Antityphoid agglutinins in African School aged children with malaria

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Summary

Background: **Salmonella antibodies are known to cross-react with other antigens including those from *Escherichia coli*. Malaria antigens may also have similar characteristics.**

***Objectives*: To establish antibody levels in malaria patients and controls and to decipher (if any), relationship(s) between significant agglutinin responses to the various antigens as outcome factor and malaria parasitaemia as exposure factor.**

***Study Setting*: University of Benin Teaching Hospital and Egor Local Government Area of Edo state, Nigeria,. between July and August, 1999.**

***Study Design*: Prospective and a case control study.**

Patients and Methods: Agglutinins against H and O antigens of Salmonella typhi, Salmonella *paratyphi* A, B and C were determined using the Widal test in 189 school aged children(5-16 years) with malaria (males, 54.0%; females, 46.0%) and 175 apparently healthy children,(52.0% males, and 48.0%) of comparable age and gender distributions. Agglutination reaction at ≥1.80 were significantly more in malaria patients (52/189 or 27.5%) than in controls (28/175 or 16.0%) ($x^2 = 7.07$; $p<0.05$). Agglutination reaction to various antigens were significantly higher in controls with malaria parasitaemia (10/36 or 27.8%) than those without parasitaemia (18/139 or 12.9%) ($x^2 = 4.57$; $p<0.02$) (OR=2.59). Significant titres were also more in subjects and controls with malaria parasitaemia (62/235 or 26.38%) than in those without (18/139 or 12.94%) ($x^2=9.32$; $p< 0.05$) (OR=2.41). At dilution of ≥1.80 children aged 9 –14 years (as compared to those ≤8 years of age) and more males (subjects and controls) had demonstrable agglutinins. At most serum dilutions, antibodies to H antigens were more commonly found in subjects and controls than agglutinins against O antigen of *Salmonella typhi*. Fifty percent of subjects and controls had demonstrable agglutination at approximate dilutions of 1:60 and 1:40 respectively. The number of subjects and controls that showed demonstrable agglutinins against *Salmonella paratyphi* antigens were less than 10.0%.

***Conclusion*: Enhanced agglutinin to DO and DH may be a phenomenon associated with childhood malaria.**

Keywords: *Anti-typhoid, Agglutinins, Malaria, Children.*

Résumé

Introduction: Anticorps salmonelloses sont connues de réagir en travers avec d'autres antigènes y compris ceux de Escherichia coli. Les antigènes du malaria pourraient également avoir des traits caractéristiques similaires.

Objectifs: Etablir les niveaux d'anticorps chez des patients atteints du malaria et des contrôles et de savoir (si ce n'est aucun), des rapports entre des réponses importantes des agglutinines aux antigènes diverses comme un facteur des résultats et parasitaemie malaria comme un facteur d'exposition.

Cadre d'étude: Centre Hospitalier Universitaire du Benin et région de l'administration départmentale d'Ego état d'Edo, Nigeria, entre juillet et août, 1999.

Plan d'étude: Etude en perspective et des cas de contrôles.

Patients et méthodes: Des agglutines contre les antigénes H et O de salmonella typhi; salmonella paratyphi A, B et C ont été décidés à travers les méthodes de sérodiagnostique de Widal et Félix chez 189 écoliers âgés (5-16 ans) atteints du paludisme (sexe masculin, 54,0%, sexe féminin 46,0%) et 175 enfants apparemment en bonne santé, (52,0% sexe masculin, et 48,0%) d'une répartition âgé et genre comparés. Réaction agglutination en ≥ 1,80 étaient remarquablement plus élevé chez les patient atteints du paludisme (52/189 ou 27,5%) plus que chez les contrôles (28/175 ou 16,0%) ($x^2 = 7,07$, $P<0,05$). Réaction agglutination aux antigènes diverses étaient sensiblement plus élevés chez les contrôles atteint de malaria parasitémie (10/36 ou 27,8%) plus que ceux sans parasitémie (18/139 ou 12,9%) ($x^2=4,57$, $P<0,02$) (ou =2,59). Des titres importants sont également plus chez les sujets et contrôles atteints du paludisme parasitémie (62/235 ou 26,38%) plus que ceux sans (18/139 ou 12,94%) ($x^2=9,32$; $P<0,05$) (ou = 2,41). Pendant la dilution de ≥ 1,80 enfants ages 9-14 ans (par rapport aux ceux ≤ âgés de 8ans) et plus du sexes masculin (sujets et contrôles) avaient des agglutines démontrables. Pendant les dilutions d'un grand nombre de sérum, des anticorps pour antigene H etaient plus ordinairement trouvés chez les sujets et contrôles plus que des agglutines contre antigène 0 de salmonella typhi. Cinquante pourcent des sujets et contrôles avaient une agglutination démontrables pendant une dilution approximative de 1: 60 et 1: 40 respectivement. Le nombre des sujets et contrôles qui avaient démontré des agglutinines démontrables contre antigènes salmonella partatyphi étaient moins de 10.0%.

Conclusion: Agglutinine améliorée par rapport au DO et

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DN pourrait êntre un phénomène lié au paludisme d'enfance.

Introduction

Typhoid and paratyphoid fevers remain important causes of morbidity and mortality in developing countries noted also, for heavy malaria burden[1]. Typhoid septicaemia is readily fulminant unless early diagnosis is made and adequate therapy offered.[2] The conventional method of culturing the offending Salmonella organism, in a bid to making diagnosis, has been supplanted in most modern societies by serologic methods that have the advantages of speed, sensitivity and specificity. However, in most tropical countries, saddled with enormous burden of the disease, recourse is often made to other methods because modern facilities are lacking.[3] Thus, despite its acknowledged shortcomings[4,5] the single Widal test on serum samples collected during active phase of the illness is commonly used in these countries including Nigeria[3] Interpretation of single antibody level, rather than rising titres in paired sera, is difficult since baseline antibody levels are influenced by such factors as age, prior exposure to salmonella antigen[5] and cross reactivity with other coliform organisms and possibly malaria.[6] Increasing cases of childhood malaria erroneously treated for typhoid, because of elevated typhoidal antibodies, were noted recently by these authors – an observation that tends to lay credence to the possibility of cross reactivity between salmonella and malaria antigens. In this work, the hypothesis that typhoidal antibody levels are increased in malaria was assessed

Methodology

The prospective study was carried out in Benin City between July and August 1999. The study subjects, aged 5.0 – 16.0 years were recruited consecutively from those attending the Outpatient paediatric facility of the University of Benin Teaching Hospital (UBTH), Benin City. They were children with clinical diagnosis of malaria (supported by demonstration of ring form of *Plasmodium falciparum* and who responded subsequently to only appropriate anti-malaria chemotherapy). Anti-malarial used depended on the existing departmental protocol and eventual response. Largely, quinine dihydrochloride; (10mg/kg/dose given 8 hourly and for a minimum of 5 days) was used. Seldomly, (arthemeter 1.6mg/kg/dose; given 12 hourly for 3 days) or halofantrine (8mg/kg/dose; 6 hourly and for 3 doses) were used where the need arose. Apparently healthy children of comparable age and gender distribution, and selected systematically, using the multi-stage random sampling technique, from Edaiken Primary School also in Benin City, served as controls. Subjects and controls were recruited following informed consent from accompanying parent(s) or guardian (in case of patients) and from the Chairman of Egor L.G.A. and Head teacher of the primary school. Patients who had received antibiotics with acknowledged efficacy against Salmonella organisms in the index illness or in the preceding two weeks (i.e. chloramphenicol, ampicillin, amoxycillin clavulanic-acid potentiated amoxycillin, cotrimoxazole, ceftazidime and ceftriaxone) or known to have received vaccination against typhoid in the proceeding 3 years were excluded. Similarly, controls that had been exposed to similar group of antibiotics and vaccines within same period were excluded as these conditions could interfere with antibody response. Also excluded were controls that had had malaria one-week prior to study.

Following clinical assessment meant to enhance diagnosis, 2.5ml of blood was obtained from each child (subject and controls) after due skin preparation. Of the 2.5ml, 0.5ml was introduced into sequestrene bottle and 2.0ml in universal bottle and allowed to clot, then thick and thin films of blood were made from the non-clotted sample for species identification and malaria parasite quantification according to the methods described by Gilles[7]. Following centrifugation, the serum from each child was subjected to sero-agglutination test using Widal test reagent (Welcome, UK). Rapid slide agglutination was carried out first, followed by tube agglutination that was used for confirmation of titres as recommended by Stokes[8]. Doubling dilutions of sera from 1:20 to 1:320 were made and tested against standard O and H antigens of *Salmonella typhi* and *Salmonella paratyphi* A,B and C. Method adopted was a modification of that described by Stokes.[8] The highest dilution that demonstrated agglutination was considered the titre. Storage of sera was at 4°C while awaiting treatment.

Sample size determination was done using the one sample situation for estimating population proportion with specified absolute precision[9]. Assumed proportion of childhood population with malaria was 30.0%. Owing to prohibitive cost of investigation only every other child originally recruited were sampled. Post treatment reviews were undertaken to determine response to anti-malarials and general well being and ensuring that inclusion criteria were met.

Statistics

The chi square test was used to assess association between proportions. Differences between such proportions were considered significant where $p<0.05$.

Results

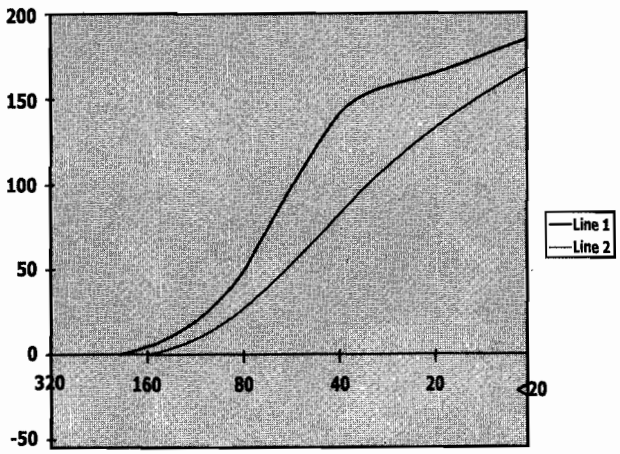
Anti-typhoid agglutinins were determined in 189 subjects; 102(54.0%) were males and 87(46.0%) females. Mean age of subjects of 8.97 ± 3.84 years did not vary significantly from the 9.71 ± 2.17 years recorded in controls. Approximately 68.0% of subjects were 6.0 – 11.0 years while the modal age of subjects was 9.0 years.

Of the 175 controls, 36(20.6%) had positive malaria parasitaemia without clinical symptoms. More males than females had asymptomatic malaria parasitaemia ($x^2 = 2.37$; $p < 0.05$). Ten (27.8%) of the 36 with malaria parasitaemia had agglutinins to DH, DO or both antigens at titres of ≥1:80 in comparison with 18(12.9%) controls without malaria parasitaemia. Agglutination reaction to DO, DH or

Table 1 Distribution of typhoid agglutinin levels in subjects and controls.

Antibody titre	Number of Subjects n(%)	Number of Controls n(%)
1:80	52(27.5)	28(16.0)
< 1:80	137(72.5)	147(84.0)
Total	189(100)	175(100)

$x^2 = 7.07;\ p < 0.02$



Line 1 – Subjects, Line 2 – Controls

Fig. 1 ***Cumulative line graph of agglutinin responses to DO, DH or both antigens in subjects and controls.***

both antigens at ≥ 1:80 dilution was significantly more in controls with malaria parasitaemia than those without it ($x^2 = 4.57$; $p<0.02$) (OR=2.59). Remarkable titres were also significantly more in subject and control with malaria parasitaemia (62/235 or 26.38%) than in those without it (18/139 or 12.94%) ($x^2=9.32$; $p< 0.05$) (OR=2.41). This implied that individuals with malaria parasitaemia had 2.41 – 2.59 times the risk of having significant agglutinins as compared to those without it.

At dilution of ≥ 1:80, (dilutions commonly remarked as suggestive of clinical disease)[2] 52/189 or 27.5% of subjects in comparison with 28/175 or 16.0% of controls demonstrated agglutination ($x^2 = 7.07$; $p < 0.02$) (Table I). More subjects and controls in the age brackets, 9 – 11 and 12 – 14 years demonstrated agglutination at titres of ≥ 1:80. Though positive titres were found more in older children, this was not statistically significant. ($x^2_1 = 0.49$; $p > 0.05$). Similarly, agglutination at titres of ≥ 1:80 was not significantly associated with gender ($x^2_1 = 0.23$; $p > 0.05$).

Forty-seven (24.9%) patients with malaria had agglutination reaction at titres of 1:80 to H, O or both typhoid antigens while only 5(2.6%) had agglutination reactions at titre of 1:160 to similar antigens. Corresponding figures for controls were 26(14.9%) and 2(1.1%) respectively. None of the subjects or controls had agglutination reaction at 1:320 dilutions (Table 11)

Table 2 Cumulative agglutination responses to DO, DH or both antigens in subjects and controls

Antibody titre (Reciprocal of)	Number of Subjects (Cumulative)	Number of Controls (Cumulative)
320	0	0
160	5	2
80	47(52)	26(28)
40	92(144)	58(86)
20	26(170)	51(137)
<20	19(189)	38(175)

Ninety-two (48.7%) and 26(13.8%) subjects had agglutination reactions to H, O or both antigens at dilutions of 1:40 and 1:20 respectively. In comparison, 58(33.1%) and 51(29.1%) controls respectively had agglutination reaction at dilution of 1:40 and 1:20 respectively. About 10% of study population and 21.7% of controls showed no demonstrable agglutination against H, O or both antigens of *Salmonella typhi.* The median or 50th centile of antibody responses to either or both antigens of subjects and controls were approximately 1:60 and 1:40 respectively (Fig.1).

Antibody responses to H and O antigens of *Salmonella paratyphi* A, B, C, were generally low for both subjects and controls. Only 2(1.1%), 7(3.7%) and 4(2.2%) subjects had demonstrable agglutinins at 1:40 dilution to the O antigen of paratyphi A, B and C respectively. Figures in relation to H antigen amongst subjects were 0(0.0%), 3(1.6%) and 7(3.7%) respectively for *S. paratyphi* A, B and C. Regarding the controls, no patient elicited response beyond 1:20 dilution against either OA or HA antigen. One (0.6%) control had agglutination to HB antigen at 1:40 dilutions while 4(2.3%) controls each had agglutination reactions to HB and HC antigens at 1:40 dilution. Thus, less than 10.0% of the study population and controls showed demonstrable agglutinins against the *S. paratyphi* antigens.

Discussion

Anti-D agglutination is known to be influenced by presence of infections caused by other coliform organisms[5]. Only recently malaria was hypothesised to have similar effect.[6] The study has shown that the proportion of individuals with enhanced agglutination reaction was significantly more amongst patients with malaria than in controls. This observation tends to support a relationship between malaria and D agglutinin reaction, thus, laying credence to the works of Alaribe et al.[6] This observation on the possible relationship between malaria and enhanced agglutination reaction is further supported by

the fact that amongst the controls, marked agglutination reaction was significantly more in those with malaria parasitaemia than those without it. Cross reactively may therefore exist between Salmonella and malarial antigens and this could offer explanation for the observed trend.

Enhanced antibody reaction in other infection due to coliform organisms had been partly explained on the basis of anamnestic response to febrile illnesses.[1] Such could have explained the pronounced response of anti-D agglutinations in patients with clinical malaria but in this study similar trend was observed among healthy controls without fever.

At titre of $\geq$ 1:80 only 27.5% of the subjects demonstrated agglutinations against *Salmonella typhi* antigens. Fifty percent of the subjects and controls had demonstrable antibodies at dilution of approximately 1:60 and 1:40 respectively. Thus, at titre of 1:100 as recommended by Agbonlahor et al,[3] and 1:160 as suggested as cut off mark by Alaribe et al,[6] fewer children had demonstrable agglutination than adults suggesting that sero-conversion may be age dependent and that a lower cut off margin may be required for diagnosis if a single titre was to be relied upon. Unlike the adult the child may have had fewer contact with the antigens and hence a weaker antibody response. This is supported by the trend noticed in the study where greater proportion of children aged 9 – 14 years, than younger ones, had demonstrable titres at dilution of $\geq$ 1:80.

Antibody responses to DH and DO were more marked than those against *S paratyphi* antigens suggesting that more children have had contact with the *Salmonella typhi* than *Salmonella paratyphi* AB or C. This trend is in consonance with the observations of Agbonlahor et al[3], Mohammed et al[10] and Olubuyide et al[11] amongst adult population. Thus, emphasis should be on antibodies to D antigens should the need arise for reliance on Widal reaction.

Antibodies against H rather than O antigens were more readily elicited in both subjects and controls, an observation that is in agreement with the works of Opara and Nweke[12] and Agbonlahor et al[3] that H antibodies, once formed, are more enduring in circulation than O antibodies. The implication of this could be that H antibodies signify chronic rather than acute infection. Emphasis may therefore be on antibodies to DO when employing Widal test to screen for acute infection in childhood. Enhanced D agglutinins may be a phenomenon associated with childhood malaria. In comparison with healthy controls, the child with malaria would mount pronounced antibody response to especially, D antigens, caution is therefore demanded in the interpretation of single Widal reactions. Allowance for higher baseline antibody levels should therefore be made in the child with malaria, should there be consideration for typhoid fever.

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