

“Malaria And Typhoid Co-Infection In India: A Diagnostic Difficulty”

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Abstract: Malaria and Typhoid remains a leading cause of morbidity and mortality globally. Various factors predispose the co infection of typhoid and Malaria. This study was planned to determine the actual number of cases suffering from typhoid malaria co-infection in our set-up and also to evaluate the efficacy of various tests for diagnosis of typhoid malaria co-infection in febrile patients in New Delhi. Overall 3010 patients were tested for Malaria and Typhoid, out of which 2260 (75%) were males and 750 (25%) were females. Out of 60/3010 cases of blood culture positive for *Salmonella typhi*, 48/60 were also positive for malaria parasite (36 *Plasmodium vivax* and 12 *Plasmodium falciparum*) by peripheral smear examination, so the rate of co-infection by using Gold Standards for both the infections was 1.59% (48/3010). No coinfection was recorded between *S. paratyphi* and malaria. On using serological techniques for estimation of typho malaria co infection, 105 samples were positive by widal and RDT (68 *Plasmodium vivax* and 37 *Plasmodium falciparum*) and rate of co infection was found to be 3.48% (105/3010). However, on comparing results of typhi dot with RDT it was observed that 120 samples were positive for typhoid and malaria (77 *Plasmodium vivax* and 43 *Plasmodium falciparum*) both by typhi dot and RDT yielding co infection rate to be 4%.

Keywords: Typhoid, Malaria, Co infection, Serological tests.

I. Introduction

Malaria remains a leading cause of morbidity and mortality globally. An estimated 3.4 billion people around the world are at risk of malaria currently [1]. Among the 1 billion people infected each year; out of which 1-3million die due to malaria [2]. In India nearly 1.6 million cases with 400-1000 deaths occur annually [3]. Typhoid fever is a serious infection resulting in about 22 million cases and 2, 16,500 deaths annually, primarily in Asia [4]. The bulk of the burden is presented from countries like India, South and Central America and sub Saharan Africa, all with growing population and poor sanitary conditions [5, 6].

Typhoid is common in malaria affected areas. Various factors predispose the co infection which includes similar epidemiological factors such as dense population, poor hygiene /sanitation practices. Additionally malaria pathogenesis leads to exhaustion of complement factors which predisposes the patient to salmonella infection and haemolysis associated with malaria leads to deposition of intracellular iron inside the liver cells favouring the growth of intracellular pathogen like salmonella. Both typhoid and malaria share rather similar signs which lead to misdiagnosis and mismanagement resulting in either under treatment or over treatment. This predisposes to disease transmission from untreated patient to new host and further irrational use of antibiotics / anti malarial results increasing surge of drug resistance [7-14]. Hence this study was planned to determine the actual number of cases suffering from typhoid malaria co-infection in our set-up and also to evaluate the efficacy of various tests for diagnosis of typhoid malaria co-infection in febrile patients in New Delhi.

II. Material and Methods

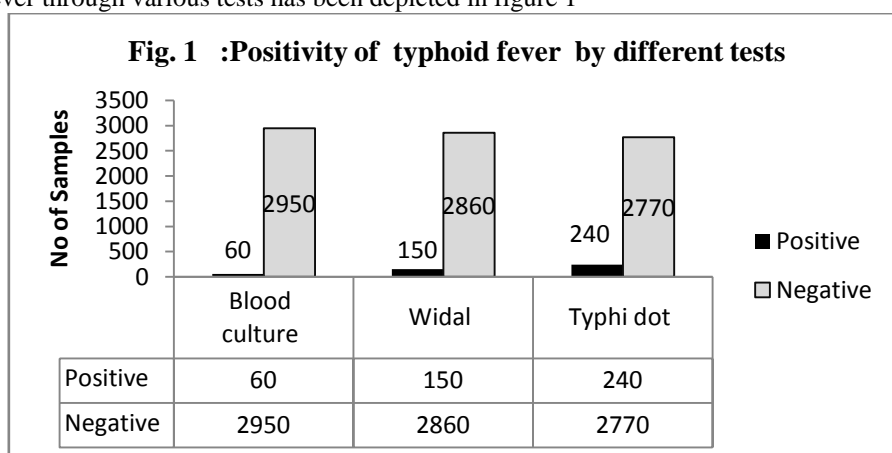
This prospective study was conducted in a tertiary care hospital, New Delhi from January 2014 to March 2016. A total of 3010 samples from the patients attending outpatient departments and admitted patients, suspected of having acute undifferentiated febrile conditions were included in the study. Patients having history of intake of anti-malarial drug or antibiotics intake, history of vaccination against typhoid were excluded. Diagnosis of typhoid fever was done by using blood culture, widal test and typhi dot test while confirmation of malaria was done by peripheral blood smear examination and rapid diagnostic test (RDT).

Briefly for blood culture 5-10 ml of blood was taken aseptically into 50 ml of brain heart infusion (BHI) broth from individuals and sub culture after 48 hours was performed on Mac Conkey agar and 5-10% of sheep blood agar (SBA). *S.Typhi/S.Paratyphi A* and *B* organisms were identified on the basis of standard cultural, microscopic and biochemical characterization. The widal agglutination test was performed on all blood samples by tube agglutination method using commercial antigen suspension (THYPHOCHECK) for the somatic

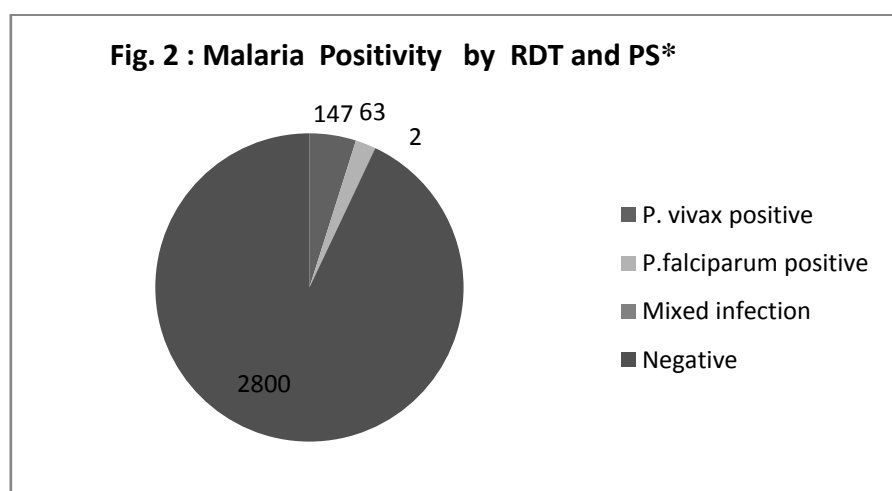
O and flagellar H antigen. Titres with TH>1:160; TO>1:80 were considered significant. Typhi dot test, which is a dot Enzyme immune assay (EIA) was performed for detection of IgM and IgG antibodies in the samples. The samples positive for IgM antibody alone or IgM and IgG both were considered positive, while samples positive for IgG alone were taken as cases of past infections. For the diagnosis of malaria, Geimsa stained thick and thin smears were examined, and rapid malaria card test was performed using commercial kit (Diya Sys Diagnosyic India Private Limited) which detects HRP-II (Histidine-rich protein II) specific to *P. falciparum* and pLDH (Plasmodium lactate dehydrogenase) and aldolase pan specific to Plasmodium species in human blood sample [15].

III. Results

Overall 3010 patients were tested for Malaria and Typhoid, out of which 2260 (75%) were males and 750 (25%) were females. The mean age was 28 ±12 years and majority of the patients (65%) were adults while 35% of the patients belonged to paediatric age group. Blood culture for typhoid bacilli was positive only in 60/3010 (1.99%) samples (52 *S. typhi* and 8 *S. Paratyphi A*), while 150/3010 (4.98%) samples showed positivity by widal test and 240/3010 (7.97%) samples were positive by typhi dot test. The details of positivity of Typhoid fever through various tests has been depicted in figure 1



The diagnosis of malaria was done using PS and RDT. Two hundred ten samples (6.97%) of 3010 were positive by RDT and 150/3010 (4.98%) by P/S. The details of the samples positive for *P. vivax* and *P. falciparum* by various tests have been shown in figure 2.



*RDT= Rapid Diagnostic Test, P/S= Peripheral Smear

Rate of co-infection was determined by Gold Standards for both the infection i.e. blood culture for typhoid and peripheral smear for malaria. The rate of co infection was also calculated taking Rapid Diagnostic Test, Typhi Dot and Widal Test combination.

Out of 60/3010 cases of blood culture positive for Salmonella typhi, 48/60 were also positive for malaria parasite (36 Plasmodium vivax and 12 Plasmodium falciparum) by peripheral smear examination, so the rate of co-infection by using Gold Standards for both the infections was 1.59% (48/3010). No coinfection was

recorded between *S. paratyphi* and malaria. On using serological techniques for estimation of typho malaria co infection, 105 samples were positive by widal and RDT (68 *Plasmodium vivax* and 37 *Plasmodium falciparum*) and rate of co infection was found to be 3.48% (105/3010). However, on comparing results of typhi dot with RDT it was observed that 120 samples were positive for typhoid and malaria (77 *Plasmodium vivax* and 43 *Plasmodium falciparum*) both by typhi dot and RDT yielding co infection rate to be 4%. The rate of typhoid malaria co infection by different combination of tests has been shown in figure 3.

Figure 3: Showing rate of co-infection by different combination of tests

	Blood culture Positive	Typhidot Positive	Widal Positive
P. vivax positive	36	77	68
P. falciparum positive	12	43	37
Total malaria positive	48	120	105
Rate of coinfection	1.59%	4%	3.48%

IV. Discussion

Malaria and typhoid fever still remain diseases of major public health importance in the tropics. Both diseases are endemic in developing countries like India. Sharing similar epidemiology, meagre hygiene the two infections are prone to infect concurrently [16]. Other clinical conditions like anaemia and haemolysis in malaria leading to iron deposition in the liver cells and complement deficiency further predisposes the salmonella infection [17].

In this present study the prevalence of co-infection rate by Gold standard technique i.e. Blood culture for typhoid and peripheral blood smear examination for malaria parasite was found to be 1.59% (48/3010). However combinations of various serological tests like Widal test for typhoid and rapid diagnostic test for malaria parasite showed co infection rate 3.38% (105/3010) and combination of Typhi dot for typhoid and RDT for malaria parasite showed co infection rate 4% (120/3010). There is paucity of data regarding the burden of co-infections from India; the literature search on “reports of co-infections from India” revealed 2 case reports, 1 from Karnataka and 1 from Andhra Pradesh in the year 2015 and 2013 [18,19], study by Shukla Snehanshu et al in the year 2014 (reported 8.5% rate of co infection), and study by Deepika verma et al 2014 which observed 1.6% of typhoid and malaria co infection by blood culture and peripheral smear examination while 8.5% by serological method [20,21]. Our findings are corroborative with the findings of these studies. Though our earlier report showed a lower co infection rate by blood culture (4%) and widal (1%) both,[22] however present study is a continuation of that study and a high no of malaria and typhoid positive cases were received in the laboratory during post monsoon months during the year 2015.

Studies from Nigeria and Ethiopia have reported co infection rate ranging from 0.5% to .8% on comparing blood culture with peripheral smear which is similar to our findings. However these studies report rate of co infection ranging from 8-28% using widal test and peripheral smear which is higher than our study and could be attributed to endemicity of Malaria in these regions [23,24]. We used Typhi dot test also to detect typho malarial co infections, which yielded co infection rate of 4%, which is higher than co infection rate detected by widal test. The possible explanation could be that thyphidot has a higher sensitivity than widal as shown by various studies [25]. Rapid Salmonella-IgM tests offer increased sensitivity, rapidity, early diagnosis and simplicity over blood culture and are widely being used for diagnosing typhoid fever. However there is a need to correlate the results of these tests with clinical profile of the patients. Since typhoid and malaria both are endemic in our country and are related to poor hygiene likelihood of co infections should always be kept in mind and reports of serological tests should be interpreted cautiously in conjunction with clinical findings.

V. Conclusion

Although co infection rates determined by serology may be due to cross reactivity however the possibility of true co infections established by blood culture and PS cannot be ignored especially considering that both the infections thrive in similar epidemiological conditions. A substantial result discrepancy is often observed among Widal test, typhi dot and blood culture for the diagnosis of typhoid fever. Hence it is important to consider history of prior antibiotic intake, previous infection and vaccination before interpreting results of serological tests. Likewise the value of peripheral smear should not be ignored when compared rapid tests and co infection should be affirmed only by using gold standards. This will help in providing accurate data on co infection and improve patient’s management by cutting down cost of treatment and eliminate drug resistance associated with misuse of antibiotics. Further, studies should be done on the other potential risk factors of malaria and typhoid fever co infection in different seasons and different study areas. The importance of improvement in sanitation and hygiene cannot be ignored to control these two important public health diseases.

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