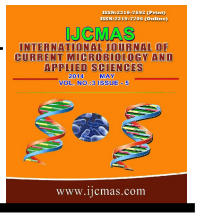
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 5 (2014) pp. 207-214**
http://www.ijcmas.com



Original Research Article

Malaria and typhoid, do they co-exist as alternative diagnosis in tropics? a tertiary care hospital experience

Shukla Snehanshu*, Pant Harshita, Sengupta Chandrim, Chaturvedi Parul, and B.L.Chaudhary

Department of Microbiology Mayo Institute of Medical Sciences,Barabanki,U.P.India.
Corresponding author

Keywords

Salmonella typhi, plasmodium parasites, Widal agglutination test, Co-infection.

A B S T R A C T

Malaria and typhoid fever are among the most endemic diseases in the tropics including India. Most cases of malaria/typhoid co-infections are based on clinical suspicion alone, so this study was carried out to determine the actual rate of co-infection of Malaria/typhoid fever and also to access the reliability of Widal test in the diagnosis of *S.typhi* .Two hundred (200) blood Samples were collected from patients with febrile illness attending teaching hospital at Mayo Institute of Medical Science Barabaki. Blood samples were subjected to microscopic examination for the identification of plasmodium parasites. Widal agglutination test was performed for the identification of antibodies to *S. typhi* and blood culture for Isolation of *S.typhi*. Of the total samples analysed, 36(18.00%) were positive for malaria, 56(28.00%) were positive for *S. typhi*, while 17(8.50%) had both typhoid and malaria, using widal agglutination test. 5(2.50%) were positive for typhoid fever by blood culture technique. The co-relational analysis has showed no specific relationship between malaria and the level of *Salmonella typhi* isolation in this research. It is therefore concluded and recommended that the assumingly high incidence of the disease will be greatly reduced if blood culture technique is routinely adopted as a base line for the diagnosis of typhoid fever. This would also reduce indiscriminate use of antibiotics without laboratory evidence that leads to drug resistant typhoid fever.

Introduction

Malaria is a protozoan disease transmitted by the bite of infected female anopheline mosquitoes. It is the most important of all tropical diseases in terms of morbidity and mortality. More than two billion people (36% of world population) are exposed to the risk of contracting malaria *al*., 2005). Each year, malaria directly causes nearly one million deaths and about 500 million clinical cases, of which 2 to 3 million constitute severe and complicated malaria (Rowe AK *et al*., 2000, Hay SI, *et al*.2004. It has recently been estimated that in India, the total DALYs (Disability

Adjusted Life Year), meaning one lost year of "healthy life" either through death or illness/disability, due to malaria were 1.86 million year (Kumar A *et al*.,2007).Typhoid fever is caused by species of Salmonella. The species and strains of Salmonella that commonly cause typhoid fever in humans are *Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B* and (Lerner and Lerner, 2003,WHO, 2003). Typhoid fever is an acute life threatening febrile illness. It has an estimated cases of about 22 million with an associated 200,000 related deaths world-wide each year (Crump *et.a,l.*, 2004).The detection of high antibody titre for Salmonella is not always indicative of current infection(s) (Samal and Sahu, 1991).

The co-infection of malaria parasite and Salmonella species is common, especially in the tropics where malaria is endemic. The common detection of high antibody titre of these Salmonella serotypes in malaria patients has made some clinicians to believe that malaria infection can progress to typhoid or that malaria always co-infect with typhoid/paratyphoid in all patients. Hence, some clinicians treat malaria and typhoid concurrently once they have high antibody titre for Salmonella serotypes, even without adequate laboratory diagnoses for malaria and vice versa.(Lerner and lerner,2003).An association between malaria and typhoid fever was first described in the medical literature in the middle of the 19th century, and was named typhomalarial fever by the United States Army (Smith;cited by Uneke1982). In the last 20 years, this relationship between malaria and salmonellae has been confirmed by additional studies from Africa that largely describe a higher incidence of non- typhoidal salmonella bacteraemia among patients with malarial parasitaemia (Bygbjerg *et al.*, 1982, Ammah *et al*., 1999).

Most cases of malaria/typhoid co-infections are based on mere assumptions or result from absolute dependability on diagnostic methods that are unreliable and non-specific, which has contributed to the assumingly high prevalence of this disease. This study was carried out in Barabaki to determine the rate of co-infection of Malaria/typhoid fever and also to access the reliability of widal test and Blood culture in the diagnosis of *S. typhi* .

Materials and Methods

Study Area

This study was carried out at Mayo Institute of Medical Science, Barabaki District of U.P. India, between July 2012 and October 2013.Barabanki region is endemic for both malaria and typhoid fever with maximum cases of malaria and typhoid being reported between month of July and November.

Study Population

The Study Population are Patients with febrile illnesses attending Teaching hospital of Mayo Institute of Medical Sciences, Barabanki, U.P, where treating clinician suspected malaria or typhoid as a differential diagnosis.

Specimen Collection

A total of 200(88 males, 112 females) blood samples were collected from patients presenting with febrile illnesses attending Mayo Institute of Medical Sciences Teaching Hospital. The samples were collected by venepuncture technique

(Carmel *et al* 1993).Blood samples were also collected from 25 apparently healthy individuals as controls. Five millilitres of blood collected by venepuncture from each person were tested for malaria parasites, Salmonella typhi O and H antibodies and also cultured for isolation of *Salmonella tyhi* Organism.). Soft tubing tourniquet was fastened to the upper arm of the patient while the puncture site was cleansed with methylated spirit (methanol) and venepunture made with the aid of a 21 gauge needle attached to a 5ml syringe. When sufficient blood had been collected, the tourniquet was released and the needle removed immediately; a large drop of the blood sample was placed on a clean, grease-free glass slide to make a thick blood film for microscopic examination while the remaining was allowed to clot and the serum obtained for serology (Widal test).

Processing of Specimens:

Blood Films: Blood for making blood films are collected into EDTA vials. A drop of blood is placed at the centre of a grease free slide, with the aids of an applicator stick. . The slide is allowed to air dried, and then stained with giemsa stain (Cheesbrough, 2005). For a thin blood films, A drop of blood is placed at one edge of the slide, another slide is placed at an angle of 45 degree at the spot where the blood is placed, with a swift movement a thin blood film is produced.

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. Films were examined microscopically for the presence of malaria parasites within red blood cells in thin films. For thick films, the ring forms, trophozoite 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.

Widal Test

Serological diagnosis was carried out using widal agglutination test on all the serum samples collected to determine the antibody titres of the sera against Salmonella H(flagella)- and O-(somatic) antigens, Commercially prepared antigen suspension (Span Diagnostic) were used. The Serological testing was done in accordance with manufacturers guidelines.

Blood culture

Blood culture was done on all those samples that were positive for both malaria and *Salmonella typhi* using blood films and widal agglutination test respectfully. The blood samples used for cultures is directly dispensed into blood culture bottle containing TSP or thioglycolate broth immediately after being collected by venepuncture from the patients with strict aseptic precautions. Since blood culture is considered to be a Gold Standard in the laboratory diagnosis of typhoid fever, isolation of *S.typhi* from the blood is truly diagnostic of typhoid fever.

Statistical analysis

The result of research was subjected to Chi-square test to determine if the relationships between the malaria parasite infection and *Salmonella typhi* are actually significant.

Results and Discussion

The result indicated 36(18%) of the total population were positive for Malaria Parasites. This further indicated that, 17 (47.22%), out of 36 malaria positive samples, were also positive for Salmonella typhi infections using widal agglutination test. The cut off points used for this study were as established Shekhar Pal *et al* (2013) **.**Table 1 Shows the distribution of malaria parasites, in relation to age/sex among patients in Barabanki The distribution pattern indicated that 30-39 age has the highest number of cases of malaria, with the female having the highest no of ten(10) infected patients. The distribution also shows that, out of the 36 positive samples, 15(17.04%) were male while 21(18.75%) were female. Table 2 shows the distribution of typhoid fever in relation to Age/Sex of patients in Barabanki. Total of 56 positive results were obtained from the analysis of 200 serum samples using widal agglutination test procedures. Male has 29(32.95%) of the positive samples while the female has 27(24.10%) of the positive patients. The highest number of typhoid fever was found in the 30-39 age group with 23 typhoid positive cases. Table3: This shows the prevalence distribution of malaria/Typhoid co-infection in Barabanki Using widal agglutination test and thick blood films. 56 blood samples were positive for widal agglutination test, while 36 blood samples were positive for Malaria parasite using thick blood films. Further analysis of the result indicated that only 17 of the total blood samples analysed were positive for both Typhoid and malaria parasites.

Despite the fact that malaria and typhoid are endemic in Barabanki, the result of this study has indicated that malaria is far more likely to caused fever than typhoid fever. Cultural diagnosis of *Salmonella typhi* has revealed the unreliability of widal agglutination test., which is basically the diagnostic procedure used in many suspected cases of typhoid fever in Barabanki and other parts of India. For an accurate and reliable diagnosis of typhoid fever, the use of Blood cultural method should highly be taken into consideration. This could be followed by stool and bone marrow (Edelman and Levine, 1986).It should be noted that bone marrow aspirate is highly difficult to obtain and culture from stool has the tendency of increasing the prevalence rate by 10-15%. This leaves the blood as an alternative and reliable method in the diagnosis of *Salmonella typhi* infection (Mbuh *et al*., 2003). In this research study, of the 200 blood samples,56 of them were positive for widal agglutination test, but the result of cultural isolation indicate that it was only 5 patients that had actually typhoid fever., Others may have malaria, brucellosis and other cross- reacting antigen. The unreliability of profile of widal agglutination test has been reported by Onuigbo(1990) and Ohanu *et al*.(2003),Mohammed *et al*.,(2010). Widal test positivity has been associated with non-typhoidal 17 fevers resulting from anamnestic reactions (Pang andPuthucheary,1983),subclinical typhoid infection in a typhoid fever endemic area(Pang and Puthucheary,1983;) ,cross-reacting antibodies produced by non-typhoidal salmonellae (Onuigbo,1990), Malaria (Onuigbo,1990; Ohanu *et al*.,2003) cirrhosis and hepatitis(Edelman and Levine 1986). This study have shown that the prevalence of typhoid fever and malaria parasite (P.falciparum) co-infection was 2.5.% using culture method and 8.5 % using widal agglutination test. This was in agreement with the work of

Table.1 Distribution of malaria parasite in relation to Age/Sex among patients in Barabanki

Age(Yrs)	Total No Tested	Male		Female		% Total Positive
		No. Tested	No. Positive	No. Tested	No. Positive	
0-9	35	14	1	21	1	2(5.71)
10-19	28	12	0	16	1	1(3.57)
20-29	35	12	4	23	5	9(25.71)
30-39	72	33	8	39	10	18(25.0)
40-49	14	9	0	5	2	2(14.28)
50-59	6	5	1	1	1	2(33.33)
>60	10	3	1	7	1	2(20.0)
Total	200	88	15(17.04%)	112	21(18.75%)	36(18.0)

Table.2 Distribution of typhoid fever patient in relation to age/sex among patients in Barabanki

Age(Yrs)	Total No Tested	Male		Female		% Total Positive
		No. Tested	No. Positive	No. Tested	No .Positive	
0-9	35	14	3	21	1	4(11.42)
10-19	28	12	5	16	2	7(25.0)
20-29	35	12	4	23	6	10(28.57)
30-39	72	33	10	39	13	23(31.94)
40-49	14	9	5	5	3	8(57.14)
50-59	6	5	1	1	2	3(50.0)
>60	10	3	1	7	0	1(10)
Total	200	88	29(32.95%)	112	27(24.10%)	56(28.0)

Table.3 Age/Sex wise distribution of malaria and typhoid fever co-infection among patients in Barabanki

Age(Yrs)	Total No Tested	Male		Female		% Total Positive
		No. Tested	No. Positive	No. Tested	No. Positive	
0-9	35	14	1	21	0	1(2.8)
10-19	28	12	0	16	0	0(0.0)
20-29	35	12	2	23	2	4(11.43)
30-39	72	33	2	39	5	7(9.72)
40-49	14	9	0	5	2	2(14.28)
50-59	6	5	1	1	1	2(33.33)
>60	10	3	1	7	0	1(10.0)
Total	200	88	7(7.95%)	112	10(8.92%)	17(8.50)

Alhassan *et al*., 2012 1.33 vs 10.33, Mbuh *et al*., 2003 and Ammah *et al*.,1999, where they had 0.5 vs 10.1% and 17 vs 47.9% prevalence using the same method as used in this research. It is pertinent to state that two malaria diagnostic approaches currently used most often, do not allow a satisfactory diagnosis of malaria. Clinical diagnosis, the most widely used approach, is unreliable because the symptoms of malaria are non-specific. Microscopic diagnosis, the established method for laboratory confirmation of malaria, presents technical and personnel requirements that often cannot be met, particularly in facilities at the periphery of the health care system. In addition, delays in the provision of the microscopy results to the clinician mean that decisions on treatment may be taken without the benefit of the results (Payne.D.1988, WHO, 2003.).

Unlike the diagnosis of malaria, typhoid fever presents a greater diagnostic challenge. Typhoid fever diagnosis is still based on clinical presentation and on diagnostic tests that are associated with numerous limitations. Blood culture, which is the gold standard for diagnosis of typhoid fever, is not routinely requested by most physicians because it is expensive and final results can only be obtained at the earliest, three days after specimen collection (Pearson and Guerrant., 2000). Although this test is highly specific, sensitivity varies from 48–78% and the yield is affected by prior antibiotic intake and stage of illness and alternative methods such as bone marrow cultures may be required even though this latter method is invasive (Gilman *et.al*., 1975). The Widal test is inexpensive and readily available in most health care settings in the tropics, but serious doubts are being raise regarding its specificity. It is now regarded as inaccurate, non-specific, poorly standardized, confusing and of limited diagnostic value (Buck *etal*., 1987, Chew, 1992).Koeleman, 1992, Choo, 1993). Cross-reactions can occur as a consequence of latent and post- infectious diseases prevalent in the tropics namely tuberculosis, pneumonia, amoebiasis, rickettsial diseases, rheumatoid arthritis and chronic active hepatitis. Koeleman, (1992). In addition, the test has to be interpreted against a baseline titer in the same geographical area since titers of diagnostic significance differ in endemic and non-endemic areas.(Buck *et al*.,1987). As a result of the diagnostic challenge associated with malaria and typhoid fever, it is very common to see patients in many parts of the tropics, undergoing both typhoid and malarial treatment even if their diagnosis has not been confirmed (Mbuh *et al*., 2003). There appears to be more typhoid fever cases in areas of drug resistant malaria and a cross-reaction between malarial parasites and salmonella antigens may cause false positive Widal agglutination test (Jhaveri *et al*.;1995, Mbuh *et al*.,2003). It seems that the outcome of the Widal reaction for patients with a clinical suspicion of typhoid and malaria depends on individual host immune responses, which become stimulated in febrile conditions associated with malaria fever. This can be accounted for by the demonstrated high prevalence of Salmonella antibodies in local healthy population and the fact that 50% of the patients had detectable levels of antibodies to the somatic antigen (Mbuh *et al*., 2003, Onuigbo, 1990).

The high prevalence of *Salmonella typhi* infections recorded in this study by the use of widal agglutination test could be attributed to haemolytic anemia and malaria parasite specific factors which

increase the susceptibility of the patients to Non- typhoidal Salmonella serotypes (NTS).as reported by Mbuh *et al*., (2003). Here it was found out that an increased risk for developing systemic NTS infection during malaria is caused by haemolytic anemia, which leads to reduced macrophage microbicidal activity.

In tropical countries like India which are endemic for both typhoid and malaria, both the disease can co-exist and difficult to differentiate on clinical suspicion alone due to overlapping clinical sign and symptoms as well as antigenic cross reactivity. The use of blood culture for the diagnosis of typhoid fever is strongly recommended. This will help improve patient's management by cutting down cost of treatment and eliminate other risks, especially drug resistance associated with misuse of antibiotics. This study does not show any specific relationship between malaria and *Salmonella typhi* isolation. The prevalence of malaria and typhoid fever co-infection in endemic areas will be greatly reduced if diagnosis of typhoid fever will be based on culture method.

References

Alhassan, H.M.,Shidali,N.N.,S.B.Manga, K.Abdullahi4,K.M.Hamid, Global Journal of Science, Engineering and Technology, Issue 2, 201, 13-20.

Ammah A, Nkuo-Akenji T, Ndip R 1999.An update on concurrent malaria and typhoid fever and in Cameroon. Trans. R.Soc. Trop.Med. Hyg., 93:127-129.

Buck RL, Escanilla J, Sangalang RP, Cabangan AB, Santiago LT. 1987 Diagnostic value of single, pre-treatment Widal test in suspected enteric fever cases in the Philippines. Trans R Soc Trop Med Hyg 1987; 81: 871–3.

Bygbjerg IC, Lanng C 1982 Septicaemia as a complication of falciparum malaria. Trans. R. Soc.Trop. Med. Hyg., 76: 705-706.

Carmel B., Kenmogne D., Copin N., Mbitsi. A. 1993. Plasmodium prevalence and parasite burden in blood donors of Brazzaville, Congo. Ann. Soc. Belg. Med. Trop. 33: 187-197.

Cheesbrough M. 2005. District laboratory practice inTropical Countries, Part 1 2nd Cambridge University Press, New York, 454.2:64-67.

Chew SK. Diagnostic value of Widal test for typhoid fever in Singapore. J Trop Med Hyg 1992; 95: 288–91.

Choo KE, Davies TME, Ismail A, Tuan Ibrahim TA, Ghazali WNW.1999 Rapid and reliable serological diagnosis of enteric fever:comparative sensitivity and specificity of Typhidot and Typhidot-M tests in febrile Malaysian children. Acta Tropica. 1999; 72:175-83.

Crump JA, Luby SP, Mintz ED 2004. The burden of typhoid fever. Bull. World Health Organ 825: 34b-53.

Elderman, R.A.and Levine, M.M.1986:Summary of international workshop on typhoidal fever. Journal Infectious Dis.8:329-349

Gilman RH, Terminel M, Levine MM, Hernandez-Menodoze P, Hornick RB. Relative efficacy of blood, urine, rectal swab, bone-marrow and rose spot cultures for recovery of Salmonella typhi in typhoid fever. Lancet 1975; 1: 1211–3.

Greenwood BM, Fidock DA, Kyle DE, Kappe SH, Alonso PL, Collins FH, *et al*. Malaria: progress, perils, and prospects for eradication. *J Clin Invest* 2008; *118:* 1266–76.

Hay SI, Guerra CA, Tatem AJ, Noor AM, Snow RW. The global distribution and population at risk of malaria: past, present, and future. *Lancet Infect Dis* 2004; *4:* 327–36.

Jhaveri K.N, Nandwani SK, Mehta PK, Surati RR, Parmar BD 1995.False positive modified Widal test in acute malaria. J. Assoc Physicians India,

43:754-755.

KoelemanJ.G1992.Retrospective Study to determine the diagnostic value of Widal test in non endemic country. Eur. J. Clin. Microbiol.Infect.Dis,pp, 167–170.

Kumar A, Valecha N, Jain T, Dash AP. Burden of malaria in India: retrospective and prospective view. Am J Trop Med Hyg 2007; 77: 69– 78.

Lerner, K.L,Lerner, B.W 2003.World of Microbiologyand Immunology, vol. 1 and 2: 5,7,25, 185-189.

Mbuh FA, Galadima M, Ogbadu L. 2003 Rate of confection with malaria parasites and Salmonella typhi in Zaria, Kaduna State, Nigeria. Ann. Afr. Med., 2:64-6

Mohammed,K.GGarba,H.S.,EgwuG.O;Agai e,B.MNata,ala,S.U,Hassan,S.,Alhassan, H.M.;Nuhu,A.M.M.Yeldu,2010:Assess ment of widal agglutination test as a diagnostic tool in Human typhoid fever, AMLSN,ASCIS,EKO 2010.

Ohanu ME, Mbah AU, Okonkwo PO, Nwagbo FS 2003. Interference by malaria in the diagnosis of typhoid using Widal test alone. West Afr. J. Med., 22: 250-252.

Onuigbo, M.A.,1990,Diagnosis of typhoid fever in Nigeria: Misuse of the widal test. Trans.R.Soc.Trop.Med.Hyg.,84

Pang T, Puthucheary S.D 1983.Significance and value of the Widal test in the diagnosis of typhoid fever in an endemic area. J Clin Pathol 1983; 36: 471–5.

Payne D 1988. Use and limitations of light microscopy for diagnosing malaria at the primary health care level. Bull. World. Health Organ.66:621-626.

Pearson R.D, Guerrant R.L 2000. Enteric fever and other causes of abdominal symptoms with fever. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases, Vedn. NewYork: Churchill Livingstone, pp. 1136-1150.

Rowe AK, Rowe SY, Snow RW, Korenromp EL, Schellenberg JR, Stein C, *et al*. The burden of malaria mortality among African children in the year 2000. *Int J Epidemiol* 2006; *35:* 691–704.

Samal K.K, Sahu C.S 1991. Malaria and Widal reaction. J. Assoc.Physicians India. 10: 745-747.[30]Smith D.C 1982a. The rise and fall of typhomalarial fever. I: origins. J.Hist. Med. Allied Sci., 37: 182-220.

Shekhar Pal, Rajat Prakash, Deepak Juyal, Neelam Sharma, Amit Rana, Sandeep Negi, .The baseline widal titre among the healthy individuals of the hilly areas in the Garhwal region of Uttarakhand, India.Journal of Clinical and Diagnostic Research [serial online]2013 03[cited:2014 Apr 6] 3 437 - 440

Smith D. C.1982. The rise and fall of typhomalarial fever.In: Uneke C. 2008 "Concurrent malaria and typhoid fever in the tropics: The diagnostic challenges and public health implications" J. Vector Borne Dis 45:133 -142.

Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature* 2005; *434:* 214–7.

Uneke C. 2008 "Concurrent malaria and typhoid fever in the tropics: The diagnostic challenges and public health implications. J. Vector Borne Dis 45:133-142.

WHO/CDC2003. Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial pathogens of Public health importance in Developing World, 103-115.