Prevalence of Malaria, Typhoid fever, and their Co-nfection among Febrile Patients attending Aboh Mbaise General Hospital Imo State

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Abstract. Malaria and typhoid fever are endemic diseases with life threatening consequences especially in Sub-Saharan Africa; and due to their geographical overlap, co-infections are very common. Their mimicking symptomatology often present with gross misdiagnosis and mistreatment. This study was carried out to determine the prevalence of malaria, typhoid fever, and their Coinfection among febrile Patients attending Aboh Mbaise General Hospital as well as to establish the advantage of stool culture over widal agglutination test in the diagnosis of typhoid fever. A total of two hundred and eighty-four (284) each of blood and stool samples were collected from patients presenting febrile conditions suggestive of malaria and typhoid fever and analyzed using parasitological, agglutination (Widal) and stool culture techniques. All isolates were identified as *Salmonella enteric* serovar *typhi* using standard microbiological techniques. Questionnaire was administered to obtain information on malaria/typhoid management practices. Out of the 284 blood and stool samples analyzed, 71(25.0%) were positive for malaria, 82(28.9%) and 21(7.4%) were positive for *Salmonella enterica* serovar *typhi for* widal and stool culture respectively*,* while 34(12.0%) were positive for coinfection of typhoid and malaria. However, prevalence of malaria parasite was not statistically significant in relation to sex ($p>0.05$), as males had 43(15.1%) prevalence and females, 28(9.9%). For *Salmonella enterica* serovar *typhi*, the prevalence was not statistically significant in relation to sex ($p>0.05$) as males had 45(15.8%) to females 37(13.0%) for widal test and males 15(5.3%) to females 6(2.1%) for stool culture. Sex was not statistically significant ($p>0.05$). For malaria/typhoid co-infection; males had 20(7.0%) co-infection rate while females had 14(4.9%) but no statistical significance ($P > 0.05$). Infections of both diseases were higher in wet season than in dry season and was statistically significant ($P < 0.05$). Symptoms were not statistically significant in both diseases ($P < 0.05$). Both malaria and typhoid were prevalent among the studied population with high rate of co-infection. The use of widal test alone in the diagnosis of typhoid fever is unreliable, misleading and should be discouraged. Culture technique still remains the gold standard in the diagnosis of typhoid fever and should be embraced.

Key words: Malaria, typhoid, Plasmodium, coinfection, Aboh Mbaise.

Introduction

Malaria and typhoid fever are endemic diseases with life threatening consequences especially in Sub-Saharan Africa (Ukaegbo et al. 2014:157-161). Malaria infection is caused by the plasmodium parasite through the bites of an infected vector, usually an anopheles mosquito. Four species of the plasmodium parasite cause malaria in humans (*P. falciparium*, *P. vivax*, *P. malariae* and *P. Ovale*) (Ukaegbo et al., 2014: 157-161).

However, *P. falciparium* is the most common causative agent and contributes to the highest fatality (Ukaegbo et al., 2014: 157-161). Typhoid fever is caused by a gram-negative bacterium called *Salmonella* typhi (Didelot et al., 2009: 61- 68). The species and strains of *Salmonella* that commonly cause typhoid fever in humans are *Salmonella paratyphi* A, *Salmonella paratyphi* B, *Salmonella paratyphi* C and *Salmonella typhi* (WHO, 2003: 103-115; Lerner and Lerner, 2003: 185-189). Infection usually occurs through ingestion of contaminated food or water (Didelot et al., 2009: 61-68). Serology tests usually measure for agglutinations of antibodies against flagella (H) and somatic (O) antigens of the bacterium (Didelot et al., 2009: 61-68). However, the detection of high antibody titre for *Salmonella* is not always indicative of current infection(s) (Lerner and Lerner, 2003: 185-189). Therefore, stool and/or blood culture from the patients is/are confirmatory (WHO, 2003: 103-115; Lerner and Lerner, 2003: 185-189). Both diseases have similar clinical manifestations such as fever, nausea, appetite loss, headache and constipation. Complications are common for both diseases and usually lead to death (Mbuh et al., 2003: 64-67).

Globally, an estimated half of the world population (3.4 billion people) lives in area at risk of malaria infections. Six countries in Sub-Sahara Africa (Nigeria, Democratic Republic of the Congo, Tanzania, Uganda, Mozambique and Cote d'ivore) account for an estimated 103 million malaria cases and 47% of the global total each year. Nigeria and the Democratic Republic of the Congo together account for 40% of the estimated total global (Global Malaria Action, 2013). Typhoid fever is an important cause of morbidity in many regions of the world with an estimated 12–33 million cases leading to 216,000 – 600,000 deaths annually (Nsutebu et al., 2003: 575-578).

The co-infection of malaria parasite and *Salmonella* species is common, especially in the tropics where malaria is endemic (Mbuh et al., 2003: 64-67). The common detection of high antibody titre of these *Salmonella* serotypes in malaria patients has made some people to believe that malaria infection can progress to typhoid or that malaria always co-infect with typhoid/paratyphoid in all patients (Mbuh et al., 2003: 64-67). Hence, 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 (Mbuh et al., 2003: 64-67). This work is therefore aimed at determining the prevalence of malaria, typhoid fever, and their Coinfection among febrile patients attending Aboh Mbaise General Hospital as well as established the advantage of stool culture over widal agglutination test in the diagnosis of typhoid fever.

Materials and methods

Study area

The study was carried out at Aboh Mbaise General Hospital located at Aboh Mbaise Local Government Area (LGA) of Imo State. It is usually attended by very low and moderate socio-economic groups and therefore very affordable and accessible to most dwellers of Ahiazu Mbaise, Ezinihitte Mbaise and Aboh Mbaise LGA.

Sample size

A total of 284 patients and 16 healthy patients were monitored as control.

Study period

This research was carried out between February 2020 and July 2020.

Study subjects/selection

Inclusion criteria

Women who met the following inclusion criteria were included in the study;

1. Patients with fever reporting for malaria and typhoid testing.

2. Patients above the age of one.

3. Patients who had malaria and/or typhoid fever who had not taken antimalarial drugs and/or antibiotics within two weeks.

4. Patients who gave written informed consent and agreed to be included in the study.

Exclusion criteria

The following were excluded from participating as subjects in the study.

1. Patients with underlying disease like HIV/AIDS, hepatitis B infection etc, because these conditions may affect the hemoglobin concentrations of affected persons.

2. Patients with any other bacterial or parasitic infections were excluded from the study to reduce the chance of false positive results.

3. Patients who did not offer an informed consent.

Study instrument i.e Questionnaire

A semi- structured interviewer-administered questionnaire was administered to all consenting participants in order to obtain information on their socio-demographic (i.e., sex, date of birth etc).

Collection of Blood and Stool Samples

Five (5) milliliters of whole blood was collected by a trained and licensed laboratory scientist from each patient by venipuncture into a clean dry glass tube. The blood was smeared immediately onto a clean grease-free slide for preparations of thick and thin blood films. Lastly, stool samples were also collected from the patients into sterile bottles. Samples were stored in test tubes and stored at 15˚C-30˚C prior to laboratory analysis as described by (Cheesbrough, 2000: 267-347).

Parasitological Examination

The collected blood samples were analyzed within 3-4 hours after collection. Thin and thick blood films were made on clean slides and labeled accordingly as recommended by (Cheesbrough, 2000: 267-347), to determine the malaria parasites. While thick films were used to detect malaria parasites in the blood samples (ring forms trophozoites and gametocytes), parasite species and morphology were determined by microscopic examination of thin films.

Briefly: a drop of blood sample was placed on the center of a slide and spread out with a corner of another slide to cover an area of about four times its original area; after which the reverse side of the slide was cleaned with cotton wool and allowed the film to air-dry for at least 30 minutes at 37ºC. The slides were immersed for 20-30 minutes in a staining jar containing Giemsa stain freshly diluted with 20 volumes of buffered water (pH 7.2). The slides were washed in buffered water pH 7.2 for 3 minutes. Thereafter, they were made to stand upright to dry in the rack for eventual examination of the slides under microscope, using oil immersion at X100 magnifications to observe Plasmodium parasites (Cheesbrough, 2000: 267-347).

The stained slides were read by two certified laboratory scientists. Discrepant parasite detection and parasite count readings between the two laboratory scientists were resolved by a re-reading of the slides or by employing a third laboratory scientists before the final result was determined. The mean parasite counts of the two readers were accepted if the discrepancy of the two readings was less than 20%. A blood smear was considered negative when the examination of 100 high power fields did not reveal asexual parasites.

Malaria parasite density count

The absolute parasite density was calculated using the formula: [No. of parasites counted x total leukocyte count] /No. of leucocytes counted (Cheesbrough, 2000: 267-

347). Multiple-blinded readings for validation of the presence or absence of parasites and of the estimated parasite density were done by certified laboratory scientist.

Widal agglutination test for Salmonella antibodies

Widal agglutination test was performed on each blood sample using the Widal agglutination kit (Biotech lab, United States) containing somatic (O) and flagella (H) antigens of *Salmonella typhi* and *Salmonella paratyphi* A-C. A negative saline control was introduced in each batch of test. The procedure used was as described by Ochei and Kolhatkar (Ochei and Kolhatkar, 2010: 692-693, 962).

Drops of sera from each patient were made on a clean tile, mixed with the antigens rocked for 3 minutes and observed for agglutination. A positive Widal test was considered as one that gave a reaction titre of 1/80 or greater in a single test. A negative saline control was introduced in each batch of test.

Isolation and Identification of Salmonella from Stool

Isolation of Salmonella from stool samples was done following standard microbiological practices (Cheesbrough, 2006). Each stool sample was enriched in Selenite F broth overnight and a loopful streaked on salmonella shigella medium the next day and incubated aerobically at 37°C overnight. Suspected colonies (colourless colonies) were sub-cultured on fresh salmonella shigella medium and incubated as reported above.

The colonies were further streaked on nutrient agar to obtain pure isolates. Colonies were subjected to gram staining, motility, hydrogen sulphide production and oxidase tests (Akoachere et al., 2009: 1-7).

Gram negative short motile rods that were oxidase negative with characteristic red slope/yellow butt reaction on Triple Sugar Iron (TSI) agar either with or without production of H_2S were confirmed as *Salmonella species* based on the API 20-E kit reactions.

The tests were performed as per manufacturer's instructions. Data interpretation was performed using the Analytical profile index (API) database (V4.1) with the apiwebTM identification software. Polyvalent antisera were used for speciation (Akoachere et al., 2009: 1-7).

Quality control

For quality control, all slides were read by a second laboratory scientist and a third reviewer settled any discrepant readings. Lastly, 16 age- matched healthy patients were monitored as controls.

Ethical Clearance

Consent was obtained from the hospital management board. The participants were provided with consent form and fact sheet containing information about the study, signed consent was obtained from the participants before the questionnaire administration and confidentiality of the information obtained from the pregnant women was assured.

Statistical analysis

Data were analyzed using statistical package for social sciences (SPSS) version 19.0 (Chicago, USA). Chi-square ($\chi 2$) was used to determine if the relationships between the Malaria parasite infection and *Salmonella serovar enterica typhi* were actually significant. The level of significance was set at $P < 0.05$.

Results

The prevalence of infected and coinfected patients in relation to sex is stated on table 1. In this study, the positivity rate was 73.2%. The male patients were more infected 120(42.3%) than the female patients 88(31.0%) but there was no statistically significant association ($P < 0.241$).

Of the 71 (25.0%) patients who were positive for malaria parasite test, the males and females showed 20 (7.0%) and 14 (4.9%) coinfection respectively with typhoid fever. The rate of infection with respect to widal test was higher in males 45 (15.8%) than females 37 (13.0%), while stool culture also showed that male patients were more infected than female patients with infection rates of 15 (5.3%) and 6 (2.1%) respectively (Table 1).

Table 1. Prevalence of infected and coinfected patients in relation to sex

SEX	No. of patients (%)	No. of infected patients (%)	Malaria +ve patients (%)	Typhoid +ve patients (%)		Coinfected patients (%)	P - Value
				Widal	Stool culture		
Male	153(53.9)	120(42.3)	43(15.1)	45(15.8)	15(5.3)	20(7.0)	P< 0.241
Female	131(46.1)	88(31.0)	28(9.9)	37(13.0)	6(2.1)	14(4.9)	
Total	284(100)	208(73.2)	71(25.0)	82(28.9)	21(7.4)	34(12.0)	

The prevalence of infected and coinfected patients in relation to age is stated on Table 2. The age group 21 – 30 had the most co-infectious rate of 10(3.5%) while the least was found in age group > 60 2(0.7%) and there was a statistically significant association (P < 0.012).

Table 2. Prevalence of infected and coinfected patients in relation to age

AGE GROUP	No. of patients (%)	No. of infected patients (%)	Malaria +ve patients (%)	Typhoid +ve patients (%)		Coinfected patients (%)	P - Value
				Widal	Stool culture		
1 -10	24(8.4)	17(6.0)	5(1.8)	7(2.5)	0	1(0.4)	P < 0.012
11 - 20	44(15.5)	30(10.6)	8(2.8)	11(3.9)	1(0.4)	4(1.4)	
21 - 30	60(21.1)	50(17.6)	19(6.7)	21(7.4)	7(2.5)	10(3.5)	
31 - 40	53(18.7)	42(14.8)	17(6.0)	17(6.0)	6(2.1)	8(2.8)	
41 - 50	51(18.0)	40(14.1)	13(4.6)	16(5.6)	4(1.4)	6(2.1)	
51 - 60	35(12.3)	19(6.7)	6(2.1)	8(2.8)	2(0.7)	3(1.0)	
>60	17(6.0)	10(3.5)	3(1.0)	2(0.7)	1(0.4)	2(0.7)	
Total	284(100)	208(73.2)	71(25.0)	82(28.9)	21(7.4)	34(12.0)	

The relationship between disease occurrence and clinical presentation in patients is found on Table 3. There was no statistically significant association among the clinical presentation of malaria/typhoid fever (p < 0.255).

Table 3. Relationship between disease occurrence and clinical presentation in patients

Clinical presentation	No. of Patients (%)	Infected patients (%)	Malaria (%)	Typhoid (%)	Malaria/Typhoid (%)	P - Value
Fever	74	51(18.0)	20(7.0)	28(9.9)	9(3.2)	0.255
Headache	66	44(15.5)	17(6.0)	26(9.2)	8(2.8)	
Fatigue	52	42(14.8)	13(4.6)	18(6.3)	6(2.1)	
Abdominal pain	35	26(9.2)	8(2.8)	12(4.2)	5(1.8)	
Joint Pain	18	21(7.4)	5(1.8)	10(3.5)	3(1.0)	
Anorexia	15	10(3.5)	3(1.0)	4(1.4)	2(0.7)	
Diarrhoea	14(5.0)	8(2.8)	3(1.0)	3(1.0)	1(0.4)	
Loss of appetite	10(3.5)	6(2.1)	2(0.7)	2(0.7)	0	
Total	284(100)	208(73.2)	71(25.0)	103(36.3)	34(12.0)	

The Month wise Prevalence of malaria/typhoid in patients is found on Table 4. The highest prevalence occurred in the month of July 54 (19.0%) while the lowest prevalence was found in the month of February10 (3.5%) and this was statistically significant (P< 0.001).

Table 4. Month wise Prevalence of infection and coinfection in patients

Months	No. of Patients (%)	Infected patients (%)	Malaria (%)	Typhoid (%)	Malaria/ Typhoid (%)	P - Value
February	20(7.0)	10(3.5)	2(0.7)	3(1.0)	1(0.4)	0.001
March	24(8.5)	21(7.4)	4(1.4)	5(1.8)	2(0.7)	
April	31(11.0)	27(9.5)	10(3.5)	10(3.5)	3(1.0)	
May	53(18.7)	38(13.4)	16(5.6)	20(7.0)	7(2.5)	
June	76(26.8)	48(16.9)	18(6.3)	30(10.6)	9(3.2)	
July	80(28.2)	54(19.0)	21(7.4)	35(12.3)	12(4.2)	
Total	284(100)	208(73.2)	71(25.0)	103(36.3)	34(12.0)	

Discussion

In this study, the culture positivity was 73.2% and this was similar to the work of Sundufu et al. (2012) in Southern Sierra Leone who recorded a culture positivity of 69%; but differs from the work of Alhassan et al. (2012: 13-20) who recorded a culture positivity of 47%. The variation in the results could be attributed to differences in the environmental conditions of the studied population (Sundufu et al., 2012: 204-207; Global Malaria Action Plan, Malaria today, 2013).

The prevalence of malaria was higher in males 15.1% than females 9.9% but there was no statistically significant association (P < 0.241). Nevertheless, another study in Sierra Leone showed that females (53.4%) were more affected than males (46.6%) (Sundufu et al., 2012: 204-207). This might be due to the fact that males are sleeping

outside their house for agricultural purpose and have greater chance to travel to malaria endemic area for crop cultivation or daily labor (Alhassan et al., 2012: 13-20).

There was significant association between age and malaria (P < 0.012) as the least prevalence was found in age group > 60 and 1-10. This might be due to low immunity against malaria infection due to old age and young age. Immunity against diseases is usually low at birth and also decreases at old age (Alhassan et al., 2012: 13-20).

This study revealed that of the 284 patients screened, 71 (25.0%) were positive for malaria whereas 82 (28.9%) to 21 (7.4%) were positive for widal and stool culture respectively. This shows that typhoid is more likely to cause fever than malaria, although both share fever in their symptomatology (Igharo et al., 2012: 49-52). This result is in agreement with the findings of Nwuzo et al. in Abakaliki, Opara et al*.* in Owerri and Igharo et al. in Ondo, who observed higher prevalent rates of typhoid than malaria in febrile patients. The prevalent rates were (21.20% typhoid vs. 13.20% malaria) (Nwuzo et al., 2014: 966-971), (42% typhoid vs. 39% malaria) (Opara et al., 2011: 5-8) and (73.9% typhoid vs. 37.6% malaria) (Igharo et al., 2012: 49-52) respectively. Nevertheless, the result varies with the findings of Igbeneghu et al*.* in Ibadan, who observed 50.4% of malaria against 4.7% of typhoid fever (Igbeneghu et al., 2009: 112-115). The variation in the results could be attributed to differences in the environmental conditions of the studied population. Factors such as poor hygiene resulting to faecal contamination, lack of potable water as well as inadequate breeding grounds for mosquitoes could have contributed to reasons why they observed higher rates of typhoid fever infections than malaria (Igharo et al., 2009: 49-52). In Aboh Mbaise, open drainages and stagnant water could have created adequate opportunities for mosquitoes to breed. In addition, most sources of drinking water in Aboh Mbaise are stream and borehole water. These surface waters could be open to faecal contamination (Opara et al., 2011: 5-8). Inadequate and unplanned toilet systems and uncontrolled defecation in the nearby bushes is the major source of faecal contamination of surface running waters with the bacteria that causes typhoid fever infections (Opara et al., 2011: 5-8).

Furthermore, the result revealed a very strong relationship between malaria and typhoid fever infections both by widal test and stool culture. However, there were considerably higher rates of concurrent malaria and typhoid fever infections by widal test (28.9%) compared to the stool culture technique (7.4%). This is to be expected as widal test, being a serological test, only proves exposure to a certain antigen; it does not tell if infection is recent or not (Keong and Sulaiman, 2006: 74-75). In addition, there could be possible cross-reactivity with other antigens having common somatic or flagella recognition proteins (Igharo et al., 2012: 49-52). Therefore, widal test which has been used for decades in Nigeria for the diagnosis of typhoid fever infections only leads to false positive and overestimated results (Keong and Sulaiman, 2006: 74-75). Cross-reactivity between malaria parasite antibodies and widal antigens has been reported to have led to over-diagnosis of typhoid fever (Nsutebu et al., 2001: 575-578). The result is in conformity with the findings of Uneke (2008: 133-142), and Keong and Sulaiman (2006: 74-75).

In addition, Ammah et al*.* in Cameroon reported that out of 200 patients with fever, 17% had concurrent malaria and typhoid fever based on bacteriological proven diagnosis as compared to 47.9% based on widal test (Ammah et al., 2009: 127-129). Samal and Sahu described 52 patients with malaria positive in the peripheral blood smear, out of whom eight cases had positive widal tests but the blood culture was negative for *Salmonella typhi* in all; all the cases were cured with antimalarial therapy (Samal and Sahu, 2010: 745-747). Other studies in agreement with this result are those of Mbuh et al*.* (0.5% culture vs. 10.1% widal test) (2003: 64-67), Igbeneghu et al*.* (0.8% culture vs.

21% widal test) (Igbeneghu et al., 2009: 112-115) and Nwuzo et al. (6.1% culture vs. 42.4% widal test) (Nwuzo et al., 2014: 966-971). However, although culture technique remains the gold standard in typhoid fever diagnosis as supported by this study, widal test is still of significant diagnostic value provided judicious interpretations of the test results are made against backgrounds of pertinent information (Uneke, 2008: 133-142).

Eighteen percent (18%) of fever patient were positive or reactive for at least one of the diseases in this study. The high rate of infection can be attributed to poor sanitation hygiene, other socio-economic factors and the optimum temperatures that favors the reproductive cycle of these bacteria or parasites (Sharma et al., 2009: 696-702). In addition, over 73.2% of all patients were infected with all three diseases.

Malaria and typhoid fever infections and coinfections were higher in the raining/wet season than drier seasons with statistically significant association ($P < 0.001$). This is mainly due to the mode of transmission and reproductive cycles of the plasmodium parasite and Salmonella bacteria (Sharma et al., 2009: 696-702). This study recorded higher malaria and typhoid infections especially in the peak periods of the raining season (July) and lower rates in the peak period of the dry season (February). Infections for both diseases were high from the months of May-July, coinciding with months of heavy rainfall. Trend analysis from this study showed a reduction in the number of malaria and typhoid cases as rainfall reduces. Higher temperatures and lack of stagnant water is the cause of the reduced prevalence of malaria typhoid cases observed in the months of February to April (Sharma et al., 2009: 696-702).

Conclusion

Typhoid fever infection was higher than malaria infection among patients in the study area. The male's patients showed higher rate of coinfection 20 (7.0%) than the females' patients 14 (4.9%) but this was not statistically significant ($p > 0.05$).

There was a substantial result discrepancy between Widal test and blood culture for the diagnosis of typhoid fever. The use of widal test alone in the diagnosis of typhoid fever is unreliable, misleading and should be discouraged. This has led to typhoid fever been over-diagnosed by widal test and many patients have been placed on antibiotics against typhoid fever when it is not called for. Therefore, Culture technique still remains the gold standard in the diagnosis of typhoid fever and should be embraced.

Lastly, malaria and typhoid fever infections and coinfections were higher in the raining/wet season than drier seasons with statistically significant association ($P < 0.05$).

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