Original Article

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Retrospective Audit of the Widal Test for Diagnosis of Typhoid Fever in Pediatric Patients in an Endemic Region

Microbiology Section

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ABSTRACT

Introduction: Although typhoid fever is confirmed by culture of *Salmonella Typhi*, Widal test is widely used in India but little information exists about its reliability.

Materials and Methods: We examined the performance of Widal test in our hospital for diagnosis of typhoid fever in children. Hundred consecutive pediatric in-patients for whom, the Widal test was requested were grouped into four categories: widal positive and clinically consistent with typhoid fever (Group 1; n=42), widal negative but clinically consistent (Group 2, n=12), widal positive but not clinically consistent (Group 3, n=12) and widal negative and also not clinically consistent (Group 4, n=34). The results were analyzed by the test performance criteria, namely, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) using culture-confirmed typhoid fever cases as the "true positives".

Results: We found that 7/100 patients had culture-proven typhoid fever. Using a cut off ≥ 50 for O agglutinins or ≥100 for H agglutinins, the Widal test gave a sensitivity of 71.43%, specificity of 47.31%, and a positive predictive value of 09.25% and a negative predictive value of 95.65%.

Conclusion: The Widal test is an easy, inexpensive and relatively non-invasive but is not reliable in our set up because of a low PPV. There is a need for a more efficient rapid diagnostic test for typhoid fever.

Keywords: *Salmonella Typhi*, Serology, Diagnosis

INTRODUCTION

Typhoid fever is endemic in India and the Widal tube agglutination test which is almost 100 years old, has been widely used in the serological diagnosis of typhoid fever in India [1]. While the Widal test has played a major role in the diagnosis of typhoid fever in the past, recent technical developments have revealed several pitfalls in its use and interpretation of its result. Classically, a fourfold rise of antibody in paired sera is considered diagnostic of typhoid fever [2]. However, paired sera are often difficult to obtain and specific antimicrobial therapy is instituted on the basis of clinical suspicion alone [3]. A single Widal test results in an unvaccinated or unexposed child may have some diagnostic relevance. However, the result of a single test has no diagnostic significance in an endemic region; in part due to difficulty in establishing a steady-state or baseline titer of Widal agglutination test as repeated exposures to *Salmonella Typhi* in endemic regions is a common occurrence [4,5]. Furthermore, due to the possibility of fever from other infectious causes, false positive reactions may occur because of cross-reactivities with other non-Salmonella organisms [6,7]. Widespread use of typhoid–paratyphoid vaccine may also cause erroneous interpretation of test results [8].

This leads to an over diagnosis of typhoid fever and limits the usefulness of Widal test as a reliable diagnostic indicator of the disease process and its management in endemic countries. The diagnosis of typhoid fever on clinical grounds is difficult, as the presenting symptoms are diverse and similar to those observed with other febrile illnesses. The definitive diagnosis of typhoid fever requires the isolation of *Salmonella Typhi* from the patient.

In our country patients often receive antibiotics prior to laboratory testing due to which bacteria can be isolated from the blood cultures only in a small fraction and culture facilities may not be freely available. Evidence-based laboratory medicine tries to combat this problem of inappropriate utilization of laboratory services by combining methods from epidemiology, biostatistics, clinical and social sciences with basic sciences to evaluate the role of investigations in clinical decision making and outcomes for patients [9].

Clinical audit is an important tool for reviewing and improving the quality of service in clinical laboratories [10, 11]. Taking into considerations the above facts we present here a retrospective audit of Widal test requests in our teaching hospital which will help us in better understanding of the usefulness of Widal test as a diagnostic indicator in our hospital, which will also have a deep impact on the management of the patients.

MATERIALS AND METHODS

This is a retrospective audit of the Widal tube agglutination test carried out from August 2012 to June 2013 at the Serology Laboratory of Department of Microbiology, Maulana Azad Medical College, Delhi, India after taking ethical clearance from Institution's ethical clearance committee. This laboratory receive samples requested for Widal test from all Departments of Lok Nayak Hospital, Delhi, India which is the associated teaching hospital of the college. Hundred consecutive pediatric (upto 12 years of age) in-patients for whom, the Widal test was requested were followed up in their respective wards in the Pediatrics Department of LN Hospital, regardless of their test results. After taking informed consent from parents we reviewed their epidemiological, clinical and bacteriological data from our previous records. All these cases were being investigated for their febrile illness and information concerning the duration of illness before admission, presenting complaints, clinical signs and symptoms and personal details of each case was recorded on a performa. Clinical signs and symptoms defined by the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA in the case definition of typhoid fever were used for clinical diagnosis of typhoid fever cases [12].

Blood samples from all cases were received in plain vaccutainer without anticoagulant in the Serology Laboratory. Blood was allowed to clot in the plain vaccutainer and serum was separated by centrifugation at 2500 rpm for 10 minutes, within one to three hours of collection. Specimens were then processed by using the commercial kit (Febrile Antigen Set; Span Diagnostic Ltd.) according to the manufacturer's instructions. Appropriate positive and negative control sera were also included. The readings for the test were taken after overnight incubation at 37°C by a clinical microbiologist with three years experience. The positivity cut-off adopted for the diagnosis of enteric fever by Widal test in paediatric patients in our laboratory (1:50 for O and 1:100 for H agglutinins) was used for this audit as well. Blood samples collected aseptically from all study participants in pediatric blood culture bottles containing Brain Heart Infusion broth were sent for culture to the Bacteriology laboratory of our Department on the day of admission to the hospital before they were started on any treatment. The blood culture bottle was incubated at 37°C aerobically for 24 hours and was then sub-cultured on 5% sheep blood agar and MacConkey agar media. After 24 hours of aerobic incubation of plates at 37°C i.e., third day, the plates were examined for the growth of bacteria. The plates showing no growth of bacteria after 48 hours were again sub-cultured on the above 2 media on the fifth day. Cultured organisms were identified by biochemical and serotyping tests which is the standard protocol of our bacteriology laboratory. Enteric fever was confirmed by the isolation of *Salmonella Typhi* from the blood culture.

All pediatric study subjects were started on empirical therapy with intravenous monocef (ceftriaxone) at a dose of 100 mg/kg body weight in two divided doses per day. Their response to this drug was noted. All the study subjects were then accordingly grouped into four categories: widal positive and clinically consistent with typhoid fever (Group 1; n=42), widal negative but clinically consistent with typhoid fever (Group 2, n=12), widal positive but not clinically consistent with typhoid fever (Group 3, n=12) and widal negative and also not clinically consistent with typhoid fever (Group 4, n=34).

The results were analyzed by the test performance criteria, namely, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Sensitivity (true-positive rate) was defined as the probability that the Widal test result would be positive when blood culture confirmed that typhoid fever was present. Specificity (true-negative rate) was the probability that the Widal test result would be negative when typhoid fever was not present. The positive predictive value was the probability that typhoid was present when the test was positive, and the negative predictive value was the probability that typhoid was not present when the test was negative. The values were calculated using culture-confirmed typhoid fever cases as the true positives and those cases from which *Salmonella Typhi* were not isolated from blood culture as the true negatives.

RESULTS

A total of 100 febrile children were enrolled for this study. The mean age of our study subjects was 5.9 ± 3.36 years (Age range 6 months to 12 years). The male to female ratio was 1.27:1 (56 males and 44 females). .

[Table/Fig-1] shows the widal titers for the four study groups. In group 1 cases no convalescent phase sample was received for paired sera tests. One sample demonstrated positivity for A_H (01%).Twenty samples (out of 42) were sent within 7 days of fever and twenty-two (out of 42) were sent after the 7th day. No sample showed positivity for *Salmonella Typhi* on blood culture after 48hrs of incubation but five (out of 42) showed positivity on the 5th day subculture. Thirty-five (out of 42) patients became afebrile within 5 days of ceftriaxone administration and four patients showed response to the same beyond the 5th day. Three patients were treated with an additional antibiotic namely azithromycin to which they responded.

Samples from Group 2 children showed O and H antibody titres<50. Eleven samples (out of 12) were sent within 7 days of fever and one (out of 12) was sent after the 7th day. Two cases exhibited blood culture positivity for *Salmonella Typhi* after 48 hrs of incubation. All the patients from this group became afebrile within 5 days of ceftriaxone administration.

[Table/Fig-2]shows the widal results for Group 3 cases and the final diagnosis arrived at in these cases considering all the laboratory

Titers	All children n=100 No. (%)	Group 1 (Widal +ve & clinically consistent) n= 42 No. (%)	Group 2 (Widal -ve & clinically consistent) n= 12 No. (%)	Group 3 (Widal +ve but not clinically consistent) n=12 No. (%)	Group 4 (Widal –ve & not clinically consistent) n= 34 No. (%)
Anti TO					
≥ 400	08(8)	07(16.67)	00(0)	01(8.34)	00(0)
=200	08(8)	08(19.04)	00(0)	00(0)	00(0)
=100	10(10)	10(23.8)	00(0)	00(0)	00(0)
=50	28(28)	17(40.47)	00(0)	11(91.67)	00(0)
<50	46(46)	00(0)	12(100)	00(0)	34(100)
Anti TH					
≥ 400	25(25)	23(54.76)	00(0)	02(16.67)	00(0)
=200	05(5)	04(9.52)	00(0)	01(8.34)	00(0)
=100	24(24)	15(35.71)	00(0)	09(75)	00(0)
=50	01(1)	00(0)	01(8.34)	00(0)	00(0)
<50	45(45)	00(0)	11(91.67)	00(0)	34(34)

[Table/Fig-1]: O and H agglutinins in the study groups

S. No.	TO	TH	Clinical diagnosis
1.	=50	=100	Pulmonary kochs
2.	≥400	≥400	Chronic liver disease
3.	=50	=100	Chronic liver disease
4.	=50	=100	Pancytopenia
5.	=50	=200	Malaria
6.	=50	=100	Malaria
7.	=50	=100	Pneumonia
8.	=50	=100	Pneumonia
9.	=50	=100	Chronic liver disease
10.	=50	≥400	Kerosene ingestion
11.	=50	=100	Liver abscess
12.	=50	=100	Chronic liver disease

[Table/Fig-2]: Widal results and final diagnosis of Group 3 cases(Widal +ve but not clinically consistent) n=12

	Culture positive (Enteric fever positive)	Culture negative (Enteric fever negative)	
Widal positive	05 (True positives)	49 (False positives)	54
Widal negative	02 (False negatives)	44 (True negatives)	46
	07	93	

[Table/Fig-3]: Distribution of cases with respect to Blood Culture and Widal test results

Sensitivity	5/7(71.43%)
Specificity	44/93(47.31%)
Positive predictive value (PPV)	5/54(9.25%)
Negative predictive value (NPV)	44/46(95.65%)

[Table/Fig-4]: Performance of widal test for enteric fever diagnosis

investigations. None of these cases showed blood culture positivity for *Salmonella Typhi*. Four samples (out of 12) were sent within 7 days of fever and eight (out of 12) were sent after the 7th day. All of them were started on ceftraixone and five of these patients became afebrile within 5 days of the drug treatment.

Group 4 (Widal –ve & not clinically consistent) consisted of patients whose clinical diagnoses were various non-typhoidal infectious diseases.

[Table/Fig-3] shows the distribution of our study participants with respect to blood culture and Widal test results.

[Table/Fig-4] shows the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Widal test using culture-confirmed typhoid fever cases as the "true positives" and all other febrile children with blood culture negative for *Salmonella Typhi* as the "true negative

DISCUSSION

Widal test has been used for over a century in developing countries for diagnosis but it has been reported to have low sensitivity, specificity and positive predictive value [13,14]. We present here an assessment of the utility of Widal test in diagnosing typhoid fever in pediatric patients of New Delhi's one of the largest and the busiest tertiary care hospital.

Fifty four cases out of the hundred enrolled in our study fulfilled the requirements for CDC's case definition of typhoid fever. Out of these only 42 cases showed a positive Widal test (Group 1). Twelve cases were Widal test positive but not clinically compatible (Group 3). This serpositivity in clinically incompatible cases may be due to presence of pre existing antibodies from previous exposures to *Salmonella Typhi* infection, subclinical infection or the presence of cross reacting antibodies due to some other non typhoidal fevers. But, still all of them were started on Ceftriaxone before receiving the results of the Widal test. Blood samples for cases with a positive result in the Widal test (n=54) were sent on the day of admission to the hospital; 44.45 % (24 samples) of which were sent within 7 days of fever and 55.56 % (30 samples) beyond the 7th day of fever. The traditional view that the test is more likely to be positive in the second week of the illness is not supported by our data, although positivity for the level of T_H (p =0.0243) was more in the second week of the illness. Statistically significant differences were not observed for T_O (p =0.0912) and A_H (p =1.00) titers between the second and the first week of illness. This finding supports the conclusions of other researchers that in endemic areas the H and 0 agglutinins appear earlier in the course of illness [15,16] and is most probably attributable to an already immunologically sensitized population.

All the cases in our study were initiated on Ceftriaxone. Such an action promotes the emergence and spread of drug resistant strains in the community. *Salmonella Typhi* is showing resistance to Ceftriaxone, Azithromycin and Ciprofloxacin from different parts of the world; also Ciprofloxacin resistance is so common that it cannot be routinely used [17]. Although it would be best to initiate specific antimicrobial only after laboratory confirmation of the disease, antibiotic therapy may be initiated among patients with high suspicion of enteric fever on clinical grounds alone. Therefore the benefits of antibiotic therapy need to be carefully weighed against the disadvantage of spread of antibiotic resistant strains. Other interesting observations to be noted in our study are that the Widal test was requested in cases without clinical evidence of the disease (Group 3 & 4 cases) and that the Widal test report had no impact on patient management as the therapy was not changed in Group 2 (Widal -ve & clinically consistent) cases after the test report.

Using a cut off ≥ 50 for O agglutinins or ≥100 for H agglutinins, the Widal test gave a sensitivity of 71.43%, specificity of 47.31%, and a positive predictive value of 09.26% and a negative predictive value of 95.65%. This is in marked contrast to the findings of other investigators, who have reported a high PPV of the Widal test in children [3, 18]. However a study from Tanzania has reported similar results with low PPV and high NPV of Widal test in children [19]. It has been argued that PPV is the most important measure of a diagnostic method since it represents the proportion of patients with positive test results that are correctly diagnosed [20]. In our study a negative result had a good predictive value for the absence of disease, but a positive result had a very low predictive value for typhoid fever.

In summary the Widal test in our set up performed fairly well in terms of sensitivity and specificity but the test was only useful for excluding the disease. Considering the low cost of Widal testing and the absence of comparably cheap tests, Widal testing is likely to remain the test of choice in many developing country settings.

CONCLUSION

The diagnosis of typhoid fever on clinical grounds is difficult, as the presenting symptoms are diverse and similar to those observed with other febrile illnesses.

The definitive diagnosis of typhoid fever requires the isolation of *Salmonella Typhi* or para*Typhi* from the patient. However, since patients often receive antibiotics prior to a laboratory diagnosis, bacteria are isolated from the blood cultures in very few cases. Besides this, the unavailability of microbiologic facilities and the long waiting time for culture results (7 to 10 days) have been identified as reasons for the preference for the Widal test.

A high rate of false positives results in over diagnosis of typhoid fever leading to a worsening of antibiotic resistance in the country. Therefore the results of this test must be interpreted with caution taking into account the patients clinical details and history of vaccination etc. thus emphasizing the importance of laboratory clinic communication. However a negative Widal test result in a child with clinical symptoms consistent with typhoid fever is useful for excluding the presence of disease and exempting them from unnecessary specific antimicrobial therapy as the negative predictive value of the test is high. Some other important questions which need to be addressed are that what should be the basis for requisition of the Widal test in an endemic region – clinical criteria laid down by the CDC or fever alone and What should be the basis of starting empirical specific antimicrobial therapy- clinical suspicion or laboratory confirmation and How does the Widal test report alter patient management in Widal test negative cases who have already been started on empirical Ceftriaxone based on clinical grounds?

A highly specific and sensitive diagnostic test is therefore urgently required to contribute to better health in endemic resource poor settings where access to highly trained laboratory workers with adequate time is rare.

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REFERENCES

[1] Pal S, Prakash R, Juyal D, Sharma N, Rana A, Negi S. The baseline widal titre among the healthy individuals of the hilly areas in the garhwal region of uttarakhand. *India. J Clin Diagn Res.* 2013; 7(3):437-40.
[2] House D, Chinh NT, Diep TS, Parry CM, Wain J, Dougan G, et al. Use of paired serum samples for serodiagnosis of typhoid fever. *J Clin Microbiol*. 2005; 43(9): 4889-90.
[3] Kulkarni ML, Rego SJ. Value of single widal test in the diagnosis of typhoid fever. *Indian Journal of Paediatrics*. 1994 Nov; 31(11): 1373-6.
[4] Olopenia LA, King AL. Widal agglutination test-100 years later: still plagued by controversy. *Postgrad Med J*. 2000; 76: 80–4.
[5] Levine MM, Grados O, Gilman RH, Woodward WE, Solis-Plaza R, Waldman W. Diagnostic value of the Widal test in areas endemic for typhoid fever. *Am J Trop Med Hyg*. 1978; 27(4):795-800.
[6] Reynolds DW, Carpenter L, Simon WH. Diagnostic specificity of Widal's reaction for typhoid fever. *JAMA*. 1970; 204: 2192-3.

[7] Pang T, Puthucheary SD. Significance and value of the Widal test in the diagnosis of typhoid fever in an endemic area. *J Clin Pathol.* 1983; 36(4): 471-5.
[8] Wicks ACB, Holmes GS. Davidson L. Endemic typhoid fever: a diagnostic pitfall. *Q J Med*. 1971; 40: 341-54
[9] Horvath AR. Evidence-based laboratory medicine. *Orv Hetil.* 2003; 144(18): 869-76.
[10] Ally SH, Ahmed A, Hanif R. An audit of serological tests carried out at clinical laboratory of Ayub Teaching Hospital, Abbottabad. *J Ayub Med Coll Abbottabad.* 2005; 17(2): 75-8.
[11] Bonini P, Plebani M, Ceriotti F, Rubboli F. Errors in laboratory medicine. *Clin Chem*. 2002; 48(5): 691-8.
[12] Case definitions for infectious conditions under public health surveillance. Centers for Disease Control and Prevention. *MMWR Recomm Rep*. 1997; 46(RR-10): 1-55.
[13] Sherwal Bl, Dhamija RK, Randhawa VS, Jais M, Kaintura A, Kumar M. A comparative study of *Typhi*dot and Widal test in patients of Typhoid fever. *JIACM.* 2004; 5:244-6.
[14] Begum Z, Hossain Md A, Shamsuzzaman AKM, Ahsan Md M, Musa AKM, Mahmud Md C, et al. Evalutaion of *Typhi*dot(IgM) for Early Diagnosis of Typhoid fever. *Bangladesh J Med Microbiol.* 2009; 3:10-3.
[15] Saha SK, Ruhulamin M, Hanif M, Islam M, Khan WA. Interpretation of the Widal test in the diagnosis of typhoid fever in Bangladeshi children. *Ann Trop Paediatr*. 1996; 16(1): 75-8.
[16] Senewiratne B, Senewiratne K. Reassessment of the Widal test in the diagnosis of typhoid. *Gastroenterology*. 1977; 73: 233-6.
[17] U. S. Department of Health and Human Services, Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States 2013, Available from:http://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508.pdf.
[18] Noorbakhsh S, Rimaz S, Rahbarimanesh AA, Mamishi S. Interpretation of the Widal test in Infected Children. *Iranian J Publ Health*. 2003; 32(1); 35-7.
[19] Ley B, Mtove G, Thriemer K, Amos B, von Seidlein L, Hendriksen I, et al. Evaluation of the Widal tube agglutination test for the diagnosis of typhoid fever among children admitted to a rural hospital in Tanzania and a comparison with previous studies. *BMC Infect Dis*. 2010;10:180.
[20] Altman DG, Bland JM. Diagnostic tests 2: Predictive values. *BMJ.* 1994; 309:10.

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