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Measurement of serum haptoglobin as an indicator of the efficacy of malaria intervention trials

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Abstract

Serum haptoglobin levels were measured by an enzyme-linked immunosorbent assay in Gambian children who participated in 3 malaria intervention trials with untreated or impregnated bed nets. In one study, in which a significant effect on clinical malaria was observed, the mean serum haptoglobin level was significantly higher in the intervention than in the control group. In the other 2 studies, in which no significant protection was observed, mean haptoglobin levels were similar in intervention and control groups. Measurement of serum haptoglobin may provide a useful indirect measure of the effectiveness of malaria control programmes.

Introduction

Low or absent levels of serum haptoglobin are found frequently in individuals who live in tropical Africa (CONSTANS *et al.*, 1981) or in some parts of Melanesia (HILL *et al.*, 1987). The cause of ahaptoglobinaemia in these populations has been the subject of considerable debate. It was suggested initially that genetic factors were responsible for the high prevalence of ahaptoglobinaemia in some populations (ALLISON, 1959), but others have argued that environmental factors are more important (CURTAIN *et al.*, 1965; GIBLETT *et al.*, 1966). There is now strong evidence that malaria and ahaptoglobinaemia are closely related. Several studies have shown an association between ahaptoglobinaemia and malaria parasitaemia in individual subjects (ROUGEMENT *et al.*, 1974; WELCH *et al.*, 1979; BOREHAM *et al.*, 1981; MONJOUR *et al.*, 1982; TRAPE *et al.*, 1985). Furthermore, the occurrence of ahaptoglobinaemia in susceptible populations can be completely, or almost completely, eliminated by the administration of malaria chemo-prophylaxis (ROUGEMONT *et al.*, 1988; BOREHAM *et al.*, 1981; TRAPE *et al.*, 1985). The way in which malaria causes ahaptoglobinaemia is uncertain. The main function of haptoglobin is to bind free haemoglobin into complexes which are then removed from the circulation. Thus, ahaptoglobinaemia might be expected during acute episodes of malaria, in which marked haemolysis may occur. However, ahaptoglobinaemia may be found in adult immune subjects resident in malaria endemic areas who have a negative blood film (TRAPE *et al.*, 1985). A possible explanation for this phenomenon is the occurrence in some aparasitaemic subjects of malaria-induced haemolytic anaemia which does not depend directly upon parasitization of red blood cells (TRAPE *et al.*, 1985).

Because of the close association between ahaptoglobinaemia and malaria, ROUGEMONT *et al.* (1988) suggested that assay of haptoglobin levels could be used as an indirect measurement of malaria infection and they have shown how, in an area of Mali, seasonal changes in the haptoglobin index paralleled those of the prevalence and intensity of malaria parasitaemia. Determination of the outcome of a malaria intervention campaign, which may involve active surveillance for clinical cases of malaria of many subjects, is time-consuming and expensive. Therefore, we have investigated whether measurement of serum haptoglobin, using a simple and rapid enzyme-linked immunosorbent assay (ELISA), could be used to give an indirect assessment of the impact of a malaria control programme.

Table 1. Summary of the design and outcome of three malaria control trials in The Gambia

	Study 1		Study 2[a]		Study 3[b]	
	Intervention Untreated nets	Control No nets	Intervention Treated nets	Control Untreated nets	Intervention Treated nets	Control Untreated nets
Clinical episodes[c]	19·8% (37/187)	27·3% (33/121)	14·0% (18/129)	21·5% (26/121)	9·8% (18/184)	20·5%** (44/215)
Splenomegaly[d]	39·6% (74/187)	40·5% (49/121)	24·8% (32/129)	24·8% (30/121)	22·3% (41/184)	36·3%** (78/215)
Parasitaemia[d]	53·5% (100/187)	48·8% (59/121)	37·2% (48/129)	37·2% (45/121)	31·0% (57/184)	36·7% (79/215)
High parasitaemia[d]	19·3% (36/187)	18·2% (22/121)	7·8% (10/129)	6·6% (8/121)	8·2% (15/184)	16·3%* (35/215)
Packed cell volume[e]	34·3% (4·4,187)	33·7% (5·2,120)	34·6% (5·0,109)	33·9% (5·1,103)	36·1% (4·8,178)	33·4%*** (4·7,211)

[a]Intervention by individual.
[b]Intervention by village. Significant differences between intervention and control results are indicated thus: $^{*}P<0{\cdot}05$, $^{**}P<0{\cdot}01$, $^{***}P<0{\cdot}001$.
[c]Incidence determined by weekly visits.
[d]Determined by a cross-sectional survey at the end of the transmission season.
[e]Mean (standard deviation and number of determinations shown in parentheses).

Material and Methods

Samples

Serum samples were obtained from children during 3 trials of different malaria control strategies in villages near Farafenni on the north bank of the River Gambia. The nature of these trials and their outcome are summarized in Table 1.

Study 1. In this trial standard bed nets were introduced into a group of villages where they had not been

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used previously and the incidence of malaria recorded in children who slept under these nets and in a group of children who lived in neighbouring control villages where nets were not used.

Study 2. Study 2 was undertaken in a large village in which nearly the whole population slept under bed nets. In this study 10% of nets in the village were impregnated with the insecticide Permethrin® (0·5 g/m^2) and incidence of malaria recorded among children who slept under impregnated nets and among a group of control children resident in the same village who slept under control, non-impregnated nets.

Study 3. Study 3 was undertaken in the same group of small villages as study 1. However, in this trial, villagers in all hamlets were issued with bed nets and nets in one-half of the villages were impregnated with Permethrin®. The incidence of malaria was then recorded among children who lived in villages in which all nets were impregnated and in those who lived in villages with control nets.

The way in which these trials were undertaken, the methods used to measure malaria morbidity, and the outcome of each study have been described elsewhere (SNOW *et al.*, 1987, 1988a, 1988b).

Measurement of haptoglobin

Serum levels of haptoglobin were measured as described previously (YERLY *et al.*, 1990). In brief, flat-bottomed polystyrene plates (Nunc, Denmark) were coated with 100 µl of immunoglobulin (Ig) G, obtained from the serum of a rabbit immunized against haptoglobin, at a concentration of 20 µg/ml. After incubation for 3 h at 37°C, plates were washed 3 times and non-specific binding blocked by incubation with 200 µl of 4% powdered milk for 2 h at 20°C and for 12 h at 4°C. 100 µl of test serum diluted 1:20 000, or of haptoglobin standard, were then added and plates incubated for 3 h at 30°C or for 12 h at 4°C. The plates were then washed 6 times and 100 µl of anti-haptoglobin Ig conjugated with alkaline phosphatase were added to each well. After a further incubation for 3 h at 20°C in the dark, plates were washed 6 times and colour developed by the addition of 100 µl of *p*-nitrophenyl phosphate dissolved in diethanolamine buffer, pH 9·8 (1 mg/ml). After incubation for 40 min in the dark the reaction was stopped by addition of 50 µl of 0·5 N NaOH, and the optical density of test and control wells was measured at 405 nm using a Flow Multiskan® photometer.

Results

Haptoglobin levels were measured in sera from 957 children aged 1–10 years old (Table 1). Results of haptoglobin assays fell into 3 groups—those below the minimum detectable level of 0·016 mg/ml (ahaptoglobinaemic sera) (130/957; 13·6%), those above the upper cut-off level of 1·0 mg/ml (351/957; 36·7%) and those in the intermediary range (476/957; 49·7%). The following analyses relate to these 3 categories.

Overall, the distribution of haptoglobin levels by group was related to age, parasitaemia, splenomegaly and packed cell volume but not to sex. Thus, there was a significant inverse relationship between ahaptoglobinaemia and age ($\chi^2=16·6$; 3 degrees of freedom; $P<0·001$). Ahaptoglobinaemia was found significantly more frequently among children who had parasitaemia than among those who did not (82/388; 21·1% compared with 48/569; 8·4%) (Mantel-Haenzel χ^2 allowing for age=30·2; $P<0·0001$) and among those who had splenomegaly compared with those who did not (98/584; 16·8% compared with 32/373; 8·6%) (Mantel-Haenzel χ^2 allowing for age=9·6; $P<0·002$). The mean packed cell volume was significantly lower in 129 children with ahaptoglobinaemia (mean 33·4%, standard deviation [SD] 5·3%) than in 779 without (34·5%, SD 4·8%) (two-way ANOVA allowing for age, $F=13·2$; $P<0·001$).

The relationship between haptoglobin levels and the results of the 3 malaria intervention studies are shown in Table 2. In study 3, in which significantly lower rates for clinical malaria, asymptomatic parasitaemia and splenomegaly were found in the intervention group, the number of children with ahaptoglobinaemia in the intervention group was significantly lower than in the control group (Mantel-Haenzel χ^2 allowing for age=9·0, $P<0·005$). In studies 1 and 2 there was no significant difference in malariometric measurements between the intervention and control groups and the distribution of haptoglobin levels in the 2 groups was very similar.

Table 2. Haptoglobin levels in children in intervention and control groups during trial of three different malaria control strategies in The Gambia

Haptoglobin (mg/ml)	Study 1		Study 2[a]		Study 3[b]	
	Intervention Untreated nets	Control No nets	Intervention Treated nets	Control Untreated nets	Intervention Treated nets	Control Untreated nets
<0·016	26·7% (50/187)	27·3% (33/121)	–	–	6·5% (12/184)	16·3%** (35/215)
0·016–1·0	54·0% (101/187)	53·7% (65/121)	41·9% (54/129)	35·5% (43/121)	53·8% (99/184)	53·0% (114/215)
>1·0	19·3% (36/187)	19·0% (23/121)	58·1% (75/129)	64·5% (78/121)	39·7% (73/184)	30·7% (66/215)

[a]Intervention by individual.
[b]Intervention by village. Significant difference between intervention and control results indicated thus: **$P<0·01$.

Discussion

The ELISA used in this study was simple, reproducible and gave clear differences between sera with high and low concentrations of haptoglobin. Ahaptoglobinaemic sera could readily be detected by the naked eye. Up to 200 tests could be done at one time; the assay is thus well suited to large-scale epidemiological studies.

No significant difference was found between mean haptoglobin levels related to sex or to ethnic group (Mandinka, Wollof or Fula), as might have been expected if haptoglobin levels were genetically controlled. In contrast, a significant negative correlation with age was found, lowest levels being recorded in the oldest children. This trend cannot be explained solely by the prevalence of malaria as, in this community, prevalence is maximal in children around the age of 5–6 years (S. J. Allen, unpublished observations). Mean haptoglobin levels were higher in both intervention and control groups in the large, predominantly Mandinka village used for study 2 than in the smaller Fula hamlets used for studies 1 and 3. This may be due in part to the fact that nets were used widely in this village before intervention and that the prevalence of malaria was lower in this village than in the smaller hamlets used for studies 1 and 3.

A significant difference in mean haptoglobin levels was found between children in intervention and control

groups in the one study (study 3) in which clear differences were found between the 2 groups by direct measurement of malariometric indices. No difference was found between intervention and control groups in the other 2 trials, in which there was no clear indication of a protective effect against malaria in the intervention group although there was a tendency, which was not statistically significant, towards a lower incidence of clinical attacks of malaria among children in the intervention group in study 2. We conclude that measurement of serum haptoglobin can give useful indirect evidence of the success, or otherwise, of a malaria intervention trial.

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Announcement

The second 'residential meeting' of the Royal Society of Tropical Medicine and Hygiene (to include the Annual General Meeting) and other European Societies of Tropical Medicine will be held at the Royal College of Physicians of Edinburgh, Scotland from Monday 5th to Wednesday 7th July 1993. Accommodation will be on the University campus or alternatively in hotels near the City centre. Full social programme including reception and banquet. Further details available shortly from the Administrator, Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W1N 4EY (Tel: 071 580 2127; Fax: 071 436 1389).