

## **RATE OF CO-INFECTION WITH MALARIA PARASITES AND SALMONELLA TYPHI IN ZARIA, KADUNA STATE, NIGERIA**

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### **Abstract**

*Background:* Typhoid and malaria co-infection is a major public health problem in many developing countries. Most of the co-infections treated are based on methods of diagnosis plagued with assumptions which possibly exaggerate the situation. Thus the aim of this work was to investigate the rate of co-infection with respect to the use of Widal test and blood culture methods for diagnosing typhoid fever in Zaria, Nigeria.

*Method:* A total of 218 blood samples were collected from patients with a clinical suspicion of malaria and typhoid fever and examined for malaria parasites and *S. typhi* infection.

*Results:* Sixty samples were positive for malaria parasites, 22 of which were positive for typhoid by the Widal test and only one by the culture method. The rate of co-infection was significantly high when typhoid was diagnosed by Widal (10.1%) than by blood culture method (0.5%). A correlation analysis showed no specific relationship between malaria parasite load and the level of Salmonella antibody titres in malaria patients ( $r = 0.05$  and  $0.08$  for somatic and flagella antigens of *S. typhi* respectively).

*Conclusion:* The incidence of typhoid and malaria co-infection will greatly reduce if the diagnosis of typhoid fever in malaria endemic areas such as Zaria is based on blood culture.

**Key words:** Malaria, typhoid fever, co-infection

### **Introduction**

The treatment of malaria and typhoid co-infection is a common phenomenon in many parts of Africa.<sup>1</sup> Malaria and typhoid remain a treat to many people in Sub Saharan Africa for several reasons: the increasing poverty, deterioration in public health services, compounded by HIV / AIDS and increasing resistance of malaria parasites to chloroquine;<sup>2</sup> the lack of portable water and widespread misuse of the Widal agglutination test for diagnosing typhoid fever,<sup>1,3,4</sup> increased requests for Widal tests as a means of making quick money by private laboratories are other factors.<sup>1,5</sup> Malaria and typhoid fever often present with mimicking symptoms especially in the early stages of typhoid<sup>1,6</sup>. Thus it is very common to see patients undergoing both typhoid and malaria treatments even if their diagnosis has not been confirmed. There are more typhoid cases in areas of drug resistant malaria and a cross reaction between malaria parasites and salmonella antigens may cause

false positive Widal agglutination test.<sup>7</sup> A reliable diagnosis of typhoid is based on culture of blood, stool and bone marrow.<sup>3</sup> Bone marrow aspiration has technical difficulties and stool cultures are positive in most patients only in the third week of infection. This leaves blood culture as the best method for diagnosing early Salmonella typhi infections in the absence of other alternatives. The objective of this study was to determine the rate of co-infection with malaria parasites and *S. typhi* in patients with respect to the use of a single Widal test and blood culture methods for the diagnosis of typhoid fever in Zaria.

### **Method**

Patients directed to the laboratory for the Widal and malaria parasite tests by the attending physician in four hospitals in Zaria were sampled for this study. Following an explanation to and consent of the patient or parent of children, a simple questionnaire was

filled with regards to age, sex and current medication. A total of 218 blood samples were collected from consecutive febrile patients between February and April 1996. Patients who had started treatment were excluded. Blood samples were also collected from 52 apparently healthy individuals as controls. Five milliliters of blood drawn by venepuncture from each person were tested for malaria parasites, *S. typhi* O and H antibodies and also cultured for *S. typhi*.

#### Parasitological examination

Giemsa-stained thick and thin blood films were prepared for each sample and parasitaemia was evaluated per microliter of blood using the thick film preparation according to standard methods<sup>8</sup>, assuming a leukocyte count of  $5400 \mu\text{l}^{-1}$  of blood established for healthy Nigerians<sup>9</sup>. Films were examined microscopically for the presence of malaria parasites within red blood cells in thin films. For thick films, the ring forms, trophozoites and gametocytes were looked for. A smear was considered negative for malaria parasites if no parasites were seen after examining at least 100 microscopic fields. The number of parasites  $\mu\text{l}^{-1}$  of blood was expressed as (parasite count X 5400)/No of leucocytes counted, which was 100.

#### Widal test

The Widal agglutination test was performed on all blood samples by the rapid slide titration method<sup>10</sup> using commercial antigen suspension (Cal-Test Diagnostic Inc. Chino, U.S.A.) for the somatic (O) and flagella (H) antigens. The slide titration test is the prevalent method of performing the Widal test in Zaria and other parts of Nigeria.<sup>4, 11</sup> A positive Widal test was considered for any serum sample with antibody titre  $\geq 1$  in 160 to the O antigen of *S. typhi*.

#### Bacteriological blood culture

Two milliliters of each blood sample were aseptically introduced into 18 ml of thioglycolate broth (DIFCO) and incubated at  $37^\circ\text{C}$  for an initial period of 48 h and sub-cultured on MacConkey agar (Oxoid). *S. typhi* organisms were identified on the basis of standard cultural, microscopic and biochemical characterization.<sup>12</sup> Inoculated blood culture media was discarded as negative if there was no growth after 7-10 days. The relationship between malaria parasite counts  $\mu\text{l}^{-1}$  of blood and *Salmonella* O and H antibody titres were determined by carrying out a correlation analysis using the Microsoft Excel Computer Package.

## Results

The results of this study are based on parasitological examination for malaria parasites and bacteriological and serological tests for the diagnosis of typhoid fever in 218 patients attending 4 hospitals and 52 apparently healthy individuals in Zaria. The patients comprised 100 females and 118 males aged between 2 and 59 years (mean = 21 years) Malaria parasites were found in 60 (27%) samples (mean parasite load =  $1.2 \times 10^4$  parasites  $\mu\text{l}^{-1}$  of blood, standard deviation =  $2 \times 10^5$  parasites), henceforth known as malaria patients.

Twenty-two of the 60 malaria patient samples were positive for typhoid by the Widal test considering a positive Widal test for any sample showing antibody titre of greater or equal to 1 in 160 against the somatic (O) antigen of *S. typhi*.<sup>13</sup> Thirty This gave a 10.1% rate of co-infection with malaria parasites and *S. typhi* when typhoid was diagnosed by the single Widal agglutination test and a 0.5% rate of co-infection with malaria parasites and *S. typhi* when blood culture was used.

In the control group, all blood culture samples were negative for *S. typhi*. Antibodies to the somatic antigen were detected in 16 (30.8%) of 52 samples. Twelve (23.1%) control samples had malaria parasites (carriers), (mean parasite load =  $1.3 \times 10^3 \mu\text{l}^{-1}$  of blood, standard deviation = 1079). Three (5.8%) carriers of malaria parasites were positive for the Widal test.

Table 1 shows the distribution of somatic and flagella antibodies of *S. typhi* in malaria patients and carriers of malaria parasites. There was no significant difference between the mean antibody titres (anti 'O' titres) in the malaria and typhoid patients ( $Z = 0.03$  and  $\alpha = 0.05$ ), typhoid and non typhoid patients, malaria patients and carriers of malaria parasites, where  $Z$  values at 5% confidence level were  $-0.03$ ,  $1.4$  and  $0.03$  respectively. The mean parasite density was however significantly higher in malaria patients than in carriers of the parasites ( $Z = 4$ ,  $\alpha = 0.05$ ).

Table 2 shows results of correlation analysis for different categories of malaria patients and carriers of malaria parasites. Although 71% of malaria positive samples had detectable levels of antibodies to the somatic antigen of *S. typhi*, parasite load and antibody levels were shown not to be mutually correlated, irrespective of age and sex of the patients. The correlation coefficient ( $r$ ) of  $-0.01$  for somatic antigen and  $0.05$  for flagella antigen shows that the level of *Salmonella* antibodies in the malaria patients was not related to the presence of malaria parasites.

Table 1: Distribution of salmonella antibodies in malaria patients (n=60) and carriers of malaria parasites (n = 12)

Antigenes	Reciprocal antibody Titre					Reciprocal antibody Titre				
	Patients					Carriers				
	≤20	40	80	160	≥320	≤20	40	80	160	≥320
O	27	8	3	18	4	6	3	0	2	1
H	4	5	16	19	16	1	2	2	5	2

O = Somatic antigen of *S. typhi*H = Flagella antigen of *S. typhi*Table 2: Correlation coefficient for malaria parasite count and *S. typhi* O and H antibody levels in malaria patients by sex and age groups and in carriers of malaria parasites

Category	Correlation Coefficient (r)	
	O antigen	H antigen
Males (n = 30)	0.129	0.033
Females (n = 30)	0.021	0.125
Age group 1-10 years (n = 6)	0.104	0.25
Age group 11-20 years (n = 6)	0.004	0.004
Age group 12-30 years (n = 29)	0.128	0.021
Age group > 30 years (n = 8)	0.081	0.082
All patients (= 60)	-0.014	0.054
Carriers of malaria parasite	0.009	0.075

O = Somatic antigen of *S. typhi*H = Flagella antigen of *S. typhi*

## Discussion

In this study cultural diagnosis of typhoid fever showed that the rate of co-infection with malaria parasites was 0.5% against 10% by the Widal test. In addition, a correlation analysis showed that the presence of malaria parasites had no specific relationship with *S. typhi* O and H antibody levels in malaria patients and carriers of malaria parasites.

In Zaria, it is common to find patients receiving typhoid malaria treatment simultaneously since medical practitioners usually rely on a single Widal test result for the diagnosis of typhoid fever. Moreover clinicians are often compelled by patient's behaviour to prescribe anti-typhoid drugs even when Widal test results are not suggestive of typhoid. In this study, the 22 malaria patients who had a positive Widal test result could have been cases of cross reactivity between *S. typhi* and malaria parasite antigen<sup>14</sup> but 31 other Widal positive samples were negative for both malaria parasites and *S. typhi*. Many patients often take anti-malaria drugs before presenting at the hospital but would not admit it when questioned. Such patients can only be identified in a

survey through testing for residual malarial drugs in their blood. These patients who tested negative for both typhoid and malaria but were treated for typhoid and malaria eventually got well after the treatment. They could have been cases of drug resistant malaria or patients suffering from self limiting infections such as transient viraemia.

Although 36.7% of malaria patient samples were positive for typhoid by the Widal test, blood culture results suggest that only 1.6% of malaria patients would be infected with *S. typhi*. It seems that the outcome of the Widal reaction for patients with a clinical suspicion of typhoid and malaria depends on individual host immune responses, which become stimulated in febrile conditions associates with malaria fever. This memory response could cause positive Widal reactions in previously sensitized patients. A false positive Widal test reaction occurs in about 35% of malaria patients<sup>6</sup> and a similar result (36.7%) was recorded in this work. This can be accounted for by the demonstrated high prevalence of Salmonella antibodies in the local healthy population and the fact that 50% of the patients had detectable levels of antibodies to the somatic antigen.

The 6.3 prevalence of typhoid in Zaria was significantly higher than the earlier report of 3.1%. This could have been due to the period of the year during which sampling was done (February-April) when places are dry, water is scarce and cultural practices favour high transmission of *S. typhi* in this environment. The high rate of typhoid and malaria co-infection associated with the Widal test (10.1%) may be responsible for the frequent treatment of mixed infections in Zaria. However, blood cultural results showed that this rate of co-infection could be reduced to only 0.5%.

In view of this significant difference and in order to rule out any case of malaria with mimicking symptoms, or the influence of anamnestic response the practical use of cultural methods for the diagnosis of typhoid fever should be emphasized in our clinical laboratories. This will also improve patient management by cutting down cost of treatment and eliminate other risks associated with misuse of antibiotics.

#### References

1. Nsutebu E, Ndumbe P. The Widal test for typhoid fever: is it useful? *Africa Health*. 2001; 23: 5-9.
2. Alnwich D. Meeting the malaria challenge. *Africa Health*. 2001; 23:18-19.
3. Edelman R, Levine M.M. Summary of an international workshop on typhoid fever. *Rev Infect Dis* 1986; 8: 329-349.
4. Edet O. Another opinion on the Widal test. *Africa Health*. 2002; 24:2.
5. Usman A. Typhoid fever- is the Widal test useful? *Africa Health*. 2002; 24: 3.
6. Petit P. L. C, Wamola I. A. Typhoid fever: a review of its impact and diagnostic problems. *East Afr Med J* 1994; 11: 183-188.
7. Frimpong E. H, Feglo P, Essel-Ahun M, Addy P. A. K Determination of diagnostic widal titres in Kumasi, Ghana. *West Afr J Med* 2000; 19: 34-38.
8. World Health Organisation. A practical handbook on severe and complicated malaria, Geneva, 1991.
9. Akinyanju O, Grossman S. A. Leucocyte counts in healthy Nigerians. *Nigerian Medical Journal* 1973; 3: 95-99.
10. Raphael S. S, Spencer F. Immunology and serology. Lynch's medical laboratory technology. Saunders, Philadelphia. 1983.
11. Mbuh F. A. Reaction of a Widal reagent in sera from suspected typhoid and non typhoid patients in Zaria. M.Sc. Thesis, Ahmadu Bello University, Zaria. 1997.
12. Cowan S.T. Cowan and Steel's manual for the identification of Medical bacteria. 1981.
13. Olubuyide T. O Factors that may contribute to death from typhoid infection among Nigerians. *West Afr J Med* 1992; 11: 112-115.
14. Alaribe A. A, Ejezie G. C. E, Ezedinachie E. N. U. The prevalence of salmonella antibody among malaria patients in Calabar. *Nigerian Journal of Parasitology (abstract)* 1995;