

PREVALENCE OF CO-INFECTION OF MALARIA AND TYPHOID IN PREGNANT WOMEN IN UYO, AKWA IBOM STATE, NIGERIA



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ABSTRACT

Malaria and typhoid fever have both become a major public health problem affecting pregnant women in tropical and sub-tropical regions of the world. Therefore the study aims to determine the prevalence of co-infection of malaria and typhoid in pregnant women attending ante-natal clinic in St. Luke's Hospital in Uyo, Akwa Ibom State, Nigeria. A total of 220 pregnant women were recruited by voluntary participation and examined for the presence of malaria parasite using giemsa staining technique and microscopy for parasites, and were evaluated for the presence of widal agglutinins. Using the cromatest widal test kits (Linear chemicals, Spain), rapid agglutinin and tube agglutination tests were performed. Out of 220 pregnant women examined, 159 (72.27%) were positive for malaria parasites, and of the 159 malaria infected pregnant women, 104 (65.40%) tested positive to typhoid with significant antibody titre ≥ 180 . Thus making the coinfection rate of 65.40%. Then, malaria parasite prevalence was significantly increased ($\chi^2 = 17.62$, $df = 3$, $p < 0.05$) and coinfection rate was as well high ($\chi^2 = 20.79$, $df = 3$, $P < 0.05$). The study show high malaria and typhoid fever coinfection among pregnant women in the study area, and this may result to far reaching effects on adverse pregnancy outcome.

INTRODUCTION

Malaria and typhoid fevers has become a major scourge afflicting people in most developing and tropical countries of the world (Isibor *et al.*, 2011). Malaria which is the most prevalent infectious disease in the tropical and sub-tropical regions of the world is of great public health importance (Umar *et al.*, 2007). The World Health Organisation report that malaria, the deadly parasitic disease is responsible for nearly 90% of dead in Africa (Ogbodo *et al.*, 2010). Malaria is caused by obligate intracellular parasites, which lives in host erythrocytes and remodel these cells to provide optimally for their own needs (Pearson and Guarrent, 2000). Pregnant women living in high malaria endemic areas normally develop protective immunity against severe disease and high density parasitemia; and this protection, however, appears to be partially lost during pregnancy (McGregor, 1984). Malaria is a common medical complication of pregnancy in malaria endemic zones of the world where about 40% of the world's population lives (WHO, 2000).

Malaria during pregnancy is associated with intrauterine growth retardation and low birth weight in infant (McGregor *et al.*, 1983). The reduction in birth weight is usually more marked in primigravidae (Brabin, 1991), but extend to second and third gravidae in areas of low malaria transmission (Nosten *et al.*, 1999). Typhoid fever, on the other hand, is highly recognised as a systemic infectious disease characterized by an acute illness, the first typhoid manifestation of which are fever, headache, abdominal pain, relative brady cardia, splenomegaly and leucopenia (Pearson and Guarrent, 2000). Typhoid which is also endemic in Africa is an acute life threatening febrile illness caused by the bacterium *Salmonella*, with an estimated 22 million cases of typhoid fever and 200,000 related deaths occurrence world-wide each year (Cheesbrough, 2005). The hallmarks of typhoid fever lasts 4-8 weeks in untreated patients and following the initial recovery, relapse occurs in about 10-20% of untreated patients

(Mbuh *et al.*, 2003). According to Khan *et al.* (2005), presenting symptoms among patients with typhoid, differs between pregnant and non-pregnant women; while the pregnant women with typhoid were more likely to be presented with cough, non-pregnant patients are reported with cases of vomiting and nausea (Akinyemi *et al.*, 2007). The most popular choice of antibiotics for the pregnant patient were third generation Cephalosporins and Ampicillin/Amoxicillin while quinolones, widely believed to be the drugs of choice for typhoid fever, were preferentially used in pregnancy due to the high risk of toxicity to fetal skeletal structures (Koeleman, 1992).

Howbeit, concurrent malaria and typhoid fever called typho-malaria was first described by an army doctor, Woodward (1883-1994) in 1862 but with fever pattern suggestive of intermittent fever (Smith, 1982; Bynum, 2002). Recent studies in Africa seem to corroborate the relationship between malaria and typhoid fever (Akinyemi *et al.*, 2007).

Pregnancy is a physiological condition that is associated with lowered immunity to infections including typhoid fever, which is due to the exaggeration on anti-inflammatory steroid hormones, associated with pregnancy state (Richard *et al.*, 2001). This situation renders pregnant women more susceptible to infections including malaria and typhoid fever. Furthermore, malaria infected pregnant women are said to be more prone to typhoid fever because of the increased haemolysis in malaria which increases the availability of iron in the tissue especially the liver, and *Salmonella* species are believed to thrive more in iron rich tissues (Kaye and Hook, 1963; Ukibe *et al.*, 2013). Therefore, it is pertinent to note that both typhoid and malaria in pregnant women present with management problems since most drugs used in the treatment of both diseases are contra-indicated in pregnancy (Ukibe *et al.*, 2013). Both malaria and typhoid fever in pregnancy are associated with adverse pregnancy outcomes such as premature deliveries, spontaneous abortions, low birth weight babies and intra-uterine foetal deaths, (Basyam, 2007; Nasem *et al.*, 2008).

This study was carried out in order to determine the prevalence of malaria and typhoid fever co-infection in pregnant women attending antenatal clinic in St. Like's Hospital in Uyo, Akwa Ibom State, Nigeria.

MATERIALS AND METHOD

Study Area

This study was conducted at St. Lukes Hospital in Uyo Local Government Area of Akwa Ibom State, Nigeria from July to October, 2013. Uyo is the state capital of Akwa Ibom State, South-south geographical zone of Nigeria. It lies within the tropical rainforest belt between the latitude 4°52' and 5°05'N and longitude of 7°52' and 8°00'E. The rainfall pattern in this area is moderately high, causing water loggings and water filled gutters which served as mosquito breeding sites and contaminated water which supported the growth of *Salmonella spp.* The study was conducted at St. Luke's Hospital located within Uyo town. The area is populated mainly by traders and students, with inadequate infrastructural development and poor environmental sanitation, which render the environment conducive for the transmission of malaria and typhoid fever.

The choice of the hospital was based on high enrolment of pregnant women attending antenatal clinic, as necessary approval and consent were obtained from appropriate authorities and hospital management, precisely the Akwa Ibom State hospital management board.

Study Population

A total of 220 pregnant women aged between 17 and 45 years; who came for routine ante-natal services were recruited by voluntary participation. Their trimester of pregnancy, gravidity, haemoglobin concentration, age and occupation was recorded from their hospital files.

Sample Collection

The method of blood collection employed was venepuncture technique (Carmel *et al.*, 1993). The venepuncture was made with the aid of a 21 gauge needle attached to a 2ml syringe. When sufficient blood was collected, the needle was removed immediately; a large drop of blood was placed on a clean, grease-free glass slide and a thick blood film was made for microscopy while the remaining was allowed to clot and the serum was obtained for serology (Widal test).

Laboratory Analysis

The blood samples prepared on glass slide was stained by Giemsa staining technique (Cheesbrough, 2005). The commercially prepared Giemsa stain was diluted 1 in 10, just before use. This was done by adding 1ml of Giemsa stain to 9ml of buffered distilled water and properly mixed. The dried blood films were flooded with the freshly diluted stain and allowed to stain for 10 minutes. The stain was then washed off with clean water. The back of each slide was wiped with cotton wool and the slides were placed in the draining rack to air dry. During the microscopic detection, one drop of oil immersion was placed on the slide and then viewed under the microscope, using 100x objective.

The ring form trophozoites and gametocytes of *plasmodium falciparum* were looked for using the standard criteria accordingly (Cheesbrough, 2000).

- + signifies 1 – 10 parasites per 100 microscopic fields
- ++ signifies 11-100 parasites per 100 microscopic fields
- +++ signifies 1 – 10 parasites in every high power field
- ++++ signifies >10 parasites in every high power field.

Widal Test Examination

Widal test was performed on all blood samples using the rapid slide agglutination method (Ochei and Kolhatat, 2008). Blood collected in plain bottles were transferred to a clean dry centrifuge and spun for 5 minutes at 1500 RCF. The supernatant (serum) was extracted and a screening test was performed on all the sera using commercial (cromatest febrile flagella antigen kit) antigen suspension for the somatic (O) and flagella (H) antigens or *Salmonella typhi*. The tests were conducted as follows:

Rapid agglutination test

A drop of the patients serum was placed on a cleantile and one drop of the antigen appropriately added unto the serum, forming 8 wells. Two other wells for positive control and saline control included for each antigen suspensions. The tile was rocked gently for 3 minutes and observed for agglutination. The negative control shows uniform suspension while the positive shows obvious agglutination before any test was recorded as positive. Any agglutination observed with patients (pregnant women) serum was tested further by the agglutination method.

Tube agglutination method

Seven test tubes were set up for each sample to be tested. Double dilutions of patient's serum in saline were prepared and the contents were properly mixed and 1 ml of the suspension was transferred into test tube No. 2. This process was continued till No. 5, from which 1ml was discarded. This provides a serial two fold dilutions of 1/20, 1/40, 1/80, 1/160, 1/320 in the tubes. One drop of appropriate serum was added to tube No. 6 while tube No. 7 served as saline control. The contents of all the test tubes were quickly mixed and then incubated for 18 hours at 37°C in a water bath. The tubes (including the control) were examined for agglutination under bright light. Dilution in tubes where agglutination was observed was recorded as the titre of the test reaction. A positive widal test was considered for any given serum sample with antibody titre of 1/80 or 1:80 for *Salmonella typhi* antigens.

Statistical Analysis

Chi-square was used to determine the level of significance at $P < 0.05$. Simple proportions and rates were used to analyse the result.

RESULTS

Out of the 220 pregnant women examined, 159 (72.27%) tested positive for malaria parasites, of these 159 malaria infected pregnant women, 104 (65.40%) tested positive for *Salmonella* spp. (typhoid). The prevalence of malaria among the pregnant women examined between July to October is shown in Table 1. Women examined in the month of July had statistically the highest prevalence of 78.08% ($P < 0.05$) while values recorded in the month of August was the least prevalent (66.17%).

Table 1: Prevalence of malaria parasites in pregnant women between July to October

Months	No. examined	No. positive (%)
July	73	57 (78.08)
August	68	
September	43	29 (67.21)
October	36	28 (77.77)
Total	220	159 (72.27)

$N = 220$; $df = 3$; $\chi^2 = 17.62$, $P < 0.05$, statistically significant

Table 2 summarizes the prevalence of co-infection of malaria positive pregnant women with typhoid (*Salmonella spp.*), while July had the highest prevalence case of 77.19% ($P < 0.05$), the least prevalence rate was recorded in October (51.85%).

Table 2: Prevalence of Co-infection of malaria positive pregnant women with Typhoid fever (*S. typhi*) between July to October

Months	No. positive for malaria	No. co-infected with typhoid (%)
July	57	44 (77.19)
August	45	30 (66.66)
September	29	16 (53.33)
October	28	14 (51.85)
Total	159	104 (65.40)

$N = 159$; $df = 3$; $\chi^2 = 20.79$, $P < 0.05$, statistically significant

Table 3 is a summary of the prevalence of malaria parasite and co-infection with typhoid in relation to gravidity (number of deliveries). Attending ante-natal clinic at St. Luke's Hospital, Uyo, Akwa Ibom State, Nigeria. Primigravidae had significantly both the highest prevalence of malaria (97.43%) and co-infection rate of 68.42%, while multigravidae recorded both the least malaria parasite prevalence and co-infection rate of 43.85% and 56.00% respectively.

Information on the prevalence of malaria/co-infection with typhoid in relation to trimester is presented in Table 4. Women in first trimester had the highest prevalence of malaria parasite (87.87%), but the least co-infection rate of 48.27%, followed by second trimester with malaria parasite prevalence of 71.81% and co-infection rate of 65.42%. On the other hand, women in third trimester had the least incidence of malaria parasite (60.52%), but recorded the highest co-infection rate of 89.95%.

Table 3: Prevalence of malaria/co-infection with typhoid in relation to gravidity among pregnant women attending St. Luke's Hospital in Uyo, Akwa Ibom State, Nigeria

Gravidity	No. examined for malaria parasite	No. positive for Malaria Parasite	% of malaria positive	Co-infection rate (%)
Primigravidae	78	76	97.43	52 (68.42)
Secundigravidae	85	58	68.23	38 (65.55)
Multigravidae	57	25	43.85	10 (56.00)

$N = 220$, $df = 2$, $\chi^2 = 26.59$, $P < 0.05$, statistically significant

Table 4: Prevalence of malaria/co-infection with typhoid in relation to trimester

Trimester	No. examined for malaria parasite	No. positive for Malaria Parasite	% of malaria positive	Co-infection rate (%)
First	33	29	87.87	14 (48.27)
Second	149	107	71.81	70 (65.42)
Third	38	23	60.62	20 (86.95)

N = 220, df = 2, $\chi^2 = 18.23$, P<0.05, statistically significant

Table 5 shows the prevalence of malaria parasite and co-infection with typhoid in relation to haemoglobin concentration. Pregnant women with haemoglobin range of 6g/dl – 8.5g/dl had the highest case of malaria parasite (88.88%) but least co-infection rate of 54.16%. Pregnant women with haemoglobin range of 9g/dl – 11.5g/dl showed similar trends (P>0.05) in the prevalence of malaria parent (80.43%) and co-infection rate of 65.76% while pregnant women with haemoglobin range of 12g/dl – 14.5g/dl recorded the least prevalence of malaria parasite (43.63%) but highest co-infection rate of 75.00%.

Table 5: Prevalence of malaria/co-infection with typhoid in relation to haemoglobin concentration

Haemoglobin concentration	No. examined for malaria parasite	No. positive for Malaria Parasite	% of malaria positive	Co-infection rate (%)
6g/dl – 8.5g/dl	27	24	88.88	13 (54.16)
9g/dl – 11.5g/dl	138	111	80.43	73 (65.76)
12g/dl – 14.5g/dl	55	24	43.63	18 (75.00)

N = 220, df = 2, $\chi^2 = 23.08$, P<0.05, statistically significant

Age range co-infection of *Plasmodium facliparium* and *Salmonella typhi* among pregnant women are summarized in Table 6. Pregnant women of 16 – 20 years were most significantly co-infected (P<0.05), while pregnant women aged 41 – 45 years were the least infected. However, infection with malaria parasite decreases with increase in case of pregnant women, while co-infection rate was high among those within the age range of 31 – 35 years.

Table 6: Prevalence of malaria/co-infection with typhoid in relation to age group of pregnant women

Age range	No. examined for malaria parasite	No. positive for Malaria Parasite	% of malaria positive	Co-infection rate (%)
16 – 20	13	13	100.00	7 (53.84)
21 – 25	54	40	74.07	26 (65.00)
26 – 30	118	86	72.88	47 (54.65)
31 – 35	28	17	60.71	15 (88.23)
36 – 40	6	3	50.00	2 (66.66)
41 – 45	1	0	0.00	0 (0.00)

The prevalence of malaria parasite and typhoid among pregnant women manifesting fever in relation to their occupation were also obtained (Table 7). Pregnant students had the highest prevalence of malaria parasite of 84.61% and co-infection rate of 77.27% (P<0.05), while civil servants experience the least in both cases.

Table 7: Prevalence of malaria/co-infection with typhoid in relation to occupation

Occupation	No. examined for malaria parasite	No. positive for Malaria Parasite	% of malaria positive	Co-infection rate (%)
Students	26	22	84.61	17 (77.27)
Traders	126	89	70.63	62 (69.66)
House wife	26	19	73.07	12 (63.15)
Civil servant	42	29	69.04	13 (44.82)

DISCUSSION

Our results reveal a high malaria prevalence rate (72.27%) in pregnant women attending antenatal clinic at St. Luke's hospital, Uyo, Akwa Ibom State. This suggests high endemicity and transmission of malaria in this area. This may rank one of the highest prevalence rates as

this finding correlates with previous reports from Onyenekwe *et al.* (2002) Mbawugo and Okoroudo (2005) Nduka *et al.* (2006) Adefioye *et al.* (2007), Bako *et al.* (2008), Oyibo *et al.* (2009), Raimi and Kanu (2010), Isah *et al.* (2011) and Ukibe *et al.* (2013). It further confirms the susceptibility of pregnant women to malaria infection which has been attributed to pregnancy induced immunosuppression (Scholarpurka *et al.*, 1990; Klufio 1992) which is essentially physiological.

This study has revealed variation in malaria prevalence rates ($P < 0.05$) between the different months, with the highest prevalence occurring in July (78.08%). This may be due to high rainfall pattern and climatic factors that favours the transmission of malaria (KCDDE, 2009). The rate of co-infection with typhoid fever was 65.40% by widal test. This suggests that typhoid is common among malaria infected pregnant women in this part of the country. According to Scholarpurka *et al.* (1990) the reduction of cellular and immoral immunity which occurs in pregnancy renders pregnant women susceptible to other infections including typhoid fever. Various prevalence rates of malaria-typhoid co-infection have been reported in other part of Nigeria (Mbuh *et al.*, 2003, Isibor *et al.*, 2011 and Ukibe *et al.*, 2013), and in Ghana (Isah *et al.*, 2011 and India, Prasanna, 2011).

The study reveals that primigravidae had the highest co-infection rate (68.42%) in the area Uyo. This confirms previous observations that in malaria endemic areas primigravidae are most vulnerable followed by secundigravidae (McGregor, 1984; Barbin, 1991). The severity of typho-malaria in this group is thought to be due to general impaired immunity plans a diminution of acquired immunity in malaria endemic areas (Takem and D' Alessandro, 2013) which rises from hormonal changes of pregnancy, reduced synthesis of immunoglobulin and reduced function of reticulo-endothelial system which in turn causes immunosuppression in pregnancy (Rasheed *et al.* 1993).

The study further showed the high prevalence rate of malaria parasite among pregnant women in their first trimester (87.87%). This consistent with the observation that the peak prevalence of *Plasmodium falciparum* infection is between 9 and 16 weeks of gestation (Barbin, 1991). Meanwhile, the co-infection rate of these pregnant women with typhoid, was very high among women in their third trimester (86.95%) followed by second trimester (65.42%) and the first trimester being the least (48.27%). It has been reported that although nausea and vomiting which are also typhoidal symptoms in pregnant women are often associated with the first trimester of pregnancy majority of the pregnant patients presented in second and third trimester. Moreso, the hallmark of typhoid fever last for about 4-8 weeks in an untreated [patient and at the first week of infection, widal test is negative (Akinyemi *et al.* 2007). According to Ngwu (2003), widal test is strongly positive with anti O and anti H antibodies from second week of infection to the final week were elevation in liver transaminase occurs.

It was indicated in the study that the prevalence of malaria decreases with increased haemoglobin concentration. Haemoglobin concentration $< 7\text{g/dl} - 8\text{g/dl}$ signifies a very severe anaemia, those with Haemoglobin concentration between $9\text{g/dl} - 11\text{g/dl}$ signifies mild anaemia. Anaemia tends to occur due to haemolysis of parasitized red blood cells and increased demands of pregnancy folate/iron deficiency (Dasai *et al.* 2007). However haemoglobin concentration of pregnant women who had co-infection with typhoid tends to increase with increase in co-infection rate. Hence haemoglobin concentration range between $12.0\text{g/dl} - 14.5\text{g/dl}$ had the highest co-infection rate of 75%. According to Bashyam (2007) short-lived red blood cells might save [patients from malaria but the extra iron they dump out support intracellular growth and survival of Salmonella. Thus, the report suggests that pregnant women with severe anaemia have reduced haemoglobin concentration and consequently reduced red blood cells, to hump out iron. This could lead to the decrease co-infection rate observed among women with low haemoglobin concentration.

Concurrently it was observed in the study that malaria prevalence rate decreased with increase in age. Pregnant women between the age range of 16 - 20 years were statistically significant ($P < 0.05$) with prevalence rate of 100%. This finding supports earlier report from Bouyou-Akotet *et al.*, 2003; where age group of < 20 years were reported to be at high risk. This is attributed to unfavourable factors such as young maternal age biological immaturity inadequate pre-natal care poverty low pre-pregnancy weight and minority status (Scholl and Hediger, 1992). To stem this awareness on malaria prevention measures during pregnancy should target young women even before they get married or preferably at schools religious and social gathering. Also, it is recorded that pregnant women within the age range of 31 - 35 years had the highest co-infection rate which could be attributed to individual immunity level.

CONCLUSION

This study revealed that malaria prevalence rate as well as co-infection of malaria and typhoid among pregnant women attending antenatal clinic at St. Luke's Hospital Uyo, Akwa Ibom State was very high. Epidemiological and demographic factors which include gravidity, trimester haemoglobin concentration age, occupation etc, all influenced and contributed to the high rate of malaria parasite prevalence as well as the high co-infection rate. This unfavourable situation poses a great challenge to governments in the fight to maintain public health and achieve the Millennium Development Goals (MDGs). It is, therefore suggested that the state government should improve environmental factors which tend to encourage high transmission of malaria and typhoid fever especially in the area of improved housing and sanitation, provision of basic amenities such as good drinking water and electricity and public awareness programme should be created in order to enlighten the people (pregnant women) on the need to use preventive measures such as the use of Insecticide Treated nets (INTs) in order to curb the threat of malaria and typhoid which pose a major challenge to pregnancy.

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Malaria and typhoid fever are both endemic in the tropics and pregnant women constitute one of the high risk groups. The objective is to determine the rate of malaria- typhoid co-infection in pregnant women attending antenatal clinics in Wuse general hospital Abuja, Nigeria. A study of malaria infection and typhoid fever on the pregnant women attending wuse general hospital and its haematological parameters was carried out between April and May, 2015 in Abuja. Blood samples were collected and examined for malaria and typhoid using widal agglutination method and P. falciparum antigens rapid test device respectively. Of the 200 sampled, 16(8%) were infected with malaria parasite while 77 (38.5%) were infected... In pregnant women the morbidity due to malaria includes anemia, fever illness, hypoglycemia, cerebral malaria, pulmonary edema, puerperal sepsis and mortality can occur from severe malaria and haemorrhage. The problems in the new born include low birth weight, prematurity, IUGR, malaria illness and mortality.Â Pregnancy-malaria and intensity of transmission: Clinical presentation and severity of malaria in pregnancy differ in areas of high transmission and low transmission due to differences in the level of immunity. In high endemic areas, acquired immunity is high, mortality is less common, asymptomatic and incidental parasitemia are not uncommon.Â Parasite densities are similar in pregnant and non-pregnant states. Results of prevalence of malaria infection among pregnant women with different genotype. The Prevalence of malaria infection in relation to blood group of pregnant women. Discussion. Distribution of malaria infection among pregnant women.Â Malaria in pregnancy is a significant health problem in Nigeria and it thus requires systematic studies. Antecedent studies have recorded various rates of malaria prevalence among pregnant women.Â Adefioye [2] also recorded 72% prevalence rate and Chukwurah [7] recorded 63.5% of prevalence of malaria parasite in pregnant women in Akwa Ibom, Nigeria.Â The result of this study revealed that pregnant women in the different age groups were infected at different parasite densities. Malaria infection during pregnancy is a significant public health problem with substantial risk for the pregnant women; her weight is mostly the result of P. falciparum infection and occurs predominantly in Africa [11 - 13]. Malaria infection makes a large but unquantifiable contribution to low birth weight in infants in the developing world, severe maternal anemia and perinatal mortality.Â Prevalence of malaria and typhoid infection among pregnant women in the study. Prevalence of Malaria and Typhoid Infection among Pregnant Women in the study is shown in Table 1 . The age groups of the pregnant women are 16-25, 26-35 and 36-45 years, most of the pregnant women tested are between age group 26-35 years 113 (66.5). Malaria and typhoid fever are both endemic in the tropics and pregnant women constitute one of the high risk groups. The objective is to determine the rate of malaria-typhoid co-infection in pregnant women attending antenatal clinics in Wuse general hospital Abuja, Nigeria. A study of malaria infection and typhoid fever on the pregnant women attending wuse general hospital and its haematological parameters was carried out between April and May, 2015 in Abuja. Blood samples were collected and examined for malaria and typhoid using widal agglutination method and P. falciparum antigens rapid test device respectively. Of the 200 sampled, 16(8%) were infected with malaria parasite while 77 (38.5%) were infected...