DOI: 10.7860/JCDR/2014/7745.4403

Original Article

Microbiology Section

Comparative Evaluation of Various Tests for Diagnosis of Concurrent Malaria and Typhoid Fever in a Tertiary Care Hospital of Northern India

DEEPIKA VERMA[1], SACHIN KISHORE[2], M.E. SIDDIQUE[3]

ABSTRACT

Background: Malaria and Typhoid are two major infectious diseases, still prevalent in most of the tropical countries including India. Millions of individuals residing in these endemic cases contact these diseases either concurrently or as acute infection superimposed on a chronic one.

Aim: Diagnosis and comparative evaluation of various tests for diagnosis of Typhoid-Malaria co-infection in patients suffering from febrile illness.

Materials and Methods: Around 800 patients of both Out Patient Department (OPD) and In Patient Department (IPD) were referred to microbiology lab for Widal test/Typhi dot IgG/IgM and Malaria card test between July 2012-September 2012. Patients found to be suffering from co-infection were further confirmed for typhoid by blood culture. Those patients who were found sterile on blood culture were further confirmed by stool culture. Patients positive by Malaria card test (either antibody or antigen or both) were confirmed by peripheral blood smear examination for malaria parasite by both thick and thin smear examination.

Result: 68 (8.5%) patients were found to be suffering from co-infection by the above tests. Blood culture revealed 15 (22%) bacterial pathogens in the widal positive patients out of which 6 (8.8%) were *Salmonella* Typhi and 3 (4.41%) were *Salmonella* Paratyphi A. Stool culture revealed 8 (11.7%) *S*. Typhi and 5 (7.35%) *S*. Paratyphi A cases. Out of 68 patients positive by Malaria card test, only 36 (52.94%) showed Malaria parasite in peripheral blood smear also. Thus the number of confirmed cases of co-infection was found to be only 1.6%.

Conclusion: The interpretation of Widal test and Malaria card test, when diagnosing concurrent malaria and typhoid fever, must therefore be done with a lot of caution. Negative or positive Widal agglutination test is neither definitive nor completely informative. Similarly erroneous interpretation of Malaria card test (especially Antibody detection card test) result may lead to prolonged treatment and economic burden on patient.

Keywords: Widal test, Typhidot, Malaria card test, Peripheral blood smear examination

INTRODUCTION

Malaria is the most important parasitic disease caused by an obligate intracellular protozoan parasite belonging to genus plasmodium. Out of the known five species infecting man *P.falciparum*, is the most virulent one, account for most malaria deaths [1]. According to WHO Malaria is endemic in 108 countries including India, and while parasite based diagnosis is increasing, most suspected cases of malaria are still not properly identified, resulting in poor disease monitoring and overuse of anti-malarial drugs [2].

On the other hand, typhoid fever is also a major public health problem in India. It is an acute systemic infection caused by the bacterium *Salmonella* Typhi.

Although the two infections are caused by very different agents and transmitted via different mechanisms, both diseases share rather similar symptoms like fever, headache and spleenomegaly. The severity of the two diseases is compounded by increasing drug resistance of the two aetiological agents [3].

An association between malaria and typhoid fever was first described in the medical literature in the middle of the 19th century, and was named typho-malarial fever by the United States Army [4]. Within the last few decades an unusually high number of illness have been diagnosed as malaria co-existing with typhoid fever. Both typhoid and malaria share social circumstances which are imperative to their transmission. Therefore, a person living in such an environment is at risk of contracting both these diseases, either concurrently or an acute infection superimposed on a chronic one.

In India, where facilities for culture are not usually available, diagnosis of typhoid fever is essentially based on Widal agglutination test and it has been argued that diagnosis of typhoid fever by Widal test alone is prone to error [5]. The typhoidal *Salmonella* antibodies are known to cross-react with other antigens including those from non typhoidal *Salmonella* and malaria antigens due to which the use of Widal test as a diagnostic tool in patients with malaria may lead to misleading results. False positive widal test results have been reported for patients with non typhoidal Salmonella, malaria and other immunological disorders [3].

Owing to the nonspecific nature of Widal agglutination test, and cross reactivity between anti malarial antibody and antibody against *Salmonella* Typhi co-infection with malaria and typhoid is often detected by rapid diagnostic tests. So, 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.

Definitive laboratory-based diagnosis is, thus, required to differentiate the two infections as well as detect co-infections [6]. So, the aim of the study was to find out the actual no. of cases suffering from typhoid malaria co-infection and comparatively evaluate various tests for diagnosis of typhoid malaria co-infection in patients suffering from febrile illness in Rohilkhand region of the country.

MATERIAL AND METHODS

This study was conducted in the Dept of Microbiology, Rohilkhand medical college and Hospital, Bareilly, U.P, India, during July 2012-

Sept. 2012. A total of 800 blood samples were collected (5ml of blood drawn by venepuncture) from consecutive febrile patients, both OPD and IPD, who were referred to microbiology lab for following investigations Widal test/Typhi dot IgG/IGM or both and Malaria card test for malaria parasite detection. Patients were explained about the study and their consent was taken. A brief history about the duration of fever, fever with chills and rigor, history of antibiotic intake, history of vaccination against typhoid, and history of similar fever within six months which was successfully treated and cured was recorded.

Widal test: The widal agglutination test was performed on all blood samples by rapid slide agglutination method using commercial antigen suspension (Span diagnostic kit) for the somatic O and flagellar H antigen. Titres with TH>1:160;TO>1:80 were considered significant in widal test.

Tyhidot IgG/IgM: The test was performed using Onsite rapid test (CTK Biotech) card according to manufacturer's instructions. Presence of IgG and IgM antibodies was visually detected by formation of separate colored band. Formation of IgG band or IgM band or both were considered Typhi Dot positive.

Malaria card test: Malaria card test was performed and both antigen (by J Mitra Antigen detection card test) and antibody (SD bioline Antibody detecting card test) were detected for each sample. These tests were performed according to manufacturer's instructions. The antigen detection card detects HRP-II (Histidine-rich protein II) specific to *P. falciparum* and pLDH (Plasmodium lactate dehydrogenase) pan specific to P. species in human blood sample [7]. Malaria antibody detecting card test detected all isotypes of antibody against the same antigens.

Patients found to be positive by any of the tests i.e., widal or typhidot or any malaria card test positive (antigen or antibody) tests were considered suffering from co-infection and were further tested for isolation of *S.* Typhi or Paratyphi A and B by bacteriological culture of blood and stool specimen and for confirmation of malaria a peripheral blood smear stained by Leishman's stain was prepared.

Bacteriological blood culture: A minimum of 10 ml of blood was asceptically introduced into Hi media blood culture bottle containing 50 ml of glucose broth from individuals found to be suffering from both malaria and typhoid fever by above rapid diagnostic tests. All blood culture bottles were incubated at 37°C for an initial period of 24 hrs and sub-cultured on MacConkey agar after 24 hrs,72 hrs and finally at 7th day. S.Typhi/S.Paratyphi A and B organisms were identified on the basis of standard cultural, microscopic and biochemical characterization. Inoculated blood culture media was discarded as negative if there was no growth after 7 days.

Bacteriological culture of stool: Patients in whom blood culture was found to be sterile a stool specimen was collected after 2 weeks and plated on 1. Mac conkey agar, 2. Deoxycholate citrate Agar. Another set of Mac conkey and DCA was plated after enrichment in Selenite F broth after 18-24 hrs. NLF colonies were identified by biochemical test and testing by antisera.

Peripheral blood smear for malaria parasite detection: A thick and thin smear (considered the gold standard) stained by Leishman's stain was prepared in patients found to be positive by Malaria card test (J. Mitra Ag detection card test and SD Bioline Antibody detecting card test). Leishman-stained thick and thin blood films were prepared for samples positive by card test. 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 [8].

Observation and Result

Out of 800 patients, 68 (8.5%) patients were found to be suffering from co- infection by the serological tests. These patients were further evaluated by the gold standard tests which is bacterial isolation by culture in case of typhoid fever and peripheral blood smear examination in case of malaria.

68 patients were found to be having significant titres by widal test. 44 patients were positive by Typhidot IgG/IgM [Table/Fig-1]. Blood culture showed growth in 15 samples out of which 9 confirmed *Salmonella* (6 *Salmonella* Typhi; 3 *Salmonella* Paratyphi) species by biochemical and serological test (shown in [Table/Fig-1]). The bacterial profile of other samples positive by widal test on blood culture is shown in [Table/Fig-2]. In 53 stool specimens collected, *Salmonella* Typhi was isolated in eight patients and *Salmonella* Paratyphi A in five patients. So out of remaining 53 patients positive by widal test but bacteriologically sterile on blood culture, 13(24.5%) patients were confirmed for typhoid by stool culture [Table/Fig-1]. On analysis of bacteriologically negative but serologically positive cases on follow up, nine patients with widal titre of TH-1:160; TO-1:80 and Typhi dot IgM positive (either IgM

Test	Widal Test	Typhdot (IgG/IgM) Positive (N=44)		
BLOOD CULTURE 1. S.Typhi 2. S.Paratyphi A	68 15 6 3	IgG 18 0 0	IgM 10 1 1	IgG/IgM 16 4 3
TEST	**Widal positive Blood culture negative (n=53)***	**Typhidot positive Blood culture negative (n=35) #**		
STOOL CULTURE 1.S.Typhi 2.S.Paratyphi A	53 13 8 5	18 1 4	8 1 0	9 6 1

[Table/Fig-1]: Comparative evaluation of Blood culture and Stool culture for serologically diagnosed Typhoid fever
*Stool culture was performed in only 68-15=53 blood culture sterile specimens.
#The typhidot profile of 44-9 (n=35), patients is herewith tabulated

Organism	Number (n=15)	Percentage (%)
1. Salmonella Typhi	6	8.8
2. Salmonella paratyphi A	3	4.4
3.E.coli	1	1.4
4.Enterobacter	1	1.4
5.Klebsiella	2	2.9
6.Citrobacter	2	2.9

[Table/Fig-2]: Bacteriological profile of blood culture positive cases (n=15)

History of	Number	Result Of Widal Test	Result of Typhi Dot
Previous history of Antibiotic intake	9	T H-1:160 TO-1:80	IgM (either IgM or both IgM/IgG)
History of vaccination	2	TH-1:160 TO-1:80 TA-1:160	Ig G
History of infection within 6 months period, successfully treated and cured	4	TH-1:160 TO-1:80	Ig G

[Table/fig-3]: Analysis of bacteriologically negative but serologically positive cases on follow up

Malaria Parasite species	Positive by card test		Positive in PBS (peripheral blood smear)
	Antigen detection card test	Antibody detection card test	
P.vivax	30	58	30
P.falciparum	8	10	6

[Table/Fig-4]: Comparative evaluation of peripheral blood smear for serologically (by card test) diagnosed Malaria cases

or IgM/IgG both) gave history of antibiotic therapy. This could be the probable reason of their cultures not showing growth of *Salmonella* species. These 9 (13.23%) patients were also found to be malaria positive on peripheral blood smear examination and malaria antigen card test, thus were considered amongst suspected cases of co-infection.

Also two patients with titre as shown in [Table/Fig-3] and Ig G positive on Typhi Dot gave history of vaccination with Salmonella TA vaccine and four patients with TH 1:160 and TO 1:80 [Table/Fig-3] had suffered from typhoid fever in recent 6 months but were successfully treated and cured. This may be the probable reason of significant titre in their serum by widal test.

Out of 800 patients, 58 patients were detected positive for *Plasmodium vivax* and 10 patients for *Plasmodium falciparum* by SD Bioline antibody detecting card test. The number of samples positive by Antigen detection card test and peripheral blood smear (gold standard) is shown in [Table/Fig-4]. Samples positive by antigen detection card test were also positive by antibody detecting card test. The 36 (52.9%) specimens positive by gold standard were considered true malaria positive.

Thus, when 800 patients with febrile illness were diagnosed alone by widal test/typhidot and malaria card test the prevalence of co-infection was 68 (8.5%). However, when typhoid was diagnosed by bacteriological culture and malaria confirmed by peripheral blood smear results confirmed cases of co-infection was found to be 13 (1.6%). 9 (1.12 %) patients of febrile illness were considered suspected (not confirmed) cases of co-infection. These 9 patients were typhi-dot M positive, but bacteriologically negative patients (h/o antibiotic intake) and positive for malaria by both peripheral blood smear and malaria antigen card test.

DISCUSSION

In the present study the prevalence of co-infection was found by culture methods and PBS for malaria parasite as only 1.6% as compared to 8.5% by serological methods. Our results were similar to the findings of *A. J.* Sundufu et al., [3] and C.J. Uneke et al., [4] who also have reported considerably higher rates of concurrent malaria and typhoid fever by Widal test as compared to the bacteriological culture technique from Africa. Confirmation of diagnosis of typhoid fever by bacterial culture and high number of positive cases by widal test shows the unreliable nature of Widal test, which is basically used in the diagnosis of typhoid fever in India. Widal test positivity has been associated with non-typhoid fevers resulting from anamnestic reactions, sub-clinical typhoid infection in a typhoid fever endemic area, cross-reacting antibodies produced by non-typhoid *Salmonella,* malaria, cirrhosis and hepatitis [3]. False positive widal test could also be due to high prevalence of *Salmonella* antibodies in the local healthy population. In a country like India with poor sanitation exposure to *Salmonella* occurs repeatedly by contaminated food and water resulting in detectable titres by widal test in the serum of even healthy persons. Individual host immune responses do play a role and may get stimulated in febrile conditions caused by other infectious agents. This memory response can cause false positive widal test in previously sensitised persons.(anamnestic response) [8]. This fact is evident in our study also where out of 68 patients found to be positive by widal test only six (8.82%) confirmed growth of *Salmonella* Typhi and 3 (4.41%) confirmed growth of S.Paratyphi A by blood culture. Other samples positive by blood culture revealed one (1.47%) E.coli, one (1.47%), Enterobacter, two (2.94%) Klebsiella and two (2.94%) Citrobacter species. Common O antigen in the Enterobactericea family and sharing of antigen between S.Typhi and Paratyphi A and non-typhoidal *Salmonella* may also contribute to over diagnosis of typhoid fever by widal test.

A reliable diagnosis of typhoid fever is based on culture of blood, stool and bone marrow. However, bone marrow aspirates are difficult to obtain and culture from stool sample delays diagnosis leaving blood culture as the reliable method in the absence of other alternatives [2].

Owing to the lack of facilities, cultures are not usually performed in the diagnosis of typhoid fever in India and so it is common to find patients receiving typhoid malaria treatment simultaneously since medical practitioners usually rely on a single widal test result for the diagnosis of typhoid fever. This not only leads to higher cost of treatment due to unnecessary expenditure but also exposes the patient to many side effects of antibiotic misuse.

In this study we also found, that out of 44 patients positive by Typhidot IgG/M, 26 patients showed positive IgM (either IgM or IgM/IgG both). Out of these *S*. Typhi and Paratyphi A was isolated in 17 (65.38 %) Typhidot M positive cases by culture of blood and stool specimens. Other workers like Zulfiqar ahmed et al., [9] and Sushma krishna et al., [10] have also found better clinical correlation of Typhidot M with enteric fever.

On analysis of bacteriologically negative but serologically positive cases on follow up we also found that significant titres of widal test can be present in serum of patients with recent history of infection which was subsequently treated and cured and in some patients who had been innocculated with Salmonella TA vaccine. Many patients took antibiotics before coming to hospital and do not admit this even when asked. Such patients may be bacteriologically negative and may not show high titres on widal test also. So, a confirmed diagnosis of enteric fever in such cases is difficult to arrive. Combining the culture with typhidot-M may significantly help in the diagnosis of those who have previously received antibiotics.

We also detected malaria parasite in 36 peripheral blood smears whereas 38 were positive by Malaria antigen detection card test while in 30 specimens positive by only Malaria antibody detection card test no parasite could be detected in film even by very meticulous examination. Similarly, two specimens positive for *P.falciparum* by Antigen card test were found to be negative by peripheral blood smear examination but this could be due to the fact that *P.falciparum* may remain hidden in the capillaries of internal organs and is not found in the peripheral blood. Sarita yadav et al., [11] had reported similar findings in her research paper. These two patients responded to anti malarial treatment on follow up and so were considered amongst suspected cases of co-infection. None the less, antigen detection card test was found to correlate well with gold standard and as suggested by WHO also, is a reliable, rapid and easy method of diagnosing malaria [2].

However, in the name of rapid tests some companies are manufacturing Malaria antibody detecting card test which is not reliable and leads to over diagnosis of malaria and anti malarial drug misuse. We also suggest and request physicians to advice Malaria antigen detection card test to patients rather than writing simply Malaria card test for a reliable diagnosis to be made.

CONCLUSION

Although there are so many cases of concurrent typhoid and malaria due to cross reactivity, true co-infection also exists and should be borne in mind as both typhoid and malaria infections thrive in similar social conditions.

Because typhoidal *Salmonella* antibodies are known to cross-react with other antigens including those from non-typhoidal *Salmonella* and malaria antigens, the use of Widal test and Malaria card (specially Antibody detecting card test) test as diagnostic tool in patients with malaria may lead to misleading results.

History of prior antibiotic intake, previous infection and vaccination are important before interpreting results of widal test and combining culture with typhidot M will significantly help in diagnosis of typhoid fever especially in patients who had previously taken antibiotics.

None the less, a proper protocol is still required to diagnose and treat Typho-malaria co infection in countries endemic to both these diseases.

REFERENCES

[1] Afoakwah R, Acheampong DO, BoampongJN, Baidoo MS, NwaefunaEK and Tefe PS. Typhoid-Malaria co-infection in Ghana. *European Journal of Experimental Biology*. 2011;1 (3):1-6.

[2] Duret B. Malaria rapid diagnostic test performance: results of WHO product testing malaria RDTs: round 2 (2009), Philipines, WHO.

[3] Sundufu AJ, James MS, Foday IK. Role of co-infection with malaria parasites and Salmonella Typhoid in Bo City, Southern Sierra Leone. *Public Health Research.* 2012; 2(6): 204-7.

[4] Uneke CJ. Concurrent malaria and typhoid fever in the tropics: the diagnostic challenges and public health implications. *J Vector Borne Dis.* 2008;45(2): 133-42.

[5] Olopoenia LA, King LA. Widal agglutination test - 100 years later: still plagued by controversy. *Postgrad Med J.* 2000;76:80–84 © The Fellowship of Postgraduate Medicine, 2000.

[6] Pradhan P. Co–infection of typhoid and Malaria . *Journal of medical laboratory and diagnosis.* 2011; 2(3): 22-6.

[7] Moody A. Rapid diagnostic tests for malaria parasites. *Clin. Microbiol. Rev. 2002; 15 (1): 66-78.*

[8] Mbuh FA, Galadima M and Ogbadu L. Rate of co-infection with malaria parasites and Salmonella Typhi in zaria, kaduna state, Nigeria. *Annals of African Medicine. 2003;2(2):*6-7.

[9] Bhutta ZA and Mansurali N. Rapid serologic diagnosis of pediatric typhoid fever in an endemic area: a prospective comparative evaluation of two dot-enzyme immunoassays and the widal test. *Am. J. Trop. Med. Hyg*. 1999; 61(4): 654-7.

[10] Krishna S, Desai S, Anjana VK, Paranthaaman RG. Typhidot (IgM) as a reliable and rapid diagnostic test for typhoid fever. *Annals of tropical medicine and public health*. 2011; 4(1): 42-4.

[11] Yadav S, Sharma M, Aparna, Chaudhary U. Comparative evaluation of pan-malaria antigen card test and blood smear for diagnosing malaria. *International journal of life sciences biotechnology and pharma research*. 2012;1(3).

PARTICULARS OF CONTRIBUTORS:

1. Assistant Professor, Department of Microbiology, Rohilkhand Medical College and Hospital, Bareilly, India.
2. Assistant Professor, Department of Microbiology, Rohilkhand Medical College and Hospital, Bareilly, India.
3. Professor and HOD, Department of Microbiology, Rohilkhand Medical College and Hospital, Bareilly, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Deepika Verma,
C/O Dr P.C. Verma, A74, Vaibhav, Suncity Vistaar, Pilibhit bypass, Bareilly-243006, India.
Phone: 8439803086, E-mail: dr_dipikaa05@rediffmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Sep 25, 2013**
Date of Peer Review: **Jan 23, 2014**
Date of Acceptance: **Mar 28, 2014**
Date of Publishing: **May 15, 2014**