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Full Length Research Paper

Parasitological, bacteriological, and cultural determination of prevalence of malaria parasite (*Plasmodium falciparum*) and typhoid fever co-infection in Abakaliki, Ebonyi State

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A total of 250 blood samples were collected from febrile patients with clinical symptoms of malaria and typhoid fever. They were examined for malaria parasite and typhoid fever using parasitological examination, bacteriological and cultural techniques respectively. The result showed that 33 (13.2%) samples were positive for malaria parasite, 53 (21.2%) were positive for typhoid fever by the widal test and 2 (0.8%) were positive by culture method. Fourteen patients (5.6%) had malaria and typhoid fever co-infection which was significantly high when diagnosed by widal test than by cultural method (0.8%). The statistical analysis showed no significant relationship with patient's age, sex and spatial distribution but significant relationship was observed with patient's occupation.

Keywords: Prevalence, *Plasmodium falciparum*, *Salmonella typhi*, parasitological, bacteriological, culture.

INTRODUCTION

Typhoid fever and malaria co-infection is a major public health problem in many developing countries of the world (Mbuh et al., 2003). Typhoid fever is an acute, life threatening febrile illness caused by the bacterium *Salmonella enterica,* with an estimated 22 million cases of typhoid fever and 200,000 related deaths occurrence world - wide each year (Crump et al., 2000).

Malaria is caused by protozoan parasites of the genus *Plasmodium*, *phylum Apicomplexa*; in humans it is caused by *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium vivax*. Moreover, *P. falciparum* is the most important cause of this disease and is responsible for about 80% of malaria infections and 90% of deaths in sub-Sahara Africa (Mendis et al., 2001). Malaria caused about 400 - 900 million cases of fever and approximately one to three million deaths annually (Breman, 2001). This represents at least one death every 30 s. Vast majority of cases occur in children under the age of 5 years and more so with pregnant women (Greenwood et al., 2005).

Malaria and typhoid fever remain a threat to many people in sub Sahara Africa for several reasons which includes increasing poverty, poor public health services, increase in HIV/AIDS spread, increase resistance of malaria parasites to wide range of antimicrobial agents and lack of portable water (Alnwich, 2001). This disease has been associated with major negative economic impact in regions where it is widely spread such as high costs of health care, working days lost due to sickness, days lost in education, decreased productivity due to brain damage from cerebral malaria, loss of investment and tourism etc (Ellis et al., 2006; Greenwood et al., 2005). In developing countries where there is a heavy malaria burden, the disease may account for as much as 40% of public heath expenditure, 30 - 50% of in-patient admission and up to 50% of outpatient visits (WHO, 2006). The present study is aimed at determining the prevalence of malaria and typhoid fever co-infection in Abakaliki Nigeria using parasitological, bacteriological and culture methods and its relationship with epidemiological factors such as age,

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sex, spatial distribution and occupation.

MATERIALS AND METHODS

Collection of blood samples: A total of 250 blood samples were aseptically collected from four hospitals in Abakaliki namely Mile 4 hospital (52), Federal Medical Centre Abakaliki (65), Ebonyi State University teaching hospital (80), EBSUTH Nwaezenyi Annex (53) within a period of six months (February 2007 - August 2007). These blood samples were collected from male and female patients with febrile illness attending these hospitals and structural questionnaire was given to these patients to obtain information on some epidemiological factors.

Serum preparation

About 1 - 2 ml of venous blood collected from patients into sterile dry tubes were allowed to clot and retracted, after which it was centrifuged at 3,500 rpm for 5 min to give a clear sera. These were stored at -20°C in a refrigerator and were analysed within 2 days of collection.

Parasitological examination of blood samples

Each blood sample was subjected to parasitological examination using whole blood commercial malaria *P. falciparum* test device (ACON laboratory Ltd San Francisco USA) with a catalog number IMA -402. A Pasteur pipette was used to transfer 10 ml of whole blood sample into a sterile test tube and 3 drops of buffer was added into the test tube and was mixed appropriately. *P. falciparum* test strip was placed into the test tube containing the whole blood sample and buffer with the arrow pointing toward the specimen and the result was taken after 10 min.

Examination of blood sample by widal testing

Widal agglutination test was performed on all blood samples by the rapid slide titration method using commercial antigen suspension (cal - Test Diagnostic inc. Chino, U.S.A) for somatic (O) and flagella (H) antigens. 50 ml of the blood serum was placed on eight rows of circles on the test cards and a drop of positive and negative serum suspension were placed beside each sample on the test slide. Each content on the slide were mixed thoroughly and spread over the entire circle and the slide was rocked gently for a minute and was observed for agglutination under light source. A positive widal test was considered for any given serum sample with antibody titer of 1:160 for *S. typhi*murium antigens.

Tube agglutination test

1 ml serial dilution of serum sample was carried out and 1 drop of appropriately well shaken suspension was added to each test tube in a given row and mix to get a dilution of 1:20,1:40, 1:160, 1:320 and 1:640. The test tubes were incubated for 24 h after which the tubes were examined for agglutination reaction.

Bacteriological blood culture test

2 ml of each blood sample was aseptically introduced into 18 ml of sterile thioglycolate broth (DIFCO) and incubated at 37°C for an initial period of 48 h and sub cultured on MacConkey agar (Oxoid, Uk) *S. typhi* organisms were identified and characterisized using standard Microbiology technique (Cheesbrough, 2002).

RESULTS

A total of 250 blood samples collected from febrile patients with clinical symptoms of malaria and typhoid fever were examined for malaria parasite *P. falciparum* and *S. typhi* using parasitological examination, bacteriological and culture test methods respectively. Sixty apparently healthy individuals were used as control and the patients composed of 123 males and 127 females aged between 0 and 70 years.

The result of our study showed that 14 (42.4%) out of 33 malaria patient samples were positive for typhoid fever by widal test by considering a positive widal test for any sample showing antibody titer of greater or equal to 1: 160 against the somatic (0) antigen of *S. typhi*, 14(5.6%) were positive for co - infection with malaria parasite (*P. falciparum* and *S. typhi*) when diagnosed using single widal agglutination test and a 0.8% prevalence of malaria parasite and *S. typhi* co - infection when diagnosed using blood culturing method. In the control group, all 60 cultured samples yielded negative for *S. typhi*. Antibodies to the somatic antigen were observed in 20 (33.3%) samples, 30 (50%) control samples yielded malaria parasite carriers while 2 (3.33%) carries of malaria parasite (*P. falciparum*) were positive for widal test.

Tables 1 - 3 shows the age distribution of *P. falciparum*, typhoid fever, malaria and typhoid fever co-infection respectively, the results after analysis with chi - square showed that the factors considered does not have any relationship with disease occurrence. Table 4 - 6 shows the results of sex distribution of malaria parasite, typhoid Fever, malaria and typhoid fever co-infection which showed that the factor has a relationship with the occurrence of the disease (Table 4), while (Table 5 and 6) showed that there is no relationship between factors considered and disease occurrence. Table 7 - 9 represents the occupational distribution of malaria and typhoid fever co-infection, malaria infection and typhoid fever and the result showed that factors considered have relationship with disease occurrence. Table 10 - 12 showed the spatial distribution of malaria parasite and typhoid fever infection and the result showed no relationship (Table 10 and 11), while Table 12 showed that the occurrence of the disease has a relationship with the factors considered.

DISCUSSION

Our present study revealed that the prevalence of typhoid fever and malaria parasite (*P. falciparum*) co-infection was 0.8% using culture method and 5.6% using widal test technique for screening for the presence of *P. falciparum* and *S. typhi*. This was in agreement with the works of Mbuh et al., 2003 and Ammah et al., 1999 where they had 0.5 vs 10.1% and 17 vs 47.9% prevalence using the same method as above respectively. More so, statistical analysis showed that age, sex, and spatial distribution have no relationship with malaria parasite and typhoid

Table 1. Age distribution of malaria parasite (*Plasmodium falciparum*) infection in Abakaliki, Ebonyi State.

Age (yrs)	Number tested	Number tested positive	% positive	Tested RR value
0 - 10	26	2	7.69	0.56
11 - 20	58	7	12.07	0.89
21 - 30	96	12	12.50	0.92
31 - 40	38	9	23.68	2.09
41 - 50	14	1	7.14	1.89
51 - 60	9	1	11.11	0.84
61 - 70	9	1	11.11	0.84
Total	250	33	13.20	

Table 2. Age Distribution of typhoid fever infection in Abakaliki, Ebonyi State.

Age (yrs)	Number tested	Number tested positive	% tested positive	Relative risk
0 - 10	26	4	15.38	0.70
11 - 20	58	15	25.86	1.31
21 - 30	96	22	22.92	1.14
41 - 50	14	4	28.57	1.42
51 - 60	9	1	11.11	0.51
61 - 70	9	1	11.11	0.51
Total	250	53	21.20	

Table 3. Age Distribution of malaria parasite (*Plasmodium falciparum*) and typhoid fever Co-infection in Abakaliki, Ebonyi State.

Age (yrs)	Number tested	Number tested positive	% tested positive
0 - 10	26	0	0
11 - 20	58	2	3.45
21 - 30	96	7	7.29
31 - 40	38	4	10.53
51 - 60	9	0	0
61 - 70	9	0	0
Total	250	14	5.60

Table 4. Sex distribution of malaria parasite (*Plasmodium falciparum*) infection in Abakaliki, Ebonyi State.

Sex	Number tested	Number tested positive	% tested positive
Male	123	10	8.13
Female	127	23	18.11
Total	250	33	13.20

Table 5. Sex distribution of typhoid fever infection in Abakaliki, Ebonyi State.

Sex	Number tested	Number positive	Tested % tested positive
Male	123	27	21.95
Female	127	26	20.47
Total	250	53	21.20

Table 6. Sex distribution of malaria and typhoid fever co-infection in Abakaliki, Ebonyi State.

Sex	Number tested	Number tested positive	% tested positive
Male	123	5	4.07
Female	127	9	7.09
Total	250	14	5.60

Table 7. Distribution of malaria / typhoid fever co-infection with respect to occupation in Abakaliki, Ebonyi State.

Occupation of patient	Number tested	Number tested positive	% tested positive
Applicant	15	2	13.33
C. servants	20	0	0
Drivers	05	0	0
Farmers	100	5	5.00
Lab. Attendant	4	1	25.00
Nurse	3	1	33.33
Students	83	2	2.41
Traders	20	3	15.00
Total	250	14	

Table 8. Distribution of malaria parasite (*Plasmodium falciparum*) infection with respect to patient's occupation in Abakaliki, Ebonyi State.

Occupation of patient	Number tested	Number tested positive	% tested positive
Applicant	15	3	20.00
C. servants	20	2	10.00
Drivers	5	3	60.00
Farmers	100	11	11.00
Lab. Attendant	4	1	25.00
Nurse	3	1	33.33
Students	83	7	8.432
Traders	20	5	25.00
Total	250	33	13.20

Table 9. Distribution of typhoid fever infection with respect to patient's occupation Abakaliki, Ebonyi State.

Occupation of patient	Number tested	Number tested positive	% tested positive
Applicant	15	6	40.00
C. servant	20	1	50.00
Drivers	5	3	60.00
Farmers	100	23	25.00
Lab. Attendant	4	1	25.00
Nurse	3	1	33.00
Students	83	12	12.46
Traders	20	4	20.00
Total	250	53	

Table 10. Spatial distribution of malaria / typhoid fever co-infection in Abakaliki, Ebonyi State.

Location	Number tested	Number tested positive	% tested positive
Urban	100	4	4.00
Rural	150	10	6.67
Total	250	14	5.60

Table 11. Spatial distribution of malaria (*Plasmodium falciparum*) co-infection in Abakaliki, Ebonyi State.

Location	Number tested	Number positive	% tested positive
Urban	100	13	13.00
Rural	150	20	13.33
Total	250	33	13.20

Table 12. Spatial distribution of typhoid fever infection in Abakaliki, Ebonyi State.

Location	Number tested	Number tested positive	% tested positive
Urban	100	15	15.00
Rural	150	38	25.33
Total	250	53	21.20

fever co-infection while patient's occupation has a relationship with disease occurrence.

In recent times, it is common to find patients receiving typhoid malaria treatment simultaneously since medical practitioners still use a single widal test result for the diagnosis of typhoid fever. In this study, 19 malaria patients who have positive widal test results could be that they had cases of cross reaction between *S. typhi* and malaria parasite antigen. This is not out of place with the findings of Alaribe et al., 1995, Mbuh et al., 2003, and Uneke, 2008. Twenty other widal positive samples were negative for both malaria parasite and *S. typhi* when the other two methods were used. Many patients often take antimalaria drugs before presenting themselves to doctors but would not accept such when asked, such patients can only be identified in a survey through testing for residual malaria drugs in their blood (Mbuh et al., 2003). These patients who tested positive for both typhoid fever and malaria but were treated for typhoid fever and malaria eventually got well after treatment. They could have been cases of drug resistant malaria parasite or patients suffering from self limiting infections such as transient viraemia (Uneke, 2008).

Furthermore, 42.4% of malaria patient blood samples were positive for typhoid by the widal test, blood culture results suggest that only 6.1% of malaria positive patients were infected with *S. typhi.* It seems that the out come of the widal reaction for patients with clinical symptoms of typhoid and malaria depends on individual host immune responses, which become stimulated in febrile conditions associated with malaria fever. This memory response could cause positive widal reactions in previously sensitized patients.

When the spatial distribution of typhoid fever infection data was analysed statistically, it was observed that the factor considered has a relationship with disease occurrence. This could be attributed to the non availability of potable water in the rural areas, poor sanitary environment, presence of water logged/swamps, presence of bushes around houses etc. Also, sex and occupations of the patients has a relationship with the disease occurrence. In view of this significant difference and in order to rule out any case of malaria with mimicking symptoms the practical use of culture methods for the diagnosis of typhoid fever is hereby advocated. This will also improve patient's management by cutting down cost of treatment and eliminate other risks associated with misuse of antibiotics. In conclusion the prevalence of malaria and typhoid fever co-infection is endemic areas such as Abakaliki will be greatly reduced if diagnosis of typhoid fever will be based on culture method.

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