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Inter-relationship Between Malaria Parasitaemia And Widal Reaction In Febrile Patients

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ABSTRACT

The inter-relationship between malaria parasitaemia and widal reaction in febrile patients was investigated. Malaria and typhoid fever (febrile illnesses) were diagnosed by microscopic malaria parasite presence and Salmonella typhi/paratyphi antibodies screened by Widal reaction with titres greater than or equal to 160 regarded as positive. Of the 797 blood samples investigated. 125 (15.7%) were strictly malaria cases, 429 (53.9%) were mixed infection. 183 (23.0%) were strictly typhoid cases while 60 (7.5%) were negative for both febrile conditions. A co-infection rate of 52.7% and 58.9% was obtained for *Salmonella typhi* 'O' and 'H' respectively and this was closely followed by *S. paratyphi* B 'O' with a co-infection rate of 41.3%. This positivity rate of *S. paratyphi* B 'O' is significant ($P<0.05$) and may indicate a possible antigenic similarity with *S. typhi* and malaria parasite.

Key words: Malaria parasitaemia. Widal test, Paratyphi antigens.

INTRODUCTION

Fever, a clinical hallmark of inflammation and the common designation of systemic temperature elevation, is produced by a short polypeptide. interleukin-1 and Tumour Necrosis Factor-alpha (TNF-) derived from macrophages during infectious disease processes and inflammatory responses, apparently reset the body's "thermostat" to permit a higher body core temperature level[1]. Malaria and Typhoid are febrile illnesses resulting in Malaria and Typhoid fever respectively. World Health Organisation has recognized malaria, a mosquito - borne febrile illness, as causing more morbidity than any other disease and has been reported to be responsible for at least 1 million deaths a year, mostly in voting children in tropical countries[2]. It is the most common cause of outpatients visit to health care facilities and it is consistently reported as one of the five main causes of death in Nigeria[3]. Typhoid and Paratyphoid fever are clinically similar acute systemic illnesses caused by infection with *Salmonella tiphi* and *Salmonella paratyphi*. Both are generally termed enteric fever[4]. Typhoid fever is one of the leading preventable global causes of death due to infectious disease, accounting for over 600,000 annual deaths worldwide[5]. Schizogony in *Plasmodiun falciparum* infection has long been shown not to occur in the peripheral blood stream but in tissues and organs where there is massive multiplication in visceral capillaries[6]. Clark and Chaudhri[7] explained that the schizonts are not found in the peripheral blood but the tissue necrosis factor-α (TNF-α) released at the burst of schizogony, acts on the peripheral, unparasitized circulating red cells causing physical and chemical changes to the red cells rendering them susceptible to macrophage phagocytosis. Macrophages become activated and the release of interleukin-l inevitably ensues[8,9]. The clinical features of enteric fever tend to be more severe with *Salmonella typhi* (typhoid fever)[4]. The organisms penetrate the ileal mucosa and spread through the regional lymph nodes, the lymphatics and blood stream infecting mononuclear macrophages in lymph nodes, bone marrow, liver and spleen[4]. The infection of macrophages stimulates the production of interleukin-1 and Tumor Necrosis Factor-α (TNF--α) thereby causing the prolonged fever[10]. The pathogenesis of both malaria and typhoid fever centre on the production of the macromolecules Interleukin-1 and Tissue Necrosis Factor-alpha consequently producing fever. The

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definitive diagnosis of malaria is the presence of just a parasite in a blood film, which may be missed because of the cyclical nature of the parasite or the Microscopists skill. The isolation of *Salmonella typhi* or *paratyphi* organisms, which is diagnostic, may also be missed due to the stage of infection. Hence the need for this study.

MATERIALS AND METHODS

Subjects: These include 797 adults with febrile illness referred by some hospitals to Alees Diagnostic Medical Laboratory center, Lagos for laboratory investigations for malaria parasites and Widal reaction tests. 5ml of blood sample was collected from each subject and 2ml was dispensed into Ethylene Diamine Tetra Acetic acid (EDTA) anticoagulant tubes and mixed by inversion. The remaining 3ml was dispensed into clean plain tubes, allowed to clot, retract and serum separated for Widal tests.

Malaria Parasite examination: Thin and thick films were made on clean slides. The films were stained with Giemsa stain using a previously known method[11]. These were examined microscopically for malaria parasites and scored as described by Cheesbrough[12] as follows:

1-10 malaria parasites seen per 100 high power fields of examination = +.

11-100 malaria parasites seen per 100 high power fields of examination = ++.

1-10 malaria parasites seen per high power field of examination = +++.

More than 10 in every high power field of examination = ++++.

Widal Screening test: Rapid slide agglutination tests were done using Commercial Antigen kit (Biotech Reagents Ltd, UK). The Kit consists of *Salmonella typhi* (D) and *Salmonella paratyphi* A, B, and C for both the somatic (O) and flagella (H) antigens. Tube titrations were done on serum samples with doubling dilutions using normal saline ranging from 1 in 20 to 1 in 640. Titre values greater than or equal to in 160 were regarded as positive and less than 1 in 160 as negative for any of the antigens.

Statistical analysis: Results were analyzed using Microsoft excel Computer assisted programme. The test for significance was chi square with p values less than 0.05 regarded as significant.

RESULTS

Of the 797 blood samples examined, 554 (69.5%) had Malaria parasitaemia. One hundred and twenty-five (15.7%) of these were strict malaria cases while 429 (53.8%) had concurrent Widal positive titres. One hundred and eighty-three (23%) were Widal positive-malaria negative and 60 (7.5%) were malaria negative-Widal negative (Table 1). Various rates of cross-reaction of Salmonella antibodies were observed in patients positive for both Malaria and Widal tests. There was little rate of cross-reaction with most of the *S. paratyphi* antibodies ($\leq$10.0%) expect *S. paratyphi* B 'O' with a significant rate of

41.3% (x^2=8.386 p<0.0S).

The cross-reaction rates for *S. typhi* were 52.7% and 58.9% for 0 and H antibodies respectively (Table 2). The Widal test positive patients were found to have more of the *S. typhi* antibodies with both '0' and 'H' being almost equal with 46.4% and 45.4% respectively (Table 3). This was closely followed by *S. paratyphi* B 'O' with 43.7%. Other *S. paratyphi* species had less than 8.0% of patients being positive for Widal test only.

DISCUSSION

Typhoid and Malaria fevers are both endemic in Nigeria and in most cases both present with a common symptom of prolonged fever difficult to differentiate clinically except through laboratory diagnosis.

A significant proportion of 69.5% malaria positivity within the febrile conditions supports the report of Salako et al (1981) that malaria constitutes the major cause of outpatients visit to health-care facilities in Nigeria[3]. The prevalence of strictly malaria cases in this study is marginally but not significantly (p>0.05) lower than the findings of investigators from Enugu, South East Nigeria who reported a rate of 22.0% (12) but significantly (<0.05) lower than the report of other investigators who obtained 27% from Zaria in Northern Nigeria[14]. The rate of concomitant infection with *S. typhi*

'O' (52.7%) was similar to the 52.6% rate obtained by Ohanu et al.[13] but significantly higher than the 10% rate obtained by Mbuh et al.[14] using titres of greater than or equal to 160. Elsewhere in the world varying rates were also obtained; 47.9% from Cameroun[15] and 32.5% from India[16]. These variations can be attributed to the environmental sanitation, tradition and hygienic level of the sources of infection of the different localities of the studies.

Table 1: Summary of overall result

Test Result	No. (%) obtained
Malaria parasitaemia only	125 (15.7)
Malaria and Widal positive	429 (53.8)
Negative for Malaria parasite only	183(23.0)
Malaria negative, Widal negative	60(7.5)
TOTAL	797

Table 2: Occurrence of Salmonella diagnostic antibodies in Malaria positive patients (n=429)

Salmonella antigen	Positive for both MP and Widal [No. (%)].
S. typhi 'O'	226 (52.7)
S. typhi 'H'	253 (58.9)
S. paralyphi A 'O'	10 (2.3)
S. paratyphi A 'H'	34 (7.9)
S. paralyphi B 'O'	177 (41.3)
S. pararyphi B 'H'	43 (10.0)
S. paralyphi C 'O'	24 (5.6)
S. paratyphi C 'H'	32 (7.5)

Table 3: Distribution of Salmonella diagnostic antibodies among the Widal positive/Malaria negative subjects (n = 183).

Salmonella antigen	Positive for Widal test only (%)]
S. typhi 'O'	85 (46.4)
S. typhi 'H'	83 (45.40
S. paratyphi A 'O'	9 (4.9)
S. paratyphi A 'H'	14 (7.7)
S. paratyphi B 'O'	80 (43.7)
S. paratyphi B 'H'	28 (15.3)
S. paratyphi C 'O'	10 (5.5)
S. paratyphi C 'H'	11(6.0)

Typhoid is transmitted through food and drinking water and so it is mainly hygiene and sanitary conditions that determine its spread[5]. The presence of concomitant infections in patients also enabled subclinical parasitaemia which could have been missed but for the malaria fever synergized by typhoid. This indicates that some individuals' habour malaria parasites without fever thus signifying effect of immune tolerance to the parasite in the locality. This observation is supported by the findings of investigators who reported that a large proportion of children in endemic areas have malaria parasitaemia without clinical symptoms[17]. The Malaria-negative, Typhoidal antibody-negative cases found in this study could have been due to other infections that can give rise to fever. These are referred to as pyrexia of unknown origin[18].

With the exception of *Salmonella paratyphi* B 'O' with a high rate of co-infection, the rates of co-infection of the other paratyphoid group were significantly low ($p<0.05$). The paratyphoid Salmonellae are known to cause milder forms of the enteric fever[5] and so their presence in Malaria patients may not have been responsible for the fever. The fact that the rate of co-infection of *S. paratyphi* B 'O' and *S. typhi* (Group D) were quite close could indicate a possible antigenic similarity between *S. paratyphi* B 'O', *S. typhi* (Group D) and malaria antigen. The manifestation of both infections is fever and before fever can be produced, the malaria parasite or typhoid bacteria must release certain chemical molecules to bring about the rise in temperature. These molecules, which are fever causing agents, have been identified to be Tumor necrosis factor - α (TNF-α) and interleukin-1[1]. Just as malaria parasites and typhi/paratyphi bacteria invoke antibody production, TNF-α and Interleukin-1 released during the course of these infections may also be immunogenic producing their antibodies. The high titre of *S. paratyphi* B 'O' along with *S. typhi*, which correlated with Malaria parasitaemia, may be due to antigen similarities with cross-reacting antibodies.

S. Paratyphi B O' has been shown to manifest itself in both infections and thus probably has antigenic similarity with *S. typhi* and malaria parasites and/or the TNF-α and Interleukin-1. *S. paratyphi* B 'O' antigen therefore can be used as an indicator or predicting factor for typhoid and malaria and the similarity linking them thus needs further investigation.

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