

False Positivity of Widal Test in Malaria

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ABSTRACT

Background: Malaria is endemic in Pakistan. Malaria generally affects One billion people each year. Malaria has a mortality rate of 1–3 million out of the one billion affected. Malaria is a mosquito-borne infectious disease and it is caused by 5 species of Plasmodium. Typhoid fever is caused by *Salmonella typhi*. The Widal test was developed by F Widal in 1896 for the purpose of aiding the diagnosis of typhoid fever. It utilises a suspension of killed *Salmonella typhi* as antigen, to detect typhoid fever in the suspected *Salmonella typhi* infected patients presenting with fever. Cross reactions are seen when antibody produced by non-typhoidal antigens reacts with typhoid antigen causing false positive results. False interpretation of the test results may lead to misdiagnosis and mismanagement of the patient, causing high morbidity and mortality as the treatment for malaria and typhoid are altogether different.

Aim: To assess frequency of false positivity of widal test in malarial patients.

Study design: Case series.

Study period and settings: June 2014 – February 2015 at King Edward Medical University / Mayo hospital Lahore

Methods: The study was conducted at King Edward Medical University / Mayo Hospital LHR. All the patients who have peripheral smear positive for malarial parasite were enrolled in the study. Peripheral Smear of the patients having positive malarial parasite were reviewed and type of malarial parasite identified. Widal test, blood culture, urine and faeces culture were done.

Result: Among the 75 patients having malarial parasite on peripheral smear 10 (13.3%) had Widal test positive. Blood culture for *Salmonella typhi* was positive in only one (1.3%) of the 10 having widal positive. **Conclusion:** Investigation to rule in/rule out malaria shall always be done in patients presenting with PUO malaria and the use of the Widal test alone for the diagnose of typhoid fever should be limited to situations where no other confirmatory supportive test, such as positive culture, is available. Due to this many cases of pyrexia of unknown origin (PUO) receive the management of typhoid fever, based upon a false-positive Widal test thus resulting in underdiagnosis of malaria.

Keywords: Widal test, malaria, typhoid antigens

INTRODUCTION

Fever is very common presentation of enteric fever, malaria and other infections. Malaria is endemic in Pakistan. Malaria affects One billion people each year. Malaria has a high mortality out of the one billion affected, One–Three million die¹. Malaria is a mosquito-borne infectious disease. It is caused by five species of plasmodium. The majority of deaths are caused by *P. falciparum* while *P. vivax*, *P. ovale*, and *P. malariae* are generally associated with milder form of malaria that is usually not fatal². Malaria is an infection which is endemic in Pakistan, with the vast majority of cases caused by *Plasmodium vivax*, recently there has been a shift to infection caused by *Plasmodium falciparum* These cases are seen especially in the southern Punjab, Baluchistan and Sindh provinces. Signs and symptoms of malaria are recurrent attacks of high grade fever, moderate to severe shivering chills and profuse sweating as the fever fall. Other symptoms include vomiting, headache and diarrhea. Acute intravascular haemolysis with passage of dark red or black coloured urine is a rare and fatal complication of malaria caused by *P. falciparum*. Malaria is a multiorgan and system infection with involvement of renal, haematological and central

nervous system adding to mortality. The involvement of various systems is seen in malarial infection, irrespective of type of malarial infection although the severity of involvement may vary. Haematological manifestations include anemia, thrombocytopenia and leucopenia. Leucocyte count may sometimes be increased or normal. Typhoid fever is caused by *Salmonella typhi*³. Typhoid fever is an important cause of morbidity in many regions of the world⁴. It is transmitted by the ingestion of food or water which has been contaminated with the feces of an infected person containing *Salmonella typhi*. Symptoms include fever, malaise, headache and cough. There is hepatosplenomegaly along with leucopenia⁵. Widal agglutination test for the diagnosis of Typhoid was devised by George Fernand Isidor Widal, a French physician almost 100 years ago (1896)⁶ This test utilises a suspension of killed *Salmonella typhi* as antigen for the detection of typhoid fever in serum from suspected *S typhi*-infected patients who present with febrile illness. Cross reactions are observed when antibody produced by non typhoidal antigens, react with typhoid specific antigen causing false positive results⁷. Several other diseases caused by non-*Salmonella* organisms like (malaria, miliary tuberculosis, dengue, endocarditis, chronic liver disease (CLD), brucellosis, etc) have shown to exhibit this cross-reactivity in regions where typhoid is endemic. These cross-reactions thus increase the error rate of the result of Widal test⁸. Non-specific polyclonal B lymphocyte stimulation due to malaria is postulated to be responsible for false positivity of widal

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test⁹. Thus false interpretation of the test result may lead to wrong diagnosis and mismanagement of the patient causing major morbidity and mortality. As the treatment for malaria and typhoid are altogether different.

MATERIAL AND METHODS

The study was conducted at King Edward Medical University/Mayo Hospital Lahore from June 2014 – February 2015. All the patients both males and females who have peripheral smear positive for malarial parasite were enrolled in the study. Patients having history of intake of antibiotics within last fifteen days were excluded from the study. Peripheral smears were made from blood collected in EDTA vial and were stained by Giemsa stain. The peripheral Smears of the patients having positive malarial parasite reviewed and type of malarial parasite identified. Widal test was done using commercially prepared antigen suspensions for S.typhi O antigen and H antigen. For Widal test agglutinin titre greater than 1:80 was considered as significant infection.

For blood culture, the blood samples were directly dispensed into blood culture bottles containing TSB(Trypticase soy broth) with SPS(sodium polyanethol sulfonate). Urine culture was done on CLED agar. Stool culture was done on MacConkey's agar.

Inclusion Criteria: Both male and female patients of all ages having positive smear for malarial parasite

Exclusion Criteria: Patients having history of intake of antibiotics within last fifteen days.

RESULTS

Total 75 patients having malarial parasite on peripheral smear 10(13.3%) had Widal test positive (Fig 1). Blood culture for Salmonella typhi was positive in only one (1.3%) of the 10 having widal positive (Tab 1). Frequency of positivity of Widal test according to species of malarial parasite was as follows: From 55 cases of Plasmodium Vivax 6(10.9%) cases were positive for Widal, out of 14 cases of Plasmodium falciparum 2 (14.2%) were positive for Widal and from 6 cases of mixed infection (both P.vivax and falciparum) 2(33.3%) had Widal test positive (Tab 2).

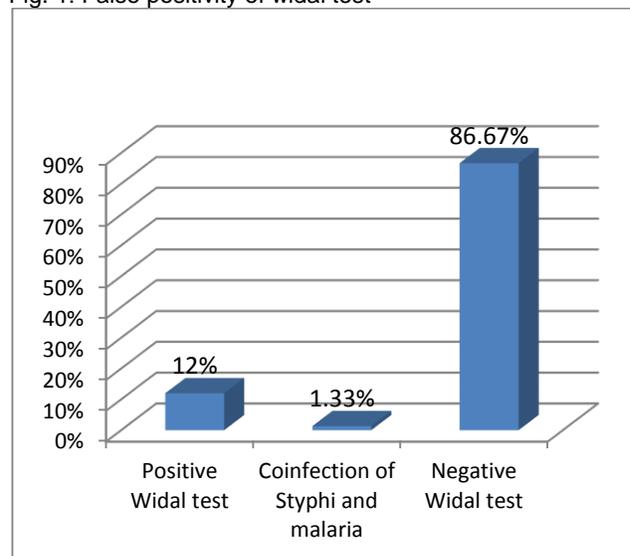
Table1: Typhoid profile

Widal Test Positive for S.typhi "O" antigen	10 (13.3%)
Blood Culture Positive for S. Typhi	01 (1.3%)
Urine and Faeces culture Positive for S. typhi	Nil

Table 2: Positive widal test in different species of plasmodium

Type of malarial parasite	n	+ve Widal Test
Plasmodium Vivax	55 (73.33%)	06(10.90%)
Plasmodium Falciparum	14 (18.7%)	02 (14.2%)
Mixed Infection (both P. Vivax and Falciparum)	06 (8%)	02(33.33%)
Total	75	10(13.33%)

Fig. 1: False positivity of widal test



DISCUSSION

Typhoid and Malaria are common causes of fever in day to day clinical practice, though caused by different etiological agents they have similar clinical presentations. Etiological diagnosis is thus very important for the proper management of these diseases. Widal test could be used judiciously in the diagnosis of typhoid fever. As It is not full proof hence bacteriological examination is invaluable. Single positive widal test can result in over diagnosis of typhoid fever. In this study, out of 75 patients having malarial parasite on peripheral smear 13.3% had positive widal test while blood culture was positive in only 1.3% of the patients having widal positive. Of the Widal positive cases 10.9% cases were of Plasmodium vivax and 14.2% were of Plasmodium falciparum. A study conducted by Ohanu showed that patients having malaria all had Widal positive with titre of more than or equal to 160 to O antigen¹⁰. A study conducted by Parkash N et al (2014) showed 18% positivity of Widal test in patients having malaria. Of the Eighteen (18) widal positive cases, Ten (77%) cases were of P. falciparum and eight (9%) cases were of P.vivax¹¹. A study conducted in Nigeria (2009) showed that 12% of patients were positive for both malarial parasite and widal test but when tested by blood culture positivity was only 0.4%¹². Onuigbo and Oguoma V.M *et al*, observed that 70% and 64.9% patients respectively who were initially diagnosed as typhoid fever on widal test results, were later on diagnosed as having malaria^{6,13}. Ammah *et al* (1999) and Ohanu ME, Mbah AU (2003) also analyzed in their study that many persons who are not suffering from typhoid infection tend to have high titres of typhoidal antibody in their serum. This could be due to poor hygiene, lack of safe water and low literacy rate. This also high lights the problem and importance of 'pure water' which plays an important role in the pathogenesis of the disease^{5,10,14}. In a study conducted in India in which of the total samples analysed, 36(18%) 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 technique¹⁵. The high level of *Salmonella* antibody giving false positive results in Widal test may be due to increasing exposure of the population to typhoid organisms at sub-clinical level or due to previous typhoid immunization. It shall also be kept in mind that quality of febrile antigen and technique affect the result of widal test, hence care should be taken to rule out malaria and other illnesses that can interfere.

CONCLUSION

Investigation to rule in/rule out malaria shall always be done in patients presenting with PUO. The use of the Widal test alone for the diagnosis of typhoid fever should be minimized to circumstances where there is no other confirmatory test, such as positive blood or stool culture, available. This also highlights the significance of blood or stool culture for diagnosis of Typhoid. Many cases of pyrexia of unknown origin (PUO) are diagnosed as having typhoid fever, on the basis of a false-positive Widal test. False positivity of the test result may lead to false diagnosis and improper management of the patient, thus resulting in morbidity and mortality related to course of illness and complications associated with malarial infection as the treatment for malaria and typhoid are altogether different and also there is indiscriminate use of antibiotics without laboratory evidence that leads to drug resistance in typhoid fever.

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