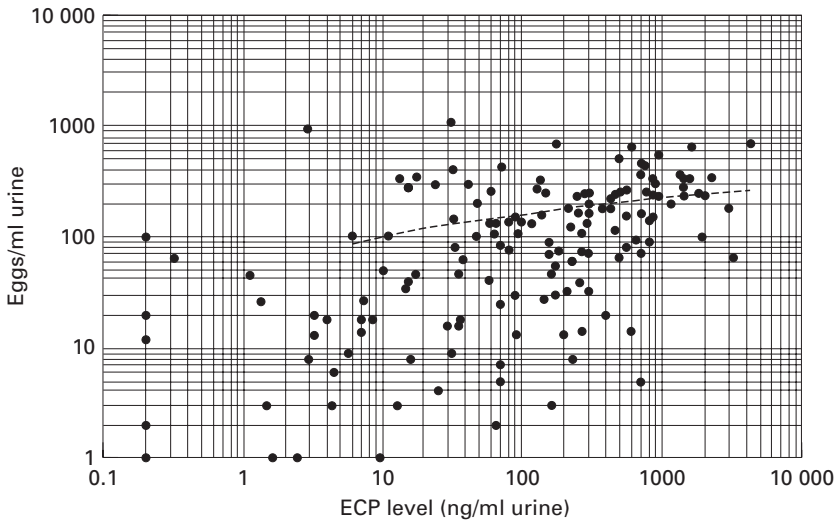

Figure 1 Scattergram of ECP levels (ng/ml) and egg counts (eggs/ml) in urine in Schistoma haematobium infected individuals ($n = 144$). Correlation coefficient *rho* = 0.435; $P < 0.0001$.

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et al. 1989). The results also showed that considerable bladder inflammation with the possibility of associated morbidity can be present even without detection of pathology by ultrasonography. Although ultrasonography has proved useful in assessing morbidity due to *S. haematobium* infection, it has some limitations. Minor degrees of bladder pathology, particularly at an early stage of infection with *S. haematobium*, cannot be detected due to inadequate image resolution (Burki *et al*. 1986; Hatz *et al*. 1992). ECP may therefore be a complementary indirect marker of morbidity, providing important information on the evolution of *S. haematobium*-associated urinary tract pathology.

In general, morbidity associated with *S. haematobium* infection in the urinary tract should be viewed in a broader context than what can be detected by ultrasonography. Signs and symptoms related to urinary schistosomiasis, such as haematuria and bladder discomfort, are equally important as they may have a negative impact on the quality of life and should therefore also enter into the overall morbidity assessment. Infected individuals without ultrasonographically detectable urinary tract abnormalities had increased ECP levels in urine, indicating marked egg-induced inflammation in the bladder. Those individuals may still be suffering from bladder discomfort although no gross pathology was detected. Hence, measurement of ECP in urine adds important information to the overall assessment of morbidity, especially in relation to bladder inflammation and its associated symptoms.

The urinary ECP levels correlated with egg counts, and the correlation became stronger when the ECP values were compared with mean egg counts of two urine samples as opposed to egg count in one urine sample only. It is likely that measurement of ECP in a single urine sample is a more reliable means of determination of infection than egg count by urine filtration, due to the major implication of the marked day-to-day variation in egg excretion (McCullough & Bradley 1973). Comparing egg counts and ECP levels between the group with ultrasonographically detectable urinary tract pathology and the group without revealed that a more distinct correlation between ECP level and egg count occurred in the latter. A possible explanation for this observation could be that in individuals without ultrasonographically detectable bladder pathology, eggs are excreted in urine with less hindrance, and therefore reflect more directly the extent of egg-induced inflammation in the bladder wall, whereas in individuals with pathology, marked tissue fibrosis prevents a high proportion of eggs from reaching the lumen of the bladder. Therefore, the egg count may, to a certain degree, disproportionately reflect the actual extent of inflammation in the latter group.

Six individuals from the control group had urinary ECP levels of > 5 ng/ml urine. They might have been infected with *S. haematobium*, but the limited number of examinations, combined with a low and variable egg excretion, may have resulted in failure to detect the eggs. It is also possible that the presence of a urinary tract or a genital infection due to other causes infection could bring about an increased ECP level in urine as a result of the involvement of the eosinophiles in the inflammatory response.

The usefulness of egg counts in measuring intensity of infection needs to be reviewed. The variation in egg excretion leads to reservations about its capacity to measure intensity of infection accurately (Vennervald *et al*. 1998). Such variation causes not only incomplete allowance for the confounding influence of intensity of infection in analysis of other variables, but also poorly matches experimental pairs in the process of developing markers of schistosomiasis morbidity. The ECP level in urine was associated with ultrasonographically detectable urinary tract pathology, in particular severe pathology. This association was less evident in individuals with light and moderate infection. However, it is not clear whether egg counts as such are a reliable reference to evaluate the characteristics of urinary ECP as a marker of morbidity. Some individuals, especially adults, will develop severe complications due to chronic schistosomiasis infection in the urinary tract regardless of decreasing intensity of infection by age (Smith & Christine 1986). In this situation egg counts may be an inadequate means to identify those individuals. More studies are needed to evaluate ECP in urine as a supplementary morbidity marker not only to identify *S. haematobium* infected individuals who have already developed severe urinary tract pathology, but also to identify those who are at risk of developing severe complications in the course of an untreated infection.

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