Value of the single Widal test in the Diagnosis of Typhoid fever.

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Abstract
The value of a single Widal test was evaluated on 100 clinically suspected typhoid fever cases and 40 age-sex matched controls, in the Department of Microbiology, Mymensingh Medical College during the period from July 2006 to June 2007. Blood culture and Widal test with rising titre were performed in all patients. Among 100 clinically suspected of typhoid fever cases, 35 were subsequently confirmed on the basis of positive blood culture for *S. typhi* and/or significant rising titre of Widal test. The sensitivity, specificity, positive and negative predictive value of a single Widal test was found as 42.85%, 85.00%, 71.42% and 62.96% respectively. Thus, an elevated levels of agglutinating O and H antibodies as measured in a single Widal test might be helpful in making a presumptive diagnosis of typhoid fever if interpreted with care.

Key words: Blood culture, Widal test, Typhoid fever

Introduction
The signs and symptoms of uncomplicated typhoid fever are non-specific and an accurate diagnosis on clinical grounds alone is difficult¹. Although a definitive diagnosis can be made by isolation of *Salmonella typhi* from blood or bone marrow², in areas of endemicity such as Bangladesh, blood cultures facilities are often unavailable and the Widal test is the only diagnostic investigation tool available. The Widal test has been in use for more than a century as an aid in the diagnosis of typhoid fever³,⁴. Widal test, which measures agglutinating antibodies against the polysaccharide, somatic ‘O’ and protein flagellar ‘H’ antigens of *Salmonella typhi*. The value of the test for the diagnosis of typhoid fever has been debated for as many years as it has been available⁵,⁶,⁸. There is no consensus concerning diagnostic criteria for interpreting the test. Serological diagnosis relies classically on the demonstration of a rising titers of antibodies in paired samples 10 to 15 days apart. In typhoid fever, however, such a rise is not always demonstrable, even in blood culture confirmed cases. This situation may occur because, the acute-phase sample was obtained late in the natural history of the disease, because of high levels of background antibodies in a region of endemicity, or because in some individuals the antibodies response is blunted by the early administration of an antibiotic⁷. Furthermore, patient management can not wait for results obtained with a convalescent–phase sample. For the practical purposes, a treatment decision must be made on the basis of the results obtained with a single acute-phase sample.

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However, its usefulness in terms of specificity and sensitivity as compared to blood culture and paired Widal test has not been studied so far in our region. In this study, compared with blood culture and Widal test for diagnosis of typhoid fever.

**Methods**

This cross-sectional comparative study was carried out in the Department of Microbiology, Mymensingh Medical College, Mymensingh, Bangladesh during one year (from June 2006 to July 2007). Blood samples from patients clinically suspected to have typhoid fever were collected for both culture and Widal test. One blood sample was collected from each patient in the first time for culture and Widal test and a second sample was collected from the patient 7-10 days after collection of the first blood sample to see rising titer of Widal test.

Blood culture was done by conventional or traditional method using trypticase soya broth (TSB) with sodium polyanethol sulfonate (SPS). Any isolated bacteria were identified according to the recommended standard protocol.

Widal agglutination test was done by rapid slide titration method, using murex reagents (Murex Biotech limited, UK) containing ‘O’ (somatic) and ‘H’ (flagellar) antigens of *Salmonella typhi* with serial dilutions of sera beginning at 1:80. In case of patients paired samples were tested when second sample were available and in all cases 1st serum samples were tested. In controls single sample were tested. Widal results were expressed as the inverse of the highest dilution expressing agglutination. The test was carried out as per manufacturer’s instruction.

1. Using a 0.2 ml pipette, 0.08, 0.04, 0.02, 0.01 and 0.005 ml of undiluted serum was delivered into a row of 3 cm diameter circles on white tile.
2. Using a dropper one drop of appropriate well-shaken antigen suspension (TO, TH, AO, BO, AH, BH) was added to each serum aliquot.
3. Mixed by stirring for a few seconds with a wooden applicator stick, proceeding from the mixture containing 0.005 ml serum to that containing 0.08 ml serum, spreading the contents to fill the circles.
4. The tile was rotated slowly and agglutination was read at one minute.

The reactions obtained are equivalent to those which would occur in a tube agglutination test with the serum dilutions of 1/20, 1/40, 1/80, 1/160 and 1/320 respectively.

This cross sectional study included 100 cases having clinical suspicion of typhoid fever. The study population was divided into four groups. Group I, culture positive typhoid fever (n=14), Group II culture negative typhoid fever cases (n=21), serum samples of patients in whom the diagnosis of typhoid fever was based on clinical suspicion and significant rising antibody titre in paired sera by Widal agglutination test but without confirmation by isolation of *S. typhi* from blood. Group III non-typhoidal febrile illness (n=20) and Group IV healthy individuals (n = 20).

**Results:**

Out of 100 cases, 14 were culture positive for *Salmonella typhi* and 62 were Widal test positive and rest of 24 were clinically diagnosed typhoid fever but blood culture and Widal test negative (Table 1).

<table>
<thead>
<tr>
<th>Category</th>
<th>No. of individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood culture positive</td>
<td>14</td>
</tr>
<tr>
<td>Positive Widal test on first sample</td>
<td>41</td>
</tr>
<tr>
<td>Significant Widal test as per rising titer among culture negative cases</td>
<td>21</td>
</tr>
<tr>
<td>Clinically suspected typhoid but both blood culture and Widal test negative</td>
<td>24</td>
</tr>
</tbody>
</table>

* Significant rising titer of TO & TH ≥ 1:160

Out of 100 clinically diagnosed typhoid fever cases 14 were blood culture positive for *S. typhi* and 41 were Widal test positive on first instance (Table2).
Table 2: Comparison of Blood culture, and Widal test on first sample among clinically suspected typhoid cases.

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood culture (n=100)</td>
<td>14 (14.00)</td>
<td>86 (86.00)</td>
</tr>
<tr>
<td>Widal test (n=100)</td>
<td>41 (41.00)</td>
<td>59 (59.00)</td>
</tr>
</tbody>
</table>

Figures in parenthesis indicate percentage. 
(p< 0.001 by Chi-square test)

The widal test was positive in 06(42.85%) out of 14 blood culture positive cases and in 09(42.85%) out of 21 culture negative typhoid cases in the first week of illness (Table3). Out of 14 culture positive cases 12(85.71) showed significant rising titer (Table 4).

Table 3: Results of Widal test on 1st sample in different study groups.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>No. of individual</th>
<th>Widal test 1st sample positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>14</td>
<td>06(42.85)</td>
</tr>
<tr>
<td>Group II</td>
<td>21</td>
<td>09(42.85)</td>
</tr>
<tr>
<td>Group III</td>
<td>20</td>
<td>04 (20.00)</td>
</tr>
<tr>
<td>Group IV</td>
<td>20</td>
<td>02 (10.00)</td>
</tr>
</tbody>
</table>

Figures in parenthesis indicate percentage. 
(p<0.05 by Chi-square test)

Group I  - Culture positive typhoid fever
Group II - Culture negative typhoid fever (Significant Widal test as per rising titer).
Group III - Febrile control (Non-typhoidal febrile illness).
Group IV - Healthy control.

Table 4 : Result of Widal test in first and second sample among confirmed typhoid cases (Group I & Group II)

<table>
<thead>
<tr>
<th>Test</th>
<th>Group I (n = 14)</th>
<th>Group II (n = 21)</th>
<th>Group I &amp; II (n = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Widal test</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>1st sample</td>
<td>06(42.85)</td>
<td>08(57.14)</td>
<td>09(42.85)</td>
</tr>
<tr>
<td>2nd sample</td>
<td>12(85.71)</td>
<td>02(14.28)</td>
<td>21(100.00)</td>
</tr>
</tbody>
</table>

Figures in parenthesis indicate percentage.

Table 5: Widal test was positive in 15(42.85%) out of 35 cases. In 40 controls 06(15%) were positive and 34(85%) were negative. Accordingly sensitivity and specificity were calculated as 42.85% and 85.00% respectively.

Table 5 : Comparative results between single Widal test and paired Widal test.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Widal test</td>
<td>42.85%</td>
<td>85.00%</td>
<td>71.42%</td>
<td>62.96%</td>
</tr>
<tr>
<td>Paired Widal test</td>
<td>85.71%</td>
<td>Not done</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N.B: Sensitivity and specificity was calculated by the following formula

Sensitivity = True positive x 100 /True positive + False negative =100 x a/a+c,
Specificity = True negative x 100 / True negative + False positive =100 x d/b+d.
PPV = Positive predictive value ; NPV = Negative predictive value

Positive predictive value = a x 100 / a+b
Negative predictive value = d x 100 / c+d

*a = True positive,  b = False positive,  c = False negative and  d = True negative.
**Discussion**

Isolation of the causative agent by culture has remained the gold standard for diagnosis of typhoid fever. Blood culture has got its limited diagnostic utility due to low sensitivity. Although the Widal test has been used for more than a century in many developing countries but it is non-specific, poorly standardized, often confusing and difficult to interpret. Moreover, sharing of O and H antigens by other Salmonella serotypes and other members of Enterobacteriaceae makes the role of Widal test even more controversial in diagnosing typhoid fever.

The most widely used serological test in typhoid fever is to detect antibody against O and H antigen of *S. typhi* by Widal test. In the present study Widal test was carried out in all the four groups of patients and controls (Table 9). The cut off value of Widal test was considered as 1: 80 for both TO and TH. Although Widal test usually become positive from second week, in this study out of 14 culture positive typhoid patients (Group I) 06 (42.85 %) had an initial TO and TH titer ≥ 160 in the first week of illness. Closely similar findings were also reported by Hatta *et al* they found 33(47.8%) out of 69 culture positive cases in the first week of illness.

Another study done by Shukla *et al.* also found that 44.2% had TO titer of ≥160 in single sample collected in the early phase of illness from patients suspected to have typhoid in an endemic area of central India. This findings were most probably attributable to a hyper immune or immunologically sensitized population which is continually exposed to *S. typhi* and other Salmonellae. This observation has practical importance as second specimens are often not sent to the laboratory. The results obtained are also of relevance to the concept that specimens which are taken in the first week of illness are of little use in the serodiagnosis of typhoid.

In this present study when paired sample were collected at 7 to 10 days interval among 14 bacteriologically proven typhoid fever cases 12(85.71%) had a significant Widal test (Table 10). This result agree closely with those of Pang & Puthuchery who found that 93.1% and Hatta *et al.* found 90.4% on paired sera of typhoid cases, gave a significant Widal reaction. The rate of positivity of Widal reaction on 2nd sample higher than 1st sample because antibody to ‘O’ and ‘H’ antigen usually begin to appear towards the end of the 1st week of fever and increase to maximum during the 3rd week.

The incidence of false negative Widal test among the bacteriologically proven cases of this study was 02(14.28%) (Table 10). This findings were similar to when compared with findings, Saha *et al.* in Bangladesh 11.3%, Noorbakhsh *et al.* in Iran 24% and Malik, 6.9% in Malaysian populations. Possible hypotheses put forward to explain this phenomenon are prior use of antibiotics, the existence of less immunogenic strains of *S. typhi*, reduced immunity from severe nutritional hypo proteinaemia.

Out of 20 non-typhoidal febrile cases 4(20%) (Table 9) showed high titer in the Widal test. This findings were closely similar with the findings of Duthie & French, they reported 23% false positive results of Widal test, Handjo *et al.* in Indonesia also found 7% non typhoidal fever showing a false positive Widal test. These raised Widal titer in non-typhoidal febrile patients was perhaps due to the fact that these persons had been infected by *S. typhi* in the past as Salmonella agglutinating antibodies may show a non-specific rise as a result of non-typhoidal fever.

Out of 20 healthy controls 2 (10%) case was positive for TH (titer ≥ 160) (Table 9). Our findings were almost similar to those of Saha *et al.* they reported 4.3% out of 300 healthy Bangladeshi children had TH titer ≥160. These raised TH titer among our healthy controls was probably due to previous exposure *S. typhi* as typhoid is endemic in our region.
Conclusion
After analyzing the findings of the present study it was concluded that although blood culture is gold standard for diagnosis of typhoid fever and rising titer of Widal test also helpful for diagnosis but elevated levels of agglutinating O and H antibodies as measured in a single Widal test might be helpful in making a presumptive diagnosis of typhoid fever if interpreted with care. Neither a "negative" Widal test rule out the diagnosis of typhoid fever in patients with signs and symptoms of the disease since a "negative" Widal test may be seen early in the course of illness.

References