High Occurrence of Typhoid Fever and Malaria Co-Infection Among Patients Clinically Diagnosed of Malaria and or Typhoid Infection in CALABAR, NIGERIA.

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ABSTRACT
Background: The occurrence of typhoid fever and malaria co-infection among patients clinically diagnosed of malaria/ and or typhoid fever was investigated in Calabar, Nigeria. Both disorders present with febrile illness**. Materials and Methods**: Venous blood collected from the study subjects were examined for the presence of *Salmonella* antibodies using the Slide Widal test (screening test) and the Tube Widal test (confirmatory test) whereas the diagnosis of malaria was based on the examination of a thin and thick blood films stained with 2% Giemsa. **Results:** The prevalence of malaria and typhoid was 26.7% and 43.3% whereas the prevalence of co-infection of typhoid and malaria was 14.6%. There was no statistically significant difference in the prevalence of malaria by age of patients ($P>0.05$, $X^2=2.934$). Males 21(30.9%) were more infected with malaria than females 19(23.2%), although there was no statistically significant difference in the prevalence of infection by gender ($P>0.05$, $X^2=0.651$). There was no statistically significant difference in the prevalence of typhoid by age of subjects ($P>0.05$, $x^2=7.3$). More females 38(46.3%) were infected with typhoid fever than males 27(39.7%) although there was no statistically significant difference in the prevalence of typhoid by gender ($P>0.05$, $X^2= 0.269$). Age ($P>0.05$, $X^2=14.66$) and gender ($P>0.05$, $X^2=7.62$) did not significantly affect the prevalence of co-infection of malaria and typhoid. Subjects aged 46-60 years had the highest co-infection rate of 5(20.0%) while those aged 1-15 years had the least infection 1(5.0%). The occurrence of co-infection among males and females were 9(13.2%) and 12(14.6%) respectively. **Conclusions**: This study confirmed a high prevalence of malaria (26.7%), typhoid fever (43.3%) and co-infection of malaria and typhoid fever (14.0%) among subjects clinically diagnosed of malaria and / or typhoid fever. This study has confirmed that relying solely on the diagnosis of typhoid fever on results of slide widal test leads to over diagnosis of the infection and unwarranted administration of antibiotics.

Key words: Malaria, Typhoid Fever, Widal, Calabar, Nigeria.

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INTRODUCTION

Malaria is one of the most common endemic parasitic infectious diseases found worldwide particularly in African and south Asia. About three billion people are at risk of infection in 109 countries. Each year, there are an estimated 250 million cases of malaria leading to approximately one million deaths, mostly in children under five years of age (1). Malaria is caused by parasites of the genus *Plasmodium.* The parasite is transmitted by female *Anopheles* mosquito. *P. falciparum* causes the most serious form of the disease. The initial symptoms vary particularly in children and may include irregular fever, malaise, headache, muscular pain, sweating, chills, nausea, vomiting and diarrhea. Most of these symptoms are induced by the release of cytokines by the host's immune system (2). Malaria infection develops via two phases; one that involve the liver phase (exoerythrocytic phase), and one that involves red bloods or erythrocytes (erythrocytic phase). When an infected mosquito pierces a person's skin to take a blood meal, sporozoites in the mosquito's saliva enter the bloodstream and migrate to the liver where they infect hepatocytes, multiplying asexually and asymptomatically for a period of 8-30 days (3).

Malaria is highly endemic in Nigeria with a stable transmission. The large population of Nigeria, its diverse weather conditions and cultures makes it difficult to implement the same malaria control measures throughout the country. Malaria is routinely diagnosed using conventional microscopic technique by the examination of stained thin and thick peripheral blood films (4) or by using a rapid diagnostic test.

Typhoid fever also known as enteric fever is an acute life-threatening illness characterized by a persistently high fever, headache, lethargy, skin rash, loss of appetite, constipation more often than diarrhoea, hepatosplenomegaly and bradycardia (5). An estimated 22 million cases with an associated 200,000 related deaths worldwide are reported to occur each year (6). Typhoid fever is a bacterial infection of the intestinal tract and occasionally the bloodstream caused by *Salmonella typhi*. *Salmonellae* are gram-negative motile bacilli. This species of *S. typhi* carries an endotoxin typical of gram-negative organisms. Common human strains include *Salmonella paratyphi* A, *Salmonella paratyphi* B, *Salmonella paratyphi* C, and *Salmonella typhi* (7). The risk of infection is greatest in developing countries with prolonged exposure to potentially contaminated food beverages and water. The transmission of *Salmonellae* to a susceptible host usually occurs via consumption of contaminated foods such as beef, poultry, and eggs. Improperly prepared fruits, vegetables, dairy products and shellfish have also been implicated as sources of S*almonella*.

There are 3 main antigens associated with *Salmonella* which are O (somatic), H (Flagellar) and Vi Antigen (vi -virulence) (8). The agent can be isolated in the blood during the first 7-10 days of infection. Blood cultures are positive for about 90% of cases (9). The rate of isolation decreases after the first one week. Isolation of *Salmonella* is a definitive proof that the septicaemia is caused by *Salmonella.* Faeces is the most common specimen used for the isolation of *Salmonella*. The organism is present in the faeces in large numbers only during its secondary entry into the intestine. Since *S. typhi* antigen is intermittently excreted in the urine of patients with typhoid fever, serially collected urine from patients with typhoid should be tested for antigen (10). The Widal test may be falsely positive in patients who have had previous vaccination or infection with S. *Typhi* (11). Besides cross-reactivity with other *Salmonella* species, the inability

of the test to distinguish between a current infection and a previous infection or vaccination against typhoid may result in false positive results. False positive Widal test results are also known to occur in typhus, acute falciparum malaria (particularly in children), chronic liver disease associated with raised globulin levels and disorders such as rheumatoid arthritis, myelomatosis and nephrotic syndrome (12).

Malaria and typhoid are endemic in many countries of the world where the prevailing environmental conditions of warm humid climate, poor sanitation habits, poverty and ignorance co-exist. Though caused by different organisms, one; gram negative bacilli, the other; protozoa, both present acute febrile illness. These two diseases have been associated with poverty and underdevelopment (12). Co-infection of Malaria parasite and *Salmonalle* species is common, especially in the tropics where malaria is endemic. The often detection of high Salmonella serotypes antibody titre in malaria patients has led some people to the eroneous believe that malaria infection can progress to typhoid/paratyphoid in all patients (6). As a fact, some people treat malaria and typhoid concurrently once they have high antibody titre for *Salmonella* serotypes, even without adequate laboratory diagnoses for malaria and vice versa (7). Although, the WHO has not recommended using Widal Test for the diagnosis of typhoid, it is the most used test in Nigeria irrespective of its low sensitivity and specificity. In this study, we set out to determine the level of co-infection of malaria and typhoid fever among subjects clinically diagnosed of malaria/ and or typhoid infection in Calabar. The other objective was to examine the relevance of slide widal test in the diagnosis of typhoid fever which is currently being practiced by most medical laboratories in Nigeria.

MATERIALS AND METHODS:

Study Area

The study was conducted at two health institutions namely; University of Calabar Teaching Hospital (UCTH) and General Hospital, Calabar. Calabar is the capital of Cross River State, Nigeria. It is located at latitude 04^0N and longitude 80^0 10^0E along the coastal plains of Nigeria (12).

Study Population

The study subjects were patients clinically diagnosed of typhoid fever and/ or malaria infections who were referred by clinicians for the laboratory confirmation of the aetiology.

Ethical Clearance and Consent.

The protocol for this study was approved by the Ethics and Research Committee of the University of CalabarTeaching Hospital, Calabar, Calabar, Nigeria.

Inclusion and Exclusion Criteria

Subjects that showed signs and symptoms of malaria and typhoid infection like high fever, chills, headache and vomiting were included while those with no related symptoms were excluded.

Determination of Sample Size:

A total of 150 samples were randomly selected.

The sample size was determined using the formula by

Daniel & Joseph 1999 (13). A co-infection rate of malaria parasites and *Salmonella typhi* infection of 10.1% reported in Zaria, Kaduna State was used for the calculation of sample size.

$$N = \frac{t^2 \times p(1-p)}{m^2}$$

Where: N = minimum sample size

P = prevalence rate 10.1% = 0.101

M = marginal of error =0.05

T = level confidence 95% =1.96

$$N = \frac{(1.96)^2 \times 0.101(1 - 0.101)}{(0.05)^2}$$

$$N = \frac{3.8416 \times 0.101 - 0.010201}{0.0025}$$

$$\frac{3.8416 \times 0.090799}{0.0025}$$

$$\frac{0.3488134384}{0.0025}$$

=139.5

The calculated sample size was 140 but a total of 150 subjects were recruited into the study to cater for attrition

Administration of Questionnaire

A well-structured questionnaire on social demographic characteristics was administered to each participant. The questions were divided into two parts. The first part was solely on socio-demographic factors, while the second part was based on the risk factors that can predispose people to malaria and typhoid fever.

Collection and processing of Blood Sample for the Diagnosis of Malaria

Two (2) *ul* and 6*ul* of capillary blood were used to make thin and thick film for the detection of malaria parasite. Thin films were fixed with absolute methanol before staining. Both thin and thick films were flooded with 2% Giemsa solution for 30 minutes. The slides were washed using buffered PH 7.2 water to remove excess stain and allowed to air dry before examination (14, 15). The stained slides were examined under a microscope with X100 objective lens using oil immersion.

Determination of Malaria Parasites density

This was quantified using the thick blood film smear. Malaria Parasites were counted against white blood cells until 200 white blood cells were counted. A White Blood cell count of 8000/*ul* of blood as recommended by WHO (2) was used to calculate the parasite density as shown in the formula below.

$$= \frac{\text{Number of Parasites counted} \times \text{WBC standard (6000)}}{\text{Total number of WBCs counted (200)}}$$

Evaluation of Antibody Titre for Typhoid Fever

The slide/tile method was used as a screening test (Qualitative test) while the tube method was used as a confirmatory test (Quantitative test) for the diagnosis of typhoid infection. About 4 ml of venous blood was collected into a plain container for the diagnosis of tyhoid fever.

Slide Qualitative Method using Serial Dilution Technique:

The antigen suspension was allowed to gain room temperature. The blood was centrifuged at 3000rpm for 5 minutes to obtain serum. The demarcated tile was properly clean and allowed to dry well for 2mins. To the first column of the demarcated white tile, a drop of antigen suspension (Cromatest Widal test kit, Batch No: 21788 by CHRONOLAB SYSTEMS S.L. Barcelona, Spain) of *Salmonella typhi* O, *Salmonella paratyhi* AO, *Salmonella paratyphi* BO, Salmonella paratyphi CO, was dropped to the 1st, 2nd, 3rd and 4th row on the tile and to the second column, a drop of antigen suspension of *Salmonella* H, *Salmonella paratyphi* AH, *Salmonella paratyphi* BH. Fifty (50ul) of serum was placed on the first and second column respectively. The suspension was mixed thoroughly. The tile was rocked manually for 2mins and agglutination was read macroscopically. Positive test gave agglutination within two minute of rocking.

Procedure for Tube Confirmatory Method

Serial dilution method was used following manufacturers instruction. The

serial dilutions used were 1:20, 1:40, 1:80, 1:160, 1:320, 1:640. The O (Somatic antigen) tube was incubated at 37ºC for 18 to 24 hours while the H (Flagella antigen) was incubated at 50ºC for 18 to 24 hours (overnight). The result was read after 24 hours of incubation. The sediments was dislodged gently and agglutination was checked macroscopically.

Data analysis

Data obtained from this study were analyzed using the Statistical Package for Social Science Program (SPSS version 22.0 Chicago). A 95% confidence interval was be used to allow for workable probability value of 0.05 (p=0.05). Proportions obtained in the study were compared using Chi-squared test.

RESULTS

On Table 1 is shown the prevalence of malaria, typhoid and co-infection of both disorders by age of patients. The prevalence of malaria, typhoid and coinfection of both was 26.7%, 43.3% and 14.0% respectively. The prevalence of typhoid increased with the increasing age of patients while a characteristic pattern was not established for malaria and co-infection. There was no statistically significant influence between age on the prevalence of malaria (X^2 = 2.934, df=4, P > 0.05), typhoid (X^2 = 3.230, df=4, P > 0.05) and co-infection of malaria and typhoid (X^2 = 7.3, df=4, P > 0.05).

The occurrence of malaria, typhoid and co-infection of malaria and typhoid among subjects diagnosed of fever is presented on Table 2. Malaria infection was more detected in males (30.9%) than female counterpart (23.2%) whereas more females (46.3%) had typhoid fever than the males (39.7%). More females had a slightly higher cases of co-infection of malaria and typhoid (14.6%) than males (13.2%). However, gender did not significantly affect the prevalence of malaria (X^2 = 0.463; P > 0.05), typhoid fever (X^2 = 7.3; P > 0.05 and co-infection of both in this study (X^2 = 0.651; P > 0.05).

The relationship between results of slide and Tube widal test is shown on Table 3. Of the 150 patients tested, 65 (43.3%) were positive by slide test. Only 16 (24.6%) of the 65 slide positive were confirmed positive by the Tube confirmatory test. There was a statistically significant difference between the rate of detection of Typhoid by the two procedures (X^2 = 0.000; P < 0.005).

Table 1 Prevalence of Malaria,Typhoid Fever and co-infection of malaria and typhoid among Patients with Fever by Age

Age in Years	No. Examined	No (%) Positive for Malaria	No (%) Positive for Typhoid fever	No(%) positive for co-infection of Malaria and Typhoid
1-15	20	8(40.0)	2(10.0)	1(5.0)
16-30	52	16(30.8)	19(36.5)	9(17.0)
31-45	39	6(15.4)	21(53.8)	4(10.3)
46-60	25	7(28.0)	14(56.0)	5(20.0)
61-75	14	3(21.4)	9(64.3)	2(14.3)
Total	**150**	**40(26.7)**	**65(43.3)**	**21(14.0)**

Table 2: Occurrence of Malaria, typhoid and co-infection of malaria and typhoid diagnosed of fever by gender.

Gender	No. Examined	No(%) Positive for Malaria	No(%) Positive for Typhoid fever	No (%) with mixed infection of Malaria and Typhoid Fever
Male	68	21(30.9)	27(39.7)	9(13.2)
Female	82	19(23.2)	38(46.3)	12(14.6)
Total	**150**	**40(26.7)**	**65(43.3)**	**21(14.0)**

Table 3 Relationship between results of Slide and Tube Widal Test.

Age in Years	No Examined	No(%) Positive for Slide Test	No(%) Positive for Tube Test
1-15	20	2(10.0)	0
16-30	52	19(36.5)	8 (15.4)
31-45	39	21(53.8)	4 (10.3)
46-60	25	14(56.0)	3 (12)
61-75	14	9(64.3)	1 (11.1)
Total	**150**	**65(43.3)**	**16** (24.6)

0 denotes no infection

DISCUSSION

Malaria and Typhoid fever infections are endemic in Calabar. The present study shows a prevalence of 26.7% for *Plasmodium* infection, 43.3% for typhoid infection with slide test and 24.6% for tube method respectively (Table 1). The findings of this study agree with the results of the 2015 National Malaria Indicators Survey (NMIS) which established a prevalence of 33.0% for malaria in Nigeria (17). The slight reduction in the prevalence of malaria reported in this study compared to the finding of the 2015 NMIS may be due to the ongoing high advocacy on the measures required to control malaria (16). This involves the use of long-lasting insecticide treated nets, early diagnosis of malaria using a parasite-based technique and prompt treatment with a nationally approved ACT. However, we consider this prevalence still high for a highly metropolitan town like Calabar. By implication, the vectors still abound while infected people are available for a productive bite. This ensures that transmission continues.

Malaria infection was more detected in males (30.9%) than in females (23.2%), although the difference in prevalence was not statistically significant. This is in agreement with the findings of Onyido *et al.*, (18) who reported the prevalence rate of 76% for males and 65% for females in Azia and Umudiaka communities in Anambra

state respectively. Male subjects are usually more involved than females in outdoor activities than females and this may have predisposed them more to mosquito bites.

Typhoid fever was more prevalent than malaria in the study. Slide Widal test was diagnosed as positive with a titre of 1/160 or more based on results of national base line studies conducted in Nigeria (16). Based on this parameter, the prevalence of typhoid fever in the study stood at 65(43.3%). Typhoid fever was more diagnosed in females 38(46.3%) than males 27(39.7%), though the difference in the prevalence of typhoid fever by gender was not statistically significant (X^2=0.269, P<0.050). This is in contrast to the work of Onyido *et al.*, (18) who reported 18.18% among the males and 81.82% among the females in Ekwulumili community, Anambra state, Southeastern Nigeria. Among the age groups, those within the age group of 31-45 years had the highest prevalence of typhoid fever (53.8%) while those aged 1-15 years had the least (10.0%) (18). This is in contrast with the report of Epidi *et al.*, (19) that reported 51.5% malaria infection rate. Higher exposure to polluted drinking water, close proximity to human waste and refuse dumps, low standards of food preparation and ignorance may have contributed to the higher prevalence in females and subjects aged 31-45 years (19).

Tube Widal test is the confirmatory test for the diagnosis of Typhoid fever. However, most laboratories in Nigeria depend on the results of Slide Test to diagnose the disease. Much resource is required to perform the Tube test. The result of this study is quit revealing. Of the 65 subjects who had a titre of >1/160, only 16(24.6%) gave a positive Tube Widal Test. This means that typhoid fever is over-diagnosed in Nigeria. Preference to Slide Widal test may be due to the reduction in turnaround time for slide result (30 minutes to 1 hour) compared to 18-24 hours for tube method and the lower cost of Slide Widal Test. The presence of other antibodies with related antigen determinants in the system as a result of other immunologic situations resulting from other infection may also led to over diagnosis of typhoid infection. The actual mechanism to explain the affiliation between malaria and Salmonella species infections are still unresolved. However, there are few exceptions which explain why malaria may predispose to Salmonella bacteremia and also sepsis. It is reported that antibody response to O antigen of *Salmonella typhi* was markedly reduced in acute episode of malaria compared with that in controls where humorals immunity is transiently impaired (18). False positive Widal test results are also known to occur in typhus, acute falciparum malaria (particularly in children), chronic liver disease associated with raised globulin levels and disorders such as rheumatoid arthritis, myelomatosis and nephrotic syndrome (18).This calls for the strict reliance on blood culture, stool culture and tube widal test as the confirmatory diagnosis of typhoid as other factors like antibodies developed as a result of other bacterial or parasitic infections may cross react and result in false positive slide widal test. Undue exposure of patients to antibiotics for the treatment of falsely diagnosed typhoid will exert drug pressure and manifest in drug resistance. However, only tube dilution method was used as a confirmatory test in this study due to time constraints and cost.

The co-infection of malaria and typhoid fever reported in the study area is high 21(14.0%). Females were more infected 12(14.6%) than males 9(13.2%). Elsewhere, (20) an overall lower co-infection rate of 5.0% of which 20.0% were males while 80.0% were females was reported. In a similar study by Agwu *et al.*,

(20) in New Delhi, 14(1%) of the study subjects were co-infected with malaria and typhoid. The findings of this study is not in agreement with the result obtained by Eze *et al.*, (21) in Enugu State, Nigeria which conclude that malaria infection had no relationship with *Salmonella* infection (21). The higher co-infection rate reported amongst females agrees with the work of Monika *et al.*, (21) who observed that most female farmers and traders spend their time in the farms and markets where they may have no other sources of drinking water and hence have to purchase and consume sachet water thereby exposing themselves to risk of infection (22)..

CONCLUSION

This study has confirmed a high prevalence of malaria (26.7%), typhoid fever (43.3%) and co-infection of malaria and typhoid fever (14.0%) among subjects clinically diagnosed of malaria and / or typhoid fever. This study has also revealed a very strong relationship between malaria and typhoid fever infections as both of them have some social circumstances and some diagnostic similarity. The study has shown that basing the diagnosis of typhoid fever on results of slide widal test leads to the over diagnosis of the disorder and unwarranted administration of antibiotics. The use of widal test alone in the diagnosis of typhoid fever is unreliable and should be discouraged.

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CONFLICT OF INTEREST

There was no conflict of interest declared

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