



Prevalence of Plasmodium and Salmonella Infections among Pregnant Women with Fever, Presented to Three Hospitals in Ogun and Lagos State, South-West Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author ETC performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript, managed the analyses of the study, managed the literature search. Author GIO designed the study. Authors SOT and BTA assisted, author ETC in the laboratory analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study was undertaken to establish prevalence of plasmodium and salmonella infections among pregnant women manifesting fever, presented to three hospitals in Lagos and Ogun state, Nigeria. To determine the rate of co-infection with respect to the use of widal test and stool culture as diagnostic tools for typhoid fever and To characterize and Identify the *S. typhi* isolates associated with co-infection.
Study Design: This research is Cross sectional. Pregnant women who were manifesting fever presented to two hospitals in Ogun State and Lagos State, Nigeria were tested by two diagnostic methods each for Plasmodium and Salmonella infections.
Place and Duration of Study: Covenant university Canaan Land, Microbiology Laboratory between October 2012 and June 2013.
Methodology: Three hundred and fifty pregnant women who came to the hospitals with

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clinical signs including fever were selected by random sampling. They were tested for plasmodium infection and for salmonella infections. For plasmodium, the lateral flow test cassettes were used to test for the different species. The blood smears were also viewed microscopically while for salmonella infections both Widal test and culture of stools were used.

Results: Of the 350 samples, 10(3%) tested positive for plasmodium. 6(2%) were positive for salmonella while 5(1%) women had both plasmodium and salmonella infections. All the 10 cases of plasmodium infections were caused by *P. falciparum* and all the 6 cases of salmonella infections were due to *S. typhi*. Fifty healthy pregnant women screened by same methods as controls were negative for both plasmodium and for salmonella.

Conclusion: Infection by *P. falciparum* and *S. typhi* and their co-infection are among causes of fever in pregnant women in Nigeria.

Keywords: Pregnant women; Malaria; Typhoid fever; Plasmodium falciparum and Salmonella typhi; co-infection.

1. INTRODUCTION

Malaria is a major public health problem especially in tropical and sub-tropical areas; It is estimated to be responsible for about 1 to 3 million deaths and 300-500 million clinical cases annually [1,2]. Typhoid on the other hand remains an important worldwide cause of morbidity and mortality and continues to be a health problem in developing countries where there is poor sanitation, poor standard of personal hygiene and prevalence of contaminated food [3-5]. Concurrent malaria and typhoid is often disregarded and untreated [6,7,8]. *Plasmodium* and *Salmonella* infections can result in serious complications and conditions such as maternal anemia, fever, fetal anemia, abortion, still-birth, and even death of the child or mother before birth or soon after delivery [9,10].

Malaria is a tropical disease caused by a parasite that is transmitted from human to human by the Anopheles mosquito [11]. The mosquito carries the parasites plasmodium and transfer it to the host through a bite, Not long after they found out that malaria is transmitted from person to person through the bite of this mosquito which needs blood for her egg, Approximately 40% of the global population is at risk of malaria infection [11].

Malaria is a preventable and treatable disease. If malaria is diagnosed and treated early, the duration of the infection can be considerably shortened, which in turn reduces the risk of complications and death [12]. The vast majority of cases are children under the age of five years and pregnant women [13]. There are five species of the parasites causing malaria. Malaria due to *Plasmodium vivax* is mild form of the disease and is generally not fatal, and this type has the widest geographic distribution globally. About 60% of infections in India are caused by *P. vivax*. This parasite has a liver stage and can remain in the body for years without causing sickness. Infection of *Plasmodium malariae* also mild and not fatal, it has been known to stay in the blood of some people for several decades. *Plasmodium ovale* is mild and not fatal; it has a liver stage and can remain in the body for years without causing sickness. *Plasmodium falciparum* is the most serious parasite. It is most common in Africa, especially sub-Saharan Africa. *Plasmodium knowlesi* causes malaria in macaques but also infect human [14].

Salmonella, a Gram- negative rod shaped bacteria of the family *Enterobacteriaceae*, causes a wide range of human diseases, such as enteric fever, gastroenteritis, endocarditic and

bacteraemia. *Salmonella* associated infections do not present with distinct clinical features, Other bacterial, viral, and even protozoan's may mimic its presentations [15]. More than 2,300 *Salmonella* enteric serotypes have been described, only *S. enteric serovar typhi*, *S. paratyphi*, *S. typhimurium*, *S. choleraesuis*, *S. hadar*, *S. virehow* and *S. dublin* among others, play important epidemiological roles. Although infections with non-typhoidal *Salmonellae* usually cause self-limited diarrhea illness.

2. MATERIALS AND METHODS

Pregnant women who had fever by the time they were presented to three hospitals in Ogun and Lagos states, Nigeria were sampled.

2.1 Description of the Study Area

The study was a cross-sectional study. This is the first research in Nigeria on coinfection of these organisms in pregnancy. Participants were sampled from Ota General Hospital, Ogun state, Covenant University Medical Center, Canaan land Ogun State and Igando General Hospital, Igando, Lagos State. 350 participants were pregnant women with fever complain, 50 control samples from women that were not pregnant.

2.2 Sample Collection

An intravenous blood sample of 5 ml for all was collected in an EDTA tube in accordance with routine clinical practice for all patients referred for laboratory investigation of malaria infection. Samples site were from 3 hospitals in Ogun state and Lagos state respectively. Three hundred and fifty pregnant women who showed fever and other symptoms suggestive of malaria or typhoid fever that were referred by clinicians after clinical and physical examinations to medical laboratory in Southwest Nigeria for malaria parasite and typhoid tests was used for the analysis. Also, blood samples were collected from 50 seemingly non pregnant healthy female individuals as controls. Those who participated in the study were given informed consent. A 5mL of blood was taken from each patient for laboratory investigations.

2.3 Sample Analysis

2.3.1 Examination of blood slides for malaria parasite

An intravenous blood sample of 5ml was collected into an EDTA tube. Blood films were made by placing a drop of blood on one end of a slide, and using a cover slide to disperse the blood over the slide's length. Thick and thin blood films were stained with 10% Giemsa for 10 minutes, thick film was heat fixed and thin film fixed with methanol. It was examined microscopically for malaria parasites. At least 5 blood films were examined for malaria parasite before reporting any film as negative.

2.3.2 Rapid diagnostic tests (RDT)

Lateral flow test or immunochromatographic assay cassette was performed using a commercially prepared buffer solution from Orchid Company. It contains two wells, blood sample well and buffer solution well. Following the manufacturers manual the test was done.

The appearance of red line of test band and control band indicates positive while just the control band indicates negative [16].

2.3.3 Serological test for typhoid fever

Widal tests was done using human plasma and a drop of antigen of the chromatest widal test kit was added, It was stirred and rocked for some minutes, following the companies manual. Widal tests were read as positive when O antibody titers were $\geq 1/80$ - $1/160$ for *S. typhi* [17].

2.3.4 Isolation and identification of salmonella species

A diagnosis of *Salmonella typhi* was confirmed by testing for the presence of the bacteria in Salmonella Shigella Agar (SSA) [42]. Stool samples from patients who tested positive by widal test were cultured on Nutrient Agar. The isolates were further sub cultured into SSA, to obtain pure cultures [17].

2.3.5 Statistical analysis

The sample design is cross sectional, Sample size was randomly selected. Descriptive statistics was used for data analysis. Spearman's correlation was used to determine their relationship, and chi square distribution was used to analyze the distributions relation to each other, Using Chi square, *P* value was therefore significant ($P=.05$). The SPSSPC 17.0 software package (SPSS Inc., Chicago, and Ill., USA) was used for data analysis.

3. RESULTS AND DISCUSSION

3.1 Results

Out of the 350 samples collected 10(3%) were positive for *Plasmodium falciparum*, 6(2%), and 5(1%) were positive both Plasmodium infection and Salmonella infection. Diagnosing plasmodium infections by the RDT method and by microscopic examination of blood smears gave same results. Also results of Widal test for salmonella infections and results of testing same patients by culturing their stools for *Salmonella typhi* were same. Chi square Calculated value for Ota=172.980, Igando=77.440, Covenant University=38.720 ($P=.05$) Tables 1–3.

Table 1. Prevalence of *P. falciparum* and *S. typhi* among pregnant women manifesting fever presented to hospitals in Lagos and Ogun states, Nigeria.

Hospitals	Positive Results			Total No. Exam
	Malaria (%)	Typhoid (%)	Co-infection (%)	
Ota	5 (2.5) *	2 (1)*	2 (1) ^a	200
Igando	4 (4) *	2 (2)*	2 (2) ^b	100
Covenant	1 (2) *	2 (4)*	1 (2) ^c	50
Total	10 (3)	6 (2)	5 (1)	350

*Coinfection is shown in the fourth roll with different alphabets abc, rolls marked * shows S. typhi and P. falciparum prevalence in the three hospitals (P=.05).*

Table 2. Results of testing for malaria by Rapid diagnostic test and by microscopic examination of blood smears

Hospitals	RDT (%)	Microscopy (%)	Total no. Exam
Ota	5(2.5)	5(2.5)	200
Igando	4 (4)	4(4)	100
Covenant	1 (2)	1(2)	50
Total	10	10	350

The second and third rolls shows *S. typhi* and *P. falciparum* prevalence by RDT and microscopy ($P=.05$).

Table 3. Results of diagnosing typhoid fever by the widal test and by culturing stools to identify *Salmonella typhi*

Hospitals	Widal (%)	Stool culture (%)	Total no. Exam
Ota	2(1)	2(1)	200
Igando	2 (2)	2(2)	100
Covenant	2 (4)	2(4)	50
Total	6	6	350

Second and third rolls shows prevalence of *S. typhi* and *P. falciparum* in the three hospitals by widal and stool culture from ($P=.05$).

3.2 Discussion

The study shows that co-infection of malaria and typhoid occurs during pregnancy [18], with *plasmodium falciparum* causing malaria and *S. typhi* identified with Analytical profile index to be the cause of Typhoid. Although, malaria and typhoid fever are said to be endemic in Nigeria, this study reveals that malaria is far more likely to cause fever than typhoid fever because all the 350 patients had fever and 10 were positive for malaria but 6 were positive to typhoid, This confirms the results of Eze et al. [19], who observed that though malaria and typhoid share similar symptoms malaria causes fever more than typhoid. Cultural diagnosis of typhoid fever should be done alongside Widal test; Culture should be based on culture of blood, stool and bone marrow, In stool culture there is chances of cross contamination which may hinder the proper laboratory results, therefore it is important to culture and keep subculturing until pure culture is isolated. On the other hand the Stool culture which is the gold standard to isolate *S. typhi* should be reconfirmed by a widal test. This agrees with Uneke, [20] who reported culture of stool as the gold standard test method for typhoid fever. However, bone marrow aspirates are difficult to obtain and culture of blood sample delays diagnosis leaving widal as the fast method to be used for urgent situation, even after widal test, it should be reconfirmed by culturing. So if malaria test and Widal test is done, the patient should be treated for typhoid and malaria. On the other hand, a true co-infection is occurred because both typhoid and malaria share social circumstances which are imperative to their transmission. This confirms what Prasanna, observed that excess iron taken to correct anemia in malaria during pregnancy may lead to fatal co-infection [21].

The study showed a low co-infection rate but significant; this is as a result of constant consumption of Intermittent Presumptive Treatment for Plasmodium species (IPTP) and proper training given to pregnant women during their weekly visit to hospitals as antenatal check up. There they are also tutored on how to avoid contaminated food and water, which reduces the risk of contaminating *S. typhi*.

The correlation test for the three hospitals surveyed, shows that there is a significant positive relationship between the co- infection prevalence of Malaria and typhoid in Ota, Igando and Covenant. That there is a stronger positive relationship in the co-infection prevalence of Malaria and typhoid between Ota and Igando at 0.921 and Igando and covenant at 0.684 compared to Ota and covenant at 0.626 all significant ($P=.05$). Chi-square empirical result was statistically significant ($P= .05$).

4. CONCLUSION

This study indicates that malaria is still the leading cause of fever characteristic of typhomalaria signs and symptoms. About one-third of the pregnant patients presenting with fever neither have malaria, typhoid nor typhoid malaria fever. Therefore, presumptive treatment of fever as malaria or typhoid fever should be discouraged. Typhoid fever was diagnosed using Widal test and stool culture. Therefore, using Widal test alone in the diagnosis of typhoid fever should be discouraged, If widal test must be used, it should be done alongside culture of stool or blood samples. Where, culture facilities are lacking and patients are positive for malaria. Malaria and typhoid should be treated because from the ten malaria positive pregnant women in this study, five had typhoid too. Only when malaria has been ruled out and there is a strong clinical suspicion of typhoid fever that is when such pregnant patients should be treated for typhoid fever alone. Co-infection of *Plasmodium falciparum* and *Salmonella typhi* has been confirmed as one of the causes of fever among pregnant women in Nigeria. Effective control measures can be determined now that this research has data of true co-infection existence in Pregnancy.

CONSENTS

All authors declare that 'written informed consent was obtained from the Pupils (or other approved parties) for publication of this case report.

ETHICAL PERMIT

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki."

ETHICAL CLEARANCE

Scientific and Ethical clearance were obtained from the Nigerian Institute of Medical Research Institutional Review Board (NIMR – IRB), and Covenant University Ethics committee for this work. The Ogun State and Lagos state Ministry of Health (Hospitals Management Board) was also informed and clearance obtained for this study. Written informed consent was obtained from patients prior to recruitment into this study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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