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Original Article

A comparative study of Widal test with blood culture in the diagnosis of typhoid fever in febrile patients

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Abstract

Background: Typhoid fever is a major health problem in developing countries and its diagnosis on clinical ground is difficult. Diagnosis in developing countries including Bangladesh is mostly done by Widal test. However, the value of the test has been debated. Hence, evaluating the result of this test is necessary for correct interpretation of the result. Objective of the study was to compare the result of Widal test with Blood Culture in the diagnosis of typhoid fever in febrile patients in Dhaka National Medical Institute Hospital.

Methods: Blood samples were collected from 270 febrile patients with symptoms clinically similar to typhoid fever and visiting Dhaka National Institute Hospital from December 2015 to April 2016. Blood culture was used to isolate S.typhi andS.paratyphi. Slide agglutination test and tube agglutination tests were used for the determination of antibody titer. An antibody titer of $\geq$1:80 for anti TO was taken as a cut of value to indicate recent infection of typhoid fever.

Results: One hundred and eighty six (68.9%) participants were females and eighty four (31.1%) were males. 7 (2.6%) cases of S. typhiand 4 (1.5%) cases of S. paratyphi were identified with the total prevalence of typhoid fever 4.1%. The total number of patients who have indicative of recent infection by O antigen is 160 (59.2%). The sensitivity, specificity, positive predictive value(PPV) and negative predictive value(NPV) of Widal test were 71.4%, 41.06%, 3.1% and 98.1% respectively.

Conclusions: Widal test has a low sensitivity, specificity and PPV, but it has good NPV which indicates that negative Widal test result have a good indication for the absence of the disease.

Keywords: Widaltest , Blood culture, Typhoid Fever.

Background

Typhoid fever is a systemic prolonged febrile illness caused by certain Salmonella serotypes including Salmonella typhi, S. paratyphi A, S. paratyphi B and S. paratyphi C. Human beings are the only reservoir host for typhoid fever, and the disease is transmitted by faecally contaminated water and food in endemic areas especially by carriers handling food. The World Health Organization (WHO) estimates about 21 million cases of typhoid fever with >600,000 deaths annually. The cases are more likely to be seen in India, South and Central America, and Africa i.e. in areas with rapid population growth, increased urbanization, and limited safe water, infrastructure and health systems.[1,2]

Accurate diagnosis of typhoid fever at an early stage is important not only for diagnosis of etiological agent, but also to identify individuals that may serve as a potential carrier, who may be responsible for acute typhoid fever outbreaks.[3] Options for the diagnosis of typhoid fever are clinical signs and symptoms, serological markers, bacterial culture, antigen detection and DNA amplification.[4,5] Blood, bone marrow and stool culture are the most reliable diagnostic methods but they are expensive techniques and some bacterial culture facilities are often unavailable.[6,7,8] In many countries including Bangladesh, the Widal test is the most widely used test in typhoid fever diagnosis because it is relatively cheaper, easy to perform and requires minimal training and equipment.[9,10]

Although Widal test has been in use for more than a century, the value of the test to diagnose typhoid fever has been debated for as many years as it has been

available.[11] It relies classically on the demonstration of a rising titer of antibodies in paired samples 10 to 14 days apart. In typhoid fever, however, such a rise is not always demonstrable, even in blood culture-confirmed cases.[11] In addition, Interpreting the test has been such a problem that different cut-offs have been reported from different places.[9,12] Furthermore, patient management cannot wait for results obtained with a convalescent-phase sample. For practical purposes, a treatment decision must be made on the basis of the results obtained with a single acute-phase sample.[7,13] So evaluating the result of a single Widal test is necessary for correct interpretation.

This study was carried out to evaluate the value of a single acute-phase Widal test result by blood culture for the diagnosis of typhoid fever in febrile patients in Dhaka National Medical Institute Hospital, Dhaka, Bangladesh in collaboration with other renowned laboratories in Dhaka City.

Study area and period: The study period was 1st December 2015 to 30th April 2016. The study was conducted inDhaka National Medical Institute Hospital,the largest private Medical College Hospital in Bangladesh, located in old Dhaka which is densely populated area in capital city.

Study design and patient population

A prospective study on febrile patients was conducted in which patients were screened for typhoid fever and suspected patients were enrolled in the study, then blood samples were collected and tested for confirmation of the disease. Patients were screened by their physician for the clinical symptom of typhoid fever which is fever of 2 or more days before admission accompanied by other clinical symptoms of typhoid fever in the absence of any other known febrile illnesses. Febrile patients whose presumptive clinical diagnosis were typhoid fever sent to the laboratory by their physician for Widal test were included in the study. However, those febrile patients who had received antibiotic treatment for their symptom within two weeks before coming to the hospital and those who diagnosed for other known febrile illness were not included in this study. By using these inclusion and exclusion criteria 277 suspected febrile patients were recruited for this study then data and blood sample were collected and analysed from270 patients.

Blood sample collection and inoculation

Using a sterile syringe and needle, about 8–10 ml of blood collected from each study subject,then dispensed into the culture medium bottle containing 45 ml of Tryptic Soya broth (OXOID, England) and then incubated at 37°C.

Sub culturing and biochemical identification

After 24 hours' incubation sub-culturing was performed from the Tryptic Soya broth on XLD agar (OXOID, England). After overnight incubation, sensitivity test was done for positive cultures while negative broth cultures were incubated for seven days and sub cultured before reported as negative. Suspected colonies obtained on the above media were screened by biochemical tests using Triple Sugar Iron agar (TSI) (BBL™), citrate utilization test, motility (Difco™), urease test (Himedia ltd. India) and lysine decarboxylation (LDC) [Difco™] test.

Widal test

Qualitative slide agglutination and semi quantitative tube agglutination (titration) were performed using febrile antigen kits of Salmonella typhi (Chromatest Febrile Antigens kits, Linear chemicals, Barcelona, Spain). The slide agglutination test is used as a screening test for the presence of anti TO antibodies in the patient's serum. For the slide agglutination test a drop of Salmonella typhi O antigen is added on a drop of serum on card and rotated at 100 rpm for one minute and reported as reactive or non-reactive. For those slide agglutinations whose results are reactive and weakly reactive titer was determined. In the tube agglutination test (titration), serum sample was serially diluted by using fresh 0.95% saline preparation from 1:20 to 1:640 for anti TO separately in 12 test tubes. Then a drop of O antigens are added in the test tubes, equal amount in all. Based on the manufacturer manual, an antibody titer of 1:80 and higher for anti TO antibodies were taken as a cut of value to indicate recent infection of typhoid fever.

Quality controls

Standard operational procedures were followed during processing of each sample and all the instruments used for sample processing were checked every morning for proper functioning. E.coli ATCC 25922 was used as a reference strain.

Data analysis

Statistical software package (SPSS Version 16) was used for the analysis of the data. Sensitivity, Specificity, Positive Predictive Value (PPV and Negative Predictive Value (NPV) were calculated for Widal test.

Results

Although 277 febrile patients from the hospital involved in the study, data from 270 patients (where 68.9% patients were female) were analysed, because three missed due to insufficient serum samples to perform Widal test, other three missed due to incomplete sociodemographic data, and one missed due to both insufficient serum sample and incomplete sociodemographic data. The study participants' age ranged from 15–80 years (M = 35.82 ± 12.4 [SD]) and most of them were 15–40 years (94.3%).

Qualitative slide agglutination Widal test

Qualitative slide agglutination Widal test was performed in the hospital laboratory as a primary screening test of serum for presence or absence of the O antigen of S.typhi. Slide agglutination reaction for O antigen showed that 127 (47.0%) of the patients had reactive agglutination result. 110 (40.7%) patients had non-reactive reaction result for O antigen of Salmonella typhi (Table-I).

Table-I: Qualitative slide agglutination reaction results of Widal test of febrile patients suspected of typhoid fever in Dhaka National Medical Institute Hospital

Reaction result	O antigen	
	Frequency	(%)
Reactive	127	(47.0)
Weakly reactive	33	(12.5)
Non reactive	110	(40.7)
Total	**270**	**(100.0)**

Semiquantitative tube agglutination test (titration)

Titer was performed for those patients whose slide agglutination test result indicated reactive and weakly reactive reactions. One hundred sixty 160 (59.3%) patients had reactive and weakly reactive reaction for anti TO antibody. The frequency distribution of titration result is presented on Table-II.

Table-II: The frequency distribution of semi quantitative tube agglutination titration test of Widal test in febrile patients suspected of typhoid fever in Dhaka National Medical Institute Hospital

Titer	O antigen		
	Frequency	% (n = 160)	% from total (n = 270)
No agglutination	40	25.0	14.8
1:20	32	20.0	11.9
1:40	15	9.4	5.6
1:80	42	26.3	15.6
1:160	21	13.0	7.8
1:320	6	3.8	2.2
1:640	4	2.5	1.5
Total	160	100	59.3

Serum from 40 (25.0%) patients with reactive (12/40) and weakly reactive (28/40) reaction of slide agglutination for anti TO antibody did not show any agglutination in tube agglutination titration test. Among those who had agglutination reaction results, 42 (15.6%) had titer of 1:80 for O antigen.There were only 4 (1.5%) patients whose titer of O antigen was 1:640 and higher.

Antibody titer of 1:80 for O antigen was taken as cut of values to indicate recent typhoid infection (positive titer). Taking O ≥ 80 as a cut of value, we found 73 (27%) patients had indicative of recent typhoid infection. The total number of patients who had indicative of recent infection by O antigen is 88 (32.6%). Among these, 20 (7.4%) patients had antibody titer indicative of recent infection by O (≥1:80) antigen tests.

The agreement between qualitative slide agglutination and semi quantitative tube agglutination test (titration) indicates that there was a moderate agreement between slide agglutination test and tube agglutination titer for O antigen (Kappa = 0.406). In doing these, weakly reactive slide agglutinations reactions were considered as reactive because their titer was determined.

Blood culture

Of the 270 blood cultures, only 7 (2.6%) S. typhi were isolated from the patients while S. paratyphi were identified from 4(1.5%) patients. The blood cultures of 51 (18.9%) patients' were positive for bacteria other than salmonella species (Table-III).

Table-III: The distribution of blood culture results of febrile patients suspected of typhoid fever in Dhaka National Medical Institute Hospital

Bacteria	Number of isolates (%)
S. typhi	7 (2.6)
S. paratyphi	4 (1.5)
Non typhoidal salmonella	7 (2.6)
Other bacteria	51 (18.9)
Negative blood culture	201 (74.4)
Total	**270 (100.0)**

Based on the above results of Widal test and blood culture for Salmonella typhi and Salmonella paratyphi, an evaluation of Widal titration results for the diagnosis of typhoid fever was performed for O (≥1:80) (Table 4 and 5).

Table-IV:The distribution of anti TO titers among culture positive febrile patients in Dhaka National Medical Institute Hospital.

Intensity of pain	Culture positive	Culture Negative	Total
Test positive (Widal test)	5	155	160
Test Negative (Widal Test)	2	108	110
Total	**7**	**263**	**270**

Table-V: The sensitivity, specificity, PPV, and NPV of titers of anti TO (≥1:80) Widal tests for diagnosis of typhoid fever from febrile patients in Dhaka National Medical Institute hospital

Measurement	O antigen (%)
Sensitivity	71.4
Specificity	41.06
PPV	3.1
NPV	98.1

Anti TO agglutination titer of 1:80 and higher were detected among 5/7 (71.4%) of culture confirmed typhoid cases byS. typhi as compared with 2/4 (50%) of S. paratyphi and 3/7 (42.9%) of nontyphoidal salmonella. 46 (27%) patients with a negative blood culture result had a positive Widal titer of anti TO. The antibody titer of culture confirmed typhoid fever caused by S. typhi is presented in Table-V. The overall patients which have positive titer for O antigen and culture confirmed typhoid fever cases were presented on Figure-I.

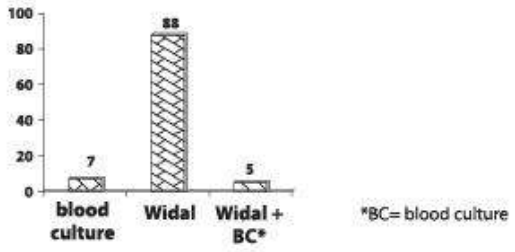

Figure-I: Diagnostic result of typhoid fever by blood culture and Widal titration of febrile patients suspected of typhoid fever in Dhaka National Medical Institute Hospital, December 2015- April 2016. *BC= blood culture.

Discussion

The sensitivity and specificity of Widal titer of anti TO 1:80 and higher in this study were about 71.4% and 41.06% respectively. This is similar with the study conducted in the endemic area of Vietnam by Olsen et al. for the evaluation of serodiagnostic assay of acute enteric fever.[14]

Another study done in Kenya has shown that Widal testing done on acute phase serum of patients suspected to have typhoid fever had limited diagnostic capability given its low sensitivity in which among all typhoid cases only 26% had diagnostic titer while 53.6% had O titer less than 1:4015.With the cut off value of anti TO ≥1:80 Widal titer in this study, Widal test had relatively good NPV (98.9%), but PPV was very low (5.7%).Positive predictive value is more important than other measure of clinical diagnostic methods because it gives the proportion of patients with positive test results that are correctly diagnosed but it is highly affected by a prevalence of the disease. In this study only 7 (2.5%) had culture proven febrile typhoid fever. So a negative Widal test result has a good predictive value for the absence of the disease but a positive result would have a low predictive value for the presence of typhoid fever.[9]

A similar study conducted in Egypt indicates that a negative result of Widal test would have a good predictive value of the disease (NPV=98%) but positive result would have a very low predictive value for typhoid fever (PPV = 5.7%).[16] Low sensitivity for Widal test may also be related to the data collection time. In this study Widal test was performed just at the admission of the patient in the hospital.False positive results of Widal titer were so high in this study (PPV = 5.7%). These false positive results may be associated with cross reacting antibodies from serum of febrile patient other than typhoid fever.

In a study conducted in Cameroon to study the prevalence of typhoid fever of febrile patients with clinically compatible symptom of typhoid fever, 45% of the patients has the true diagnosis of malaria but only 2.5% of the patients had culture proven typhoid fever4.On the other hand, the presence of Widal agglutination under condition of negative malaria smear, negative S. typhi culture and without prior immunization against typhoid suggests that other infections may also share common antigenic determinant with S. typhi.[11] Typhus, C. neoformance meningitis, immunological disorder and chronic liver disease are best example for this.[5]

A similar study conducted in Nigeria in apparently healthy students indicates a higher significant titer of antibody for anti TO antibody of S.typhi.[17] This may

have two negative outcomes in the patient and also in the community. One is that patients are treated (mismanaged) for salmonella having another febrile disease which in turn results in the development of drug resistance.[18] The other is the highly fatal disease of febrile illness such as malaria, non typhoidal salmonellosis, endocarditis and urinary tract infection might be missed.[5]

False negative results were also found in our study. 2 cases among seven culture confirmed typhoid fever cases had a negative titer. The false negative Widal test results were there probably because blood was collected too early in the disease processes, or inoculated bacterial load is inadequate to induce the antibody production.[11] Previous antibiotic treatment may also contribute to negative Widal agglutination test but there was no patient who explained taking antibiotic within two weeks before coming for the diagnosis during this study.

The positivity of slide agglutination and tube titration in this study was about 49.3% and 38% respectively. Similar positive results were obtained by slide agglutination reaction. Statistically there was moderate agreement (kappa = 0.406) between slide agglutination and tube agglutination titer of anti TO. A study conducted in Jimma, south-western Bangladesh, indicated fair agreement (kappa = 0.225) for anti TO.[19] The current study was conducted in febrile patients while Mamo and his colleagues conducted on healthy population, and this could be one reason for the agreement differences. But still the agreement of slide agglutination and tube titration was very low.

The slide agglutination test is rapid and is used as a screening procedure. An initial positive screening test requires the determination of the strength of antibody. But in many developing countries where the disease is endemic a laboratory professional performs the test, makes diagnosis and reports as positive or negative (reactive and non-reactive).[11] This is also the case of Dhaka National Medical Institute Hospital where this study was conducted. Normally the result of Widal test should be reported as either of 'agglutination' or 'no agglutination' and if agglutination is present, in titers (1:20, 1:40…) rather than in reactive or non-reactive terms. This type of reporting may be misleading and contribute to the incorrect interpretation of the test result by the physicians.[11]

In addition to S.typhi and S.paratyphi other bacteria were identified from blood culture of the febrile patients who had positive or negative Widal titer. In the current study seven (2.6%) cases of non typhoidal salmonella and 51 (18.8%) cases of other bacteria were identified from blood culture. The result of non typhoidal salmonella (2.6%) was similar to a study done in Tanzania; the study identified 2.9% of non typhoidal salmonella from blood culture.[9,20] Positive Widal titers were also seen in cases of nontyphoidal salmonella and in blood culture positive cases for other bacteria. 3 out of 7 (42.9%) of nontyphoidal salmonella cases and 17 of the 51 (33.3%) other bacteria positive cultures had a positive titer of anti TO.[21]

Conclusion

The qualitative slide agglutination tests had a moderate agreement with standard tube agglutination test (titration). Therefore, laboratories should perform the standard laboratory procedure of Widal test and follow the standard reporting instead of in 'reactive' and 'non-reactive' terms. The sensitivity, specificity, PPV and NPV of Widal test were 71.4%, 41.06%, 3.1% and 98.1% respectively. A high antibody titer development is also seen in nontyphoidal febrile infections. In addition,Widal test in the laboratory should also be performed using O antigen of S.paratyphi A, S.paratyphi B and S.paratyphi C. Nevertheless, using Widal test as the only laboratory test for the diagnosis of typhoid fever will result in misleading diagnosis.

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