CO-INFECTION OF MALARIA AND TYPHOID FEVER IN A TROPICAL COMMUNITY

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ABSTRACT

A study was carried out on patients clinically diagnosed of malaria or typhoid or both, at Nnewi Anambra State, Nigeria, to investigate the level of association between malaria and typhoid fever infections. The stool culture was used as an additional diagnostic test for typhoid fever. The study indicated that out of 256 patients, 29(14.36 %) were diagnosed with concurrent malaria and typhoid fever based on bacteriological method as compared to 147 (57.42 %) base on serological method. Plasmodium falciparum was the only Plasmodium species isolated. Furthermore, 42.59 % were likely to have been falsely diagnosed of having concurrent malaria and typhoid fever using serology. Our study indicated that out of 202 (78.90 %) malaria positive patients, 13(6.44 %); 12(5.94 %) and 3(1.49 %) had concurrent malaria co-existing with Salmonella typhi, S. paratyhpi and S. typhimurium respectively. Malaria was positively associated with typhoid fever (P < 0.05) being more pronounced using serological diagnosis. The difference in the Plasmodium falciprum parasteamia and Salmonella antibody titre was only significant using Widal test. Diagnosis of typhoid fever in malaria positive patients using Widal test solely may lead to misleading and unreliable results.

Keywords: Co-infection, *Plasmodium*, *Salmonella,* Malaria, Typhoid fever

INTRODUCTION

Malaria is one of the most important of the tropical diseases. Over 2 billion people are at risk of infection, over a quarter of a billion cases occur annually, and at least a million people die annually as a result of the disease alone or in combination with other conditions (TDR, 2005)

Four single celled protozoan parasites of the genus *Plasmodium* that cause malaria in human are *Plasmodium falciparium*, *P. ovale, P. malariae and P. vivax.* Malaria transmission is mainly by inoculation through the bite of infected blood feeding female *Anopheles* mosquito from human to human and transfusion of infected blood. Typhoid fever (enteric fever) is a bacterial disease caused by Gram-negative bacteria of the genus *Salmonella*. *S. typhi, S. paratyphi* A, *S. paratyphi* B and *S. paratyphi* C are implicated in the human typhoid fever. These subspecies are identified by serologic markers on polysaccharide somatic (O) and protein flagella (H) antigens (Cook, 1996).

Salmonella infection is acquired by feccal-oral-route. Humans are the only true reservoir of *S. typhi* as convalescent or chronic and carriers always serve as the ultimate source of infection (Olubuyide, 1992). *S. typhi* can survive for several weeks in fresh, salt and brackish water, ice, dust, dried sewage, on clothing and can multiply in milk products (Cook, 1996). Flies and other insects can carry infective agents from infected faeces or other infected materials to food and drinks (Olubuyide, 1992).

Malaria and typhoid fever are endemic fibrile diseases with overlapping signs and symptoms notably fever, confusional state, jaundice, diarhoea, vomiting, headache etc. There have been reports of malaria co-infection with typhoid fever (Gopinath *et al.,* 1995; Ammah *et al.,* 1999), and some bacteria strains have been isolated from blood cultures of Nigerian children with cerebral malaria (Prada *et al.,* 1993). An association between malaria and typhoid fever was first described in 1862 in North America as an entity called typho-malaria fever (Smith, 1982). In Africa *Escherichia Coli, Pseudomonas aeruginosa* and *Salmnonella* species were implicated as the cause of septicemia in complicated falciparum malaria (Bygbjerg and Lang, 1982). It is on these reasons that this study was undertaken to assess the level of association between malaria and typhoid fever in Nnewi North local government, Nigeria.

MATERIALS and METHODS

Study Area: Nnewi is located in the rainforest belt and lies between Lat. 6.03^0N and Long. 6.9^0E. Nnamdi Azikiwe University Teaching Hospital (NAUTH) is located in Nnewi North Local Government. Nnewi is a semi-urban area densely populated, comprising farmers, traders, civil servants and few health workers. Sanitation is poor with very poor drainage system.

Sampling: 256 patients whose ages range from 1 to >35 years with 5 years interval per age group were examined for malaria and typhoid fever.

Table 1: Age distribution of malaria and typhoid fever infection among NAUTH Patients

Age group (years)	Number examined	Number positive		
		Malaria	Typhoid fever	
			Widal test	Culture
1-5	19	17(89.47)	5(26.32)	0.(0.00)
6-10	26	21(80.71)	12(46.15)	2(7.69)
11-15	32	31(96.88)	24(75.00)	6(18.75)
16-20	46	33(82.50)	27(67.15)	8(20.00)
21-25	52	43(82.69)	37(71.15)	12(23.08)
26-30	42	33(78.57)	34(57.14)	9(21.14)
31-35	30	17(56.67)	14(46.67)	1(3.33)
>35	15	7(46.67)	4(26.67)	0(0.00)
Total	256	202(78.91)	147(57.42)	38(14.84)

Key: *Number in parenthesis = percentage*

Table 2: *Plasmodium falciparum* and *Salmonella* species in NAUTH Patients

Age in years	Number examined	*Plasmodium falciparum*	*Salmonella typhi*	*Salmonella typhimurium*	*Salmonella paratyphi*
1-5	19	17(89.47)	0(0.00)	0(0.00)	0(0.00)
6-10	26	21(80.71)	2(7.69)	0(0.00)	0(0.00)
11-15	32	31(96.88)	2(6.25)	0(0.00)	4(12.50)
16-20	46	33(82.50)	5(12.50)	1(2.50)	2(5.00)
21-25	52	43(82.69)	4(7.69)	2(3.85)	6(11.54)
26-30	42	33(78.57)	3(7.14)	1(2.38)	5(11.90)
31-35	30	17(56.67)	1.(3.33)	0(0.00)	0(0.00)
>35	15	7(46.67)	0.(0.00)	0(0.00)	0(0.00)
Total	256	202(78.91)	17(6.64)	4(1.56)	17(6.64)

Key: *Number in parenthesis = percentage*

Males and females ratio was approximately equal. Nnewi was chosen for this study because of availability of patients in large numbers in the NAUTH and the general poor personal hygiene and poor sanitary condition of the environment.

This study was conducted from January to August to embrace the two seasons. Samplings of patients were based on doctors' provisional diagnosis, of either plasmodiasis, malaria, typhoid fever or pyresia of unknown origin. Capillary blood was used for thick and thin film preparation. Where not possible, venous blood was collected into EDTA anticoagulated container.

Venous blood was also collected into clean dry glass test tube and allowed to clot, which serum was used for widal test. Clean, dry wide-mouthed and transparent container was used for collection of faecal specimen for culture.

Thick and thin blood films were made on separate clean grease free glass slide, air-dried and stained with freshly prepared 1 in 10 dilution of Giemsa stain for 10mins**.**The stained slide was washed, air-dried, covered with immersion oil and examined under the microscope using X100 objective lens (WHO, 1991).

Parasite numbers were counted and density determined using the method of Greenwood and Armstrong (1991).

Serums of patients were mixed with standard *Salmonella* antigen suspension in equal volume for O and H antigens.

50 healthy individuals without history of fever were screened to ascertain the baseline *Salmonella* antibody titre (1:40) and (1:80) for O and H *Salmonella* antigens respectively (Tanyigna *et al.,* 1999). A four fold rise or greater in the baseline antibody titre (1:160) was considered significant.

Stool samples were asceptically inoculated into selenite –F- enrichment broth and incubated overnight at 37^0C. The (SF) culture was sub-cultured using *Shigella-Salmonella* Agar (SSA) or DCA and incubated at 37^0C overnight. Suspected colonies were inoculated into Kliger Iron Agar (KIA) slants and the characteristic fermentation patterns for *S. typhi* and other salmonellae were sought after incubation of the slants at 37^0C overnight.

Correlation and chi-square, (X^2) were used in the statistical analysis of data.

RESULTS

Out of 256 patients, 202(78.90 %) had malaria; 147(57.42 %) by serology and 38(14.84 %) by culture had typhoid fever. Age groups (11-15), (16-20), (21-25) and (26-30) years were mostly infected with malaria and typhoid fever (Table 1).

Analysis of the co-infection of typhoid fever and malaria was statistically analyzed and it was found that typhoid fever was independent of malaria ($P<0.05$).

Although both infections were independent, there was a significant correlation between typhoid fever and malaria ($P<0.05$). This correlation was more pronounced when Widal test was used than culture method. *S. typhimurium* with overall prevalence of 4 (1.56 %) was isolated in the age groups; 16 – 20, 21 – 25 and 26 – 30; while *S. typhi* 17 (6.64 %) was isolated in all age groups except 1-5 and >35 years. *S. typhi* and *S. paratyphi* had equal prevalence (Table 2).

Table 3: Malaria and typhoid fever parasites association

Salmonella isolate	Malaria Positive N=202			Malaria Negative N=54		
	Male	Female	Total	Male	Female	Total
S. *typhi*	7(3.47)	6(2.97)	13(6.44)	2(3.70)	2(3.70)	4(7.41)
S. *paratyphi* A S. *paratyphi* B S. *paratyphi* C	5(2.48)	7(3.47)	12(5.94)	3(5.56)	2(3.70)	(5(9.26)
S. *typhimurium*	3(1.49)	1(0.50)	4(1.98)	0(0.00)	0(0.00)	0(0.00)
Total	15(7.43)	14(6.93)	29(14.36)	5(9.26)	4(7.41)	9(16.47)

Key: *Number in parenthesis = percentage*

The difference in prevalence with respect to age group was statistically significant ($P < 0.05$).

Furthermore, our study indicated that out of the 202 (78.91 %) malaria positive patients, 29(14.36 %) had typhoid fever bacteriologically confirmed. Both sexes were approximately equally co-infected. On the other hand, out of the 54(21.09%) malaria negative patients, 9(16.67%) were bactreriologically confirmed positive for typhoid fever (Table 3).

There was no significant difference in infection rate ($P > 0.05$), 88(43.56 %) patients had malaria parasite density/parasite count of 1000 to 50,000 parasites per micro litre of blood. Among these patients 48(32.65 %) and 12(31.44 %) had typhoid fever confirmed serologically and bacteriologically respectively. 114 (56.44 %) of malaria positive patients had parasite density of above 50,000 per micro litre of blood (Table 4).

Table 4: Relationship between *Plasmodium falciparum* Parasite density and *Salmonella* antibody titres

P. *falciparum* Density	Malaria Positive	Typhoid fever Widal test	Positive culture
1000 – 50,000	88(43.56)	48(32.65)	12(31.58)
above 50,000	114(56.44)	99(67.35)	26(68.42)
Total	202 (78.91)	147 (57.42)	38 (14.84)

Key: *Number in parenthesis = percentage*

Among these patients were 99(67.35 %) and 26(68.42 %) typhoid fever positive confirmed by Widal test and culture respectively. The disparity in the infection rates were statistically not significant by bacteriological method ($P > 0.05$) and significant by serology ($P<0.05$). Malaria infection was higher in the rainy season than in the dry season (Table 5).

Table 5: Malaria and typhoid fever infection in relation to months of the year

Months	Malaria positive n=202	Typhoid fever n = 38
January	11(5.45)	2(5.26)
February	14(6.93)	1(2.63)
March	17(8.43)	3(7.89)
April	19(9.41)	4(10.52)
May	23(11.38)	3(7.89)
June	34(16.83)	8(21.05)
July	44(21.78)	12(31.58)
August	40(19.80)	5(13.16)

Key: *Number in parenthesis = percentage*

DISCUSSION

Our study indicated a positive association between malaria and typhoid fever which is more pronounced when typhoid fever was diagnosed serologically. Further analysis showed that typhoid fever and malaria were independent of the other ($P > 0.05$).

Statistical analysis of the difference in *P. falciparum* parasitaemia in relation to *Salmonella* antibody titre was significant using serological method ($P < 0.05$) and not significant using bacteriological method ($P > 0.05$). The disparity in the degree of association of *S. typhi; S. paratyphi* and *S. typhimurium* with *P. falciparum* were statistically not significant ($P > 0.05$).

Our study showed that 42.59 % of the patients may have been falsely diagnosed as having concurrent malaria and typhoid fever when serology was used alone to diagnose typhoid fever. In Cameron out of 200 patients symptomised with fever, 17% were bacteriologically and 47.9 % serologically diagnosed of having concurrent malaria and typhoid fever and 32.5 % had malaria co-existing with *S. typhimurium* (Ammah *et al.,* 1999).

In Kenya, Berkley *et al.* (1999) reported overall incidence of bacteraemia (7.8 %) in children with severe malaria and 12.0 % in children fewer than 30 months of age. There has been a reported case of concurrent falciparum malaria and *Salmonella* bacteraemia in travelers from developing countries (Gopinath *et al.,* 1995; Jensenius *et al.,* 1998; Julia *et al.*, 2000). In Nigeria, 16% of blood culture from children with cerebral malaria was positive for Gram negative bacteria (Prada et al., 1993).

This work agreed with that of Ammah *et al.* (1999) except that a very low proportion of the patients were to *S. typhimurium* positive. *S. typhimurium* which causes gastroenteritis, is usually self-limiting and 1-7 days duration.

The high prevalence of typhoid fever serologically determined as strongly associated with malaria should be unreliable since Widal test is sensitive but non-specific and salmonellae share antigens. Therefore, there may have been cross-reaction with *S. typhimurium* which in some cases account for the high prevalence of *Salmonella* antibodies in most patients.

Widal test is capable of detecting *salmonella* antibodies in vaccinated or previously exposed individuals. Widal test had been found to be positive in acute malaria patients (Jhaveri *et al.,* 1995).

In their study, it was reported that widal test was positive in 14.58 % and 10.41 % or malaria patients for *Salmonella* O and H titres respectively and on 4 weeks follow up, most of the positive test became negative; it was then concluded in their study that non-specific polyclonal B-lymphocyte stimulation due to malaria was responsible for this phenomenon. Several other explanations had been given about the malaria and typhoid fever association.

Malaria is a predisposing factor due to its heamolytic character (Kanra *et al.,* 2000). Malaria predisposes to bacterial super-infection possibly through its effect on immune responsiveness. Under special conditions of severe malaria notably heamolysis and impairment of leucocyte and macrophage function due to phagocytosis of parasite and subsequent malaria pigment accumulation, immunosuppression; the invading bacteria would proliferate leading to bacteraemia and septicemia (Prada *et al.,* 1993). Malaria and typhoid fever are tropical diseases. Poverty mal-nutrition, poor sanitary status, poor personal hygiene, poor health facilities, poor social service and low level of education are among the factors that make tropical areas disease laden. Mal-nutrition gives room to susceptibility to infection.

Most people are ignorant of the causative agents, means of transmission, spread and acquisition of some diseases. Typhoid fever is acquired from contaminated water, food, ice creams but the presence of a symptomatic carriers worsen the situation as unusual prolonged outbreak of typhoid fever from 1988 to 1994 in Terrassa (Barcelona, Spain) was caused by a causal food handler who was a carrier (Xercavins *et al.,* 1997).

Despite the fact that malaria and typhoid fever are indistinguishable regarding their clinical signs and symptoms and there are some overlaps in their pathology, *Plasmodium* and *Salmonella* are not of the same phylum, cannot share antigens nor have same method of transmission. This association has been co-incidental as both diseases are endemic in Nnewi and environs.

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