ANTIGENIC CROSS REACTION BETWEEN MALARIA PARASITES AND TYPHOID ORGANISMS IN PATIENTS WITH FEVER OF UNKNOWN ORIGIN IN OWERRI MUNICIPAL IMO STATE

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Abstract: This study reports result of malaria parasite test, Widal test and bacteriological test was carried out on 205 samples. Of these, 160 were cases of patients presenting malaria-like symptoms while 45 were healthy controls. Out of the 160 patients studied, 117(73.1%) had acute malaria, while 18(40%) out of the 45 control sample were malaria parasite carriers. Widal test was done to determine those positive for anti-salmonella antibodies, Widal titers > 1/160 which is considered very specific for diagnosis of typhoid fever. This test was positive for salmonella 'O' and 'H' titers in 57(48.7%) and 3(16.7%) of malaria patients and carriers respectively. Statistical analysis showed that there is a relationship between malaria parasite load and the level of salmonella antibody titres. Bacteriological test was then carried out to determine the isolation rate of *S. typhi.* Only 3(2.6%) of the malaria patient samples were positive for typhoid by the blood culture method. There were no isolation in the carrier group. On four weeks follow up, after malaria treatment, 38 of the 54 samples showing widal positive without *S. typhi* isolate were retested and 29(76.3%) become negative. It was postulated that the stimulation of the immune system due to acute malaria infection could be responsible for this phenomenon. The rate of co-infection with malaria parasites and *S. typhi* was high when typhoid was diagnosed by widal (29.3%) than by blood culture method(1.50%). The incidence of typhoid and malaria co-infection will greatly reduce if the diagnosis of typhoid fever in malaria endmic areas as Owerri is based on blood culture.

Keyword: Antigenic cross reaction, malaria parasite and S.typhi in patients

INTRODUCTION

Human malaria, caused by four protozoan parasites of the genus *Plasmodium,* is a disease responsible for inordinate mortality, morbidity, and economic loss worldwide. Malaria infects up to 500 million people per year and is responsible for almost 3 million deaths annually (Snow *et al.,* 2005). Plasmodium parasites responsible for human malaria are obligately dependent on mosquitoes of the genus *Anopheles* for transmission from one human host to the next, with *Anopheles gambiae* being the primary vector in Africa (Fontenille and Simard 2004).

Typhoid fever, or commonly just typhoid, is a common worldwide illness, is caused by *Salmonella typhi and* transmitted by the ingestion of food or water contaminated with the faeces of an infected person (Giannella 1996). The

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bacteria then perforate through the intestinal wall and are phagocytosed by macrophages. The organism is a Gram-negative short bacillus that is motile due to its peritrichous flagella. The bacterium grows best at 37 °C/99 °F – human body temperature.

Malaria and typhoid fever are among the most endemic diseases in the tropics. Both diseases have been associated with poverty and underdevelopment with significant morbidity and mortality. Uneke (2008)and Mbuh *et al.*, (2003) has reported cases of co-infection with both *Salmonella typhi* and the *Plasmodium species*. In the last two decades, this relationship between the two diseases has been substantiated by studies from Africa and India (Ammah *et al.*, 1999).

Malaria and typhoid remain a threat to many people in Sub Saharan Africa for several reasons: the increasing poverty, deterioration in public health services, compounded by HIV / AIDS and increasing resistance of malaria parasites to chloroquine (Alnwch, 2001), the lack of portable water and widespread misuse of the Widal agglutination test for diagnosing typhoid fever (Nsutebu and Ndumbe 2001).

One of the current issues in malaria therapy is the Confusion of malaria fever with typhoid fever(Tropical medicine, 2009). There have been a lot of report on co-infection between malaria and typoid fever (Ammah, *et al.*, 1999; Mbuh 2003, Uneke, 2008). There are more report of typhoid cases in areas of drug resistant malaria (Frimpog *et al.*, 2000).

Of course, haemolysis which occur in malaria may predispose to gram-negative organism as what has been seen in haemolytic disease caused by sickle-cell disease and bartonellosis (Kaye & Hook 1963). Frimpong, *et al.*, (2000) observed that a cross reaction between malaria parasites and salmonella antigens may cause false positive Widal agglutination test.

Although the signs and symptoms of malaria and typhoid fever do overlap, it was observed in Pakistan that subjects with dual infection had significantly higher rates of nausea, vomiting, abdominal pain, and diarrhoea, all common presenting features of enteric fever (Khan *et al.*, 2005). Furthermore, it was noted that unlike the intermittent fever pattern generally seen with malaria, patients with dual infection tended to exhibit a continuous fever more typical of enteric fever (Khan *et al.*, 2005).

In Owerri, there is much talk about typhoid , even with the high level of education and sanitation observed there. In fact, according to medical laboratory records, the commonest disease presentation in the hospitals/health centers or laboratories today is typhoid fever. Also the tradomedical homes (alternative medicine practitioners), have carved out their own method of making a living through the management of typhoid.

It became necessary, therefore, to find out the actual situation in Owerri-being a tropical and malaria/typhoid endemic area(Ukpai & Ajoku, 2001).

MATERIALS AND METHODS

THE STUDY AREA

The study was carried out in Owerri, the capital of Imo state, Nigeria. It is located between latitude 5°34^{l} and 5°15^{l} N and longitude 7°30^{l} E. It has a daily temperature range of between 20°C to 30° C and a very high relative humidy of about 75% reaching 85% during the rainy season.

Owerri municipal has five major divisions, which include Amawom, Umuoronjo, Umuodu, Umuonyeche, Umuoyima. It has two flowing waters, Otamiri and Nworie streams, These are rarely visited or used for domestic work,

because of the avaliability of bore holes and tap water. It is an Urban area, densely populated with strangers. Majority of the inhabitants are igbos and enlightened. The major occupation is white collar jobs and trading.The level of social amenities is below average, however, sewage disposal has improved compared to what it used to be in the late 1900's and early 2000's. Generally, the standard of cleanliness and hygiene has improved. Heaps of rubbish are no longer seen in every nook and cranny due to the effort of the government agency (ENTRACO).

DATA COLLECTION POINTS

The samples for this study were collected from three hospitals and one medical laboratory in Owerri. These includes: one Government hospitals (Imo state General hospital), two private hospitals (International Christian hospital and Pamela hospital & Maternity.) and a private laboratory (Step 1 Medical laboratory which also provides service for many other clinics in Owerri and other parts of Imo State).

SAMPLE COLLECTION

Consent and assistance were obtained from the Medical Directors of each Hospital and the Director of the Medical laboratory where samples were collected to carry out this study.

Patients, directed to the laboratory for the Widal and malaria parasite tests by the attending physician, were sampled for this study. Following an explanation to and consent of the patient or parent of children, a simple questionnaire was filled with regards to age, sex and current medication.

A total of 205 blood samples were collected; 160(78 %) samples were collected from consecutive febrile patients and 45(22%) apparently healthy individuals as controls. Patients who had started treatment were excluded.

SAMPLE ANALYSIS

Blood drawn by vene-puncture from each person was tested for malaria parasites, *S. typhi* O and H antibodies and also cultured for *S. typhi*

Bacteriological blood culture

Ten mililitres (10ml) of venous blood were collected. Five mililitres was placed in a sterile universal container and the blood allowed to clot. The serum was then removed to perform a Widal test. The remaining blood sample (5ml) was added to a bottle of bile-salt broth which is incubated at 37°C for an initial period of 48h. This was examined and sub-cultured on Blood agar, MacConkey agar (Oxoid) and Deoxycholate citrate agar.(Dugiud, *et al.*, 1984 and Cheesbrough, 2002)

Only samples that were positive for Anti-Salmonella antibodies (Titre≥1/160) that were sub-cultured. Such subjects were asked to come back after two days. Those from whose samples *S. typhi* were not isolated were asked to come back after 4 weeks (1month) of malaria treatment for a retest.

S. typhi organisms were identified on the basis of standard cultural, microscopic and biochemical characterization (Cheesbrough, 2000). Inoculated blood culture media were discarded as negative if there were no growth after 7 days.

Widal test

The Widal agglutination test was performed on all blood samples by the rapid slide titration method using commercial antigen suspension for the somatic (O) and flagella (H) antigens (Cheesbrough, 2000). A positive Widal test was considered for any serum sample with antibody titre greater or equal to 1 in

160 for somatic (O) and flagella (H) antibodies. [Titre ≥1/ 160 to the O & H antigen of *S. typhi*].

Parasitological examination

Giemsa-stained thick and thin blood films were prepared for each sample and parasitaemia was evaluated per microliter of blood using the thick film preparation according to standard methods (WHO,1991) . Films were examined microscopically for the presence of malaria parasites within red blood cells in thin films. For thick films, the ring forms, trophozoites and gametocytes were looked for. A smear was considered negative for malaria parasites if no parasites were seen after examining at least 100 microscopic fields. The number of parasites µl-1 of blood was taken.

RESULTS

The Rate of Occurance of Malaria parasite infection and *Salmonella typhi* infection in the Study Area.

In this study malaria parasites were found in 135(65.9%) out of 205 subjects. One hundred and sixty (78%) samples were from patients presenting malaria-like symptoms (henceforth known as FUO samples). Out of this 117(73.1%) samples had malaria parasites with mean parasite load of 12000µl in 1of blood (malaria patients) while 18(40.0%) of the 45 control samples had malaria parasites (carriers) with mean parasite load of 1300µl in 1drop of blood.

Table 1 shows the details of occurance of malaria parasite infection in the study area.

Table 1: The Rate Of Malaria Parasite Infection in the Study Area

	FUO Samples		Control samples		Total	
Survey points	No. Examined	No infected(%)	No Examined	No infected(%)	No Examined	No Infected(%)
General.Hosp	32	22(68.8)	13	3(23.1)	45	25(55.6)
Pamela Hosp	24	23(95.8)	6	0(0.0)	30	23(76.7)
ICH	40	30(75.0)	0	0(0.00)	40	30(75.0)
Step 1Lab.	64	42(65.6)	26	15(57.7)	90	57(63.3)
Total	160	117(73.1)	45	18(40.0)	205	135(65.9)

Statistical analysis with chi square test at 5% level of significance show that there is a significant difference in the rate of infection between feverish patients samples(FUO) and the control samples. Generally the rate of infection in the study area is significantly high.

The widal test result (Table 2), shows that 83(51.9%) out of the 160 FUO samples were infected with *S. typhi*. While 13(28.9%) out of the 45 control samples were infected. Generally, rate of infection was 46.8% by the widal test. This was significantly high.

Table 2: Rate of *Salmonella typhi* infection in study Area using Widal test Method

	FUO Samples		Control samples		Total	
Survey points	No Examined	No Infected(%)	No Examined	No Infected(%)	No Examined	No Infected(%)
General Hosp.	32	14(43.8)	13	2(15.4)	45	16(35.56)
Pamela Hosp.	24	13(54.2)	6	2(23.3)	30	15(76.7)
ICH	40	26(65.0)	0	0(0.00)	40	26(75.0)

Step 1 Lab.	64	30(46.9)	26	9(34.6)	90	39(63.3)
Total	160	83(51.9)	45	13(28.9)	205	96(46.8)

Salmonella typhi was isolated from only 8(8.3%) out of the 96 blood cultures examined given an overall isolation rate of 3.9%, as shown in Table 3. Which is of no significance according to statistical analysis at 5% level of significance(P <05).

Table 3: Rate of *Salmonella typhi* infection in the study Area using Blood culture method.

	FUO Samples		Control samples		Total	
Survey points	**No Examined**	**No Infected(%)**	**No Examined**	**No Infected(%)**	**No Examined**	**No Infected(%)**
General Hosp.	32	3(21.4)	13	0(0.0)	45	3(18.8)
Pamela Hosp.	24	2(15.4)	6	0(0.0)	30	2(13.3)
ICH	40	0(0.0)	-	-	40	0(0.0)
Step 1 Lab.	64	3(10.0)	26	0(0.0)	90	3(7.7)
Total	160	8(5.0)	45	0(0.0)	205	8(3.9)

Salmonella antibodies were present (28.9%) even in apperently healthy individual, as shown in Table 3 . But all blood cultures of the control samples were negetive for *S. typhi.*

The Rate of Occurance of Anti-salmonella Antibody.

Table 4: Distribution Of Salmonella Antibodies Among Case With Acute Malaria

Parasite & Carriers (Antigenic Cross Reaction)

	FUO SAMPLES			CONTROL SAMPLES			TOTAL		
Survey points	**No Exam.**	**No. with acute malaria**	**No. +ve for sal-Ab (%)**	**No Exam.**	**No. of MP carrier**	**No. +ve for sal-Ab(%)**	**No Examined**	**No. With MP**	**No +ve for sal-Ab(%)**
General Hosp.	32	22	9(40.9)	13	3	1(33.3)	45	25	10(40.00)
Pamela	24	23	10(43.5)	6	0	0(0.00)	30	23	10(43.49)
I C H	40	30	11(36.7)	0	0	0(0.00)	40	30	11(36.67)
Step 1 - Lab.	64	42	21(50.0)	26	15	2(13.3)	90	57	23(40.35)
Total	**160**	**117**	**57(48.7)**	**45**	**18**	**3(16.7)**	**205**	**135**	**54(40.00)**

The relationship between acute malaria parasite infection and positive salmonella antibody is illustrated in Table 12. Out of the 117 persons having Acute malaria parasite infection 57(48.7%) were positive for Salmonella antibody. In the control group, 3(16.7%) out of the 18 malaria parasite carriers, were positive for

salmonella-antibody. Statistical analysis showed that there is a relationship between malaria parasite infection and the presence of Anti-salmonella antibody

TABLE 5: Distribution Of Salmonella Antibodies Among Apparently Healthy Individual (Control Samples)

Survey points	N E	No with +ve salm Ab (%)	No. Cultured	No. With Isolate of *S. typh*
General. Hosp	13	2(13.4)	2	0(0.0%)
Pamela Hosp	6	2(33.3)	2	0(0.0%)
ICH Hosp	0	0(0.00)	0	0(0.00)
Step 1 Lab	26	9(34.6)	9	0(0.0%)
Total	45	13(28.9)	13	0(0.0%)

Comparison Of Diagonistic Methods.

Comparing Widal Agglutination Test and Blood Culture method.

TABLE 6: The Rate Of *S. typhi* Infection In The Study Area According To The Widal Test And Blood Culture Method

Ages in years	No Examined	No. Infected (%)	
		Widal test method	Blood culture method
1-10	20	10(50.0)	2(10.0)
11-20	35	18(51.4)	0(0.0)
21-30	60	31(51.7)	3(5.0)
31-40	30	15(50.0)	2(6.7)
41-50	20	15(75.0)	1(5.0)
51-60	15	4(26.7)	0(0.0)
>60	25	2(8.0%)	0(0.0)
Total	**205**	**96(46.8)**	**8(3.9)**

The result of *s.tyhpi* infection using the widal test method, that the infection rate rises with increase in years. It gets to the peak at age group 41-50yrs and than falls rapidly with increase in years, the lowest being 8% in ages above 60years.With the blood culture method, the rate of infection was highest in age group 1-10years (10%) while ages 31-40yrs had 6.7% rate of infection. Five percent (5%) infection rate was recorded for both age groups 21-30 and 41-50yrs. Other age group had 0.0% rate of infection.

In the overall, the rate of typhoid infection was high (46.8%) in the study area, when widal method is used than when blood culture was used (3.9%).

DISCUSSION

Due to a combination of factors including poor sanitation and health care infrastructure, Malaria and Typhoid fever remain a major public health problem in most resource poor countries (Ojo & Mafiana, 2001). In developed countries, the incidence of cases and death has been greatly decreased by a combination of improved sanitation and hygiene, vaccines, anti malaria and antimicrobial chemotherapy and effective vector control. However, in this study area, the rate of infection for malaria parasites (65.9%) is higher than that for typhoid(46.8%) and (3.9%) using both widal test and blood culture methods respectively for diagnosing typhoid infection. Statistical analysis showed that the rate of malaria infection is significantly high. The high prevalent rate of malaria in the study area(65.9%) showed that malaria infection is still endemic in this area, not withstanding all the control method in place. This confirms what WHO reported (WHO, 1993), that the control of malaria is becoming increasingly difficult and that the epidemiological situation is likely to continue to deteriorate over the next few years. But this rate is a little lower than the 75%, that was reported in 2001 by Ukpai & Ajoku from this study area. This could be as a result of the cleaner environment and increasing aweraness on the control and prevention of the infection. However, the high infection rate could be due to factors such as the amount of rainfall, relative humidity and temperature. All these weather conditions favour the breeding of the vector.

In Nigeria there have been reports of sporadic outbreaks of typhoid but these cases have not always been confirmed leading to paucity in quality data on the prevalence of typhoid in many parts reporting outbreaks (Abayomi, 2009). For many decades now isolation of *S. typhi* from a blood culture from patients has been regarded as a definitive diagnosis for typhoid (Hoffmam *et. al.*,1984). However many laboratories in developing countries lack adequate infrastructure to perform culture and many depend on the cheaper serological tests for diagnosis of typhoid. In the current study using blood culture as a gold standard criterion for the diagnosis of typhoid it was observed that throughout the study, the incidence of typhoid in the Area was low (3.9%). The incidence rate is much lower than the one gotten(46.8%;) when widal test was used to diagnose typhoid for thesame population size. Statistical analysis showed a significant difference in the use of the different methods for the diagnosis of typhoid,($P= 0.05$). This implies that the use of blood culture method, where the causative organism for typhoid is isolated, gives a realistic result. Its use should therefore be encouraged to avoid the consequences of misdiagnosis.

This study also revealed that malaria patients (samples with acute malaria parasites; FUO) were more positive for Anti-salmonella antibodies 57(48.7%) than the malaria carrier patients (control samples with malaria parasite; CS), which is 3(16.7%). Statistical analysis showed that there is a significant relationship between malaria parasites infection and the distribution of salmonella antibodies among malaria patients and carrier (Table 4). This findings is in line with the work of Jhaveri *et al.*, (1995), reporting that

malaria parasites gives a false positive widal test result. This suggests that the presence of malaria parasites in the blood could induce the immune system to produce antibodies that can react with salmonella antigens, giving a false positive widal result. It can also be deduced that the presence of malaria parasites determines the level of *S. typhi* O and H antibody in the blood. This was made clearer, in the fact that 76.3% cases of the malaria patients positive for salmonella antibodies, who came back after 4 weeks of malaria treatment were no longer positive for the antibodies.

The 48.7% malaria patients who had a positive widal test result could be showing cross reactivity between *S. typhi* and malaria parasite antigen as previously observed by Alaribe, *et. al.*(1995).

However, The other widal positive samples which were negative for both malaria parasites and *S. typhi* could be due to patients who are used to self medication before presenting at the hospital. This agrees with what Mbuh, *et. al.* (2003) noted, having observed that many patients often take anti-malaria drugs before presenting at the hospital but would not admit it when questioned. But some of these have been identified in a survey through testing for residual malaria drugs in their blood.

While the Widal test has played a major role in the diagnosis of typhoid fever in the past, recent technical developments have revealed several pitfalls in its use and interpretation of its result. Many cases of fever of unknown origin receive the diagnosis of typhoid fever, based upon a false-positive Widal test result rather than a positive culture of *S. typhi*. These limit the usefulness of the test as a reliable diagnostic indicator of the disease process. In the present study, presence of Widal agglutinin under conditions of positive malaria smear, negative *S. typhi* culture and negative prior typhoid immunisation, would suggest that malaria parasite may have some undefined antigenic determinants similar to *S. typhi* which can induce antibody production. This could explain the febrile condition seen in some of these patients. On the other hand, the presence of Widal agglutinin under conditions of negative malaria smear, negative *S. typhi* culture and negative prior immunisation against typhoid fever suggests that other infectious agents, in addition to Salmonella and malaria parasite, may also share common antigenic determinant with *S typhi.*

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Recommendation

In view of this confusion and in order to rule out any case of malaria with mimicking symptoms, or the influence of anamnestic response the practical use of cultural methods for the diagnosis of typhoid fever should be emphasized in our clinical laboratories. This will also improve patient management by cutting down cost of treatment and eliminate other risks associated with misuse of antibiotics.

Futhermore, this study observed that there is an antigenic cross reaction between malaria parasites and typhoid organisms which could be the reason for the too much talk of malaria and typhoid co-infection in the area. Culture of *S. typhi* from blood is considered as a gold standard in the diagnosis of typhoid and is therefore higly recommended. The Widal test would still be useful as a rapid diagnostic method so long as laboratory workers

followed manufacturer's instructions for use on sera from patients whose clinical examination raised suspicion of typhoid. As some other disease conditions such as malaria, non-typhoidal salmonelae rheumatoid arthritis, chronic liver disease, nephrotic syndrome and ulcerative colitis may show similar symptoms and produce high "O" antibody titres (Cheesbrough, 2000).These should also be evaluated as differential diagnosis in order to reduce cases of false positive Widal test results. It is also recomended that other-rapid identification tests such as the IgM dipstick, which detects IgM antibodies against whole cell serotype *S. typhi*, be evaluated for use in Nigeria as the test has been found to be more sensitive than the Widal test in several studies. (Kariuki, *et al.*, 2004).

Malaria still remains the disease of major public health importance in the tropics. It has proven stubborn to control methods like antimalaria drugs and insecticide resistance.The most promising control method in view is that of genetic manipulation of the insect vector. This involves introducing foreign genes into the mosquito enabling it to block one or more developmental stages of malaria parasites within itself. The plasmoduim cycle is interrupted and so the transmission.

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