Significance and value of the Widal test in the
diagnosis of typhoid fever in an endemic area

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SUMMARY The diagnostic value of the Widal test was assessed in an endemic area. The test was done on 300 normal individuals, 297 non-typhoidal fevers and 275 bacteriologically proven cases of typhoid. Of 300 normal individuals, 2% had an H agglutinin titre of 1/160 and 5% had an O agglutinin titre of 1/160. On the basis of these criteria a significant H and/or O agglutinin titre of 1/320 or more was observed in 93–97% of typhoid cases and in only 3% of patients with non-typhoidal fever. Of the sera from typhoid cases which gave a significant Widal reaction, the majority (79.9%) showed increases in both H and O agglutinins and 51 of 234 (21.8%) of these sera were collected in the first week of illness. The significance and implications of these findings are discussed.

Typhoid fever remains an important public health problem in Southeast Asia. The definitive diagnosis of the disease requires the isolation of Salmonella typhi from the blood, faeces, urine or other body fluids. In the developing countries, facilities for isolation and culture are often not available especially in smaller hospitals and diagnosis relies upon the clinical features of the disease and the detection of agglutinating antibodies to S typhi (the Widal test). The Widal test has been used very extensively in the serodiagnosis of typhoid fever and, in developing countries particularly, remains the only practical test available. Numerous studies, however, have produced data which have cast serious doubts on the value of the Widal test in the diagnosis of typhoid fever. Several factors have contributed to this uncertainty. These include poorly standardised antigens, the sharing of antigenic determinants with other Salmonellae and the effects of treatment with antibiotics and previous immunisation with TAB vaccine. Another major problem relates to the difficulty of interpreting Widal test results in areas where S typhi is endemic and where the antibody titres of the normal population are often not known. Furthermore, in areas where fever due to infectious causes is a common occurrence the possibility exists that false-positive reactions may occur as a result of non-typhoidal fevers.

The purpose of the present study is to re-evaluate the diagnostic value of the Widal test in an endemic area and to assess some of the factors which may be of importance in the interpretation of the test.

Material and methods

CONTROLS AND PATIENTS

Serum samples for the Widal test were collected from three groups:

Group 1
Three hundred normal, healthy individuals from the medical student population of the University of Malaya and blood bank donors at the University Hospital, Kuala Lumpur.

Group 2
Two hundred and seventy-five patients attending the University Hospital, Kuala Lumpur over an eight-year period (1974–1982) with a definitive diagnosis of typhoid fever as indicated by the isolation of S typhi from the blood and/or faeces.

Group 3
Two hundred and ninety-seven patients with non-typhoidal fever consisting of patients with leptospirosis (6), dengue fever/dengue haemorrhagic fever (45), subacute bacterial endocarditis (20), endemic typhus (133) and scrub typhus (93). Leptospirosis was diagnosed serologically by the microscopic agglutination test, dengue fever by serodiag-
nosis using the complement fixation test or by virus isolation, bacterial endocarditis by the isolation of Streptococcus viridans from the blood and endemic and scrub typhus by the standard Weil-Felix test using cross-reacting Proteus antigens' i.e. OX19/OX2 for endemic typhus and OXK for scrub typhus (Wellcome Reagents Ltd, Kent, England).

**ISOLATION OF SALMONELLA TYPHI**
Isolation of *S. typhi* from the blood and faeces was carried out using standard procedures. Blood specimens were cultured on blood and chocolate agar following overnight incubation at 37°C in Robertson's cooked meat medium and Liquoid broth. Fresh faeces were plated directly on MacConkey and deoxycholate-citrate agar (DCA) and also into selenite F broth which was subcultured after overnight incubation at 37°C. Further identification was based on biochemical reactions and agglutination with specific antisera (Wellcome Reagents Ltd, Kent, England).

**WIDAL TEST**
The Widal test was done using a slightly modified version of the microagglutination method." The following procedure was used: serial dilution of sera (in normal saline) was performed starting at a dilution of 1/20. For O agglutinins, one drop of serum was added to wells of a microtitre plate followed by one drop of antigen (total volume 0·05 ml), incubated overnight at 37°C and then for two hours at 0–4°C before reading. H agglutinins were tested by adding four drops of serum to four drops of antigen (total volume 0·2 ml) to pointed glass tubes (Dreyer's tubes) and incubating at 52°C for two hours followed by overnight incubation at room temperature. Antigen suspensions were diluted 1/20 before use and comprised stained suspensions of H and O antigens from *Salmonella typhi*, *Salmonella paratyphi A* and *Salmonella paratyphi B* (Wellcome Reagents Ltd, Kent, England).

**Table 1**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Type of antigen</th>
<th>Agglutinin titres (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/20</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>H</td>
<td>61</td>
</tr>
<tr>
<td><em>S. paratyphi A</em></td>
<td>H</td>
<td>78</td>
</tr>
<tr>
<td><em>S. paratyphi B</em></td>
<td>H</td>
<td>63</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>O</td>
<td>6</td>
</tr>
<tr>
<td><em>S. paratyphi A</em></td>
<td>O</td>
<td>94</td>
</tr>
<tr>
<td><em>S. paratyphi B</em></td>
<td>O</td>
<td>64</td>
</tr>
</tbody>
</table>

**Table 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>No in group</th>
<th>Widal test result*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Significant</td>
</tr>
<tr>
<td>Normal</td>
<td>300</td>
<td>0</td>
</tr>
<tr>
<td>Typhoid fever</td>
<td>275†</td>
<td>256 (93·1%)</td>
</tr>
<tr>
<td>Other fevers</td>
<td>297</td>
<td>9 (3·0%)</td>
</tr>
</tbody>
</table>

*Significant result: H and/or O titre of ≥1/320 (v *S. typhi*).  
Non-significant result: H and/or O titre of <1/160 (v *S. typhi*).  
†Adjusted percentage—see text under Results.  
‡Paired serum specimens were obtained in only 61 cases (22%).

**Results**
The level of H and O agglutinins in the normal population is presented in Table 1. For *S. typhi* it can be seen that the majority tested (61%) had an H agglutinin titre of <1/20 with 2% having a titre of 1/160 (Table 1). For O agglutinins, 34% had a titre of 1/80 and 5% had a titre of 1/160 (Table 1). Based on these data it was decided that an H and/or O agglutinin titre of ≥1/320 would be significant and indicative of typhoid fever. Using such criteria the levels of H and O agglutinins in bacteriologically proven typhoid cases and cases of non-typhoidal fever was determined (Table 2). Results show that 93·1% of typhoid fever cases had a significant Widal test result (Table 2). In contrast, only 3% of patients with non-typhoidal fever showed a significant Widal
Widal test in endemic areas

Table 3  H and O agglutinins v Salmonella typhi in 256 bacteriologically proven cases of typhoid fever

<table>
<thead>
<tr>
<th>Type of antigen</th>
<th>Agglutinin titres (%)</th>
<th>&lt;40</th>
<th>40</th>
<th>80</th>
<th>160</th>
<th>320</th>
<th>640</th>
<th>1280</th>
<th>&gt;1280</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td></td>
<td>3.9</td>
<td>2.4</td>
<td>1.9</td>
<td>5.5</td>
<td>17.2</td>
<td>32.0</td>
<td>20.7</td>
<td>16.4</td>
</tr>
<tr>
<td>O</td>
<td></td>
<td>0.8</td>
<td>0</td>
<td>0.4</td>
<td>5.1</td>
<td>18.0</td>
<td>23.8</td>
<td>32.0</td>
<td>19.2</td>
</tr>
</tbody>
</table>

*Total No of sera tested = 256 (sera with a significant Widal test result ie H and/or O agglutinin titres of ≥1/320).

Table 4  Date of onset and Widal test result in bacteriologically proven cases of typhoid fever

<table>
<thead>
<tr>
<th>Test result</th>
<th>Day of onset illness</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sign. H titre only</td>
<td>0-7</td>
<td>15 (6.4%)</td>
</tr>
<tr>
<td>Sign. O titre only</td>
<td>8-14</td>
<td>32 (13.7%)</td>
</tr>
<tr>
<td>Sign. H and O titres</td>
<td>14</td>
<td>187 (79.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>51 (21.8%)</td>
<td>234</td>
</tr>
</tbody>
</table>

*No of sera tested = 234 (sera with a significant Widal test result ie H and/or O agglutinin titres of ≥1/320).

Discussion

An attempt has been made to assess the diagnostic value of the Widal test in an area where S typhi is endemic by taking into consideration several factors which have led to confusion in the interpretation of the test.

This study clearly show that in an endemic area such as Malaysia, S typhi agglutinins against both H and O antigens may be present in the normal population at titres of up to 1/160. This finding is in agreement with a study carried out in Sri Lanka, another endemic area, where agglutinin titres of up to 1/80 were discovered in the normal population. It is clear that any interpretation as to the significance of a Widal test result must be made against this "baseline" information. For example, titres of 1/50 and 1/100 on a single specimen, which are considered significant in non-endemic areas, are of no diagnostic significance in areas where S typhi is endemic. On the basis of these findings we have set our own laboratory guidelines of H and/or O agglutinin titres of ≥1/320 as being of diagnostic significance. By using this criterion, a diagnostic titre of agglutinins was obtained in 93–97% of typhoid fever cases. In contrast only 3% of sera from patients with other non-typhoidal fevers showed a significant Widal reaction. Our results agree closely with those of Senewiratne and Senewiratne who found that 94% of typhoid cases and only 1% of non-typhoidal fevers gave a significant Widal reaction. It is also relevant to note that the above study was done on 53 typhoid cases as opposed to the 275 reaction (Table 2). Of the 19 typhoid fever cases which gave a normal Widal test result, eight had been treated with full courses of antibiotics (mainly chloramphenicol), two had concomitant infections and one was less than 1 month old. If these 11 cases were excluded from the group, a rate of 97% would be obtained for typhoid cases which gave a significant Widal reaction (Table 2). Of the 256 sera with a significant Widal test, 86% had H and 94% had O agglutinin titres of ≥1/320 and approximately 16–20% had agglutinin titres of ≥1/1280 (Table 3). A more detailed analysis indicated that 6-4% of these sera had a significant H titre only, 13-7% had a significant O titre only and 79-9% had significant titres for both antigens (Table 4). Furthermore, 51 of 234 (21.8%) of these sera were collected in the first week of illness, 46-6% in the second week and 31.6% at more than two weeks (Table 4); in all cases the majority of sera exhibited significant titres against both H and O antigens (Table 4). Further analysis of data on the Widal test results in 297 cases of non-typhoidal fevers showed that no significant Widal reactions were observed in the sera of patients with dengue fever/dengue haemorrhagic fever (45 cases), subacute bacterial endocarditis (SBE) (20 cases) or leptospirosis (6 cases) and only 3-4% of sera from patients with endemic (133 cases) and scrub typhus (93 cases) gave a significant Widal reaction. With respect to these last two groups, the diagnostic titres obtained in the Weil-Felix test for the specimens of concern were always much higher than the cross-reacting titres observed with the Widal test (data not shown).
studied in the present report. These data are in marked contrast to the results obtained in other studies. Brodie\textsuperscript{11} in his study of the 1964 Aberdeen outbreak involving 403 cases of bacteriologically proven typhoid cases reported that H agglutinins did not develop in 15% of patients tested and O antibodies did not develop in as many as 41%. Wicks\textit{et al}\textsuperscript{3} found the Widal test to be completely negative in 26 of 123 (21.1%) Rhodesian patients with positive blood cultures. Sen and Saxena\textsuperscript{3} also concluded that serological tests are of little value in the diagnosis of typhoid fever. Several factors mentioned previously may have contributed to this discrepancy. Firstly, the widespread practice of empirical usage of antibiotics such as ampicillin, amoxycillin and cotrimoxazole could delay and inhibit the formation of agglutinins. This was noted in the present study. Secondly, and as illustrated in the present report, titres of H and O agglutinins need to be interpreted against a baseline titre in normal individuals living in the same geographical location. Thirdly, strain variations—for example, different phage types, in the various areas could result in differences in the antibody response. \textit{S. typhi} is a well known intracellular pathogen and a less "immunogenic" strain—that is, a strain with increased capacity to remain within cells, could produce a lower antibody response. Fourthly, it is also probable that in endemic areas, where the population is permanently "immunologically sensitised" due to constant exposure, the response to infection is more rapid, reaches higher levels and is less likely to be affected by antibiotic use when compared to non-endemic areas. Finally, other factors which may also be important include poorly standardised antigen preparations, previous immunisation with TAB vaccine and technical differences. In relation to the last point, the Widal test performed on the same serum specimen in four laboratories gave widely different results.\textsuperscript{12}

With regard to the possibility of false-positive reactions, the present study has shown that the large majority of sera from proven cases of other febrile illnesses common in the region—for example, dengue fever/dengue haemorrhagic fever, leptospirosis, rickettsial typhus, did not give a significant Widal reaction. This finding does not, of course, discount the possibility of false-positive reactions occurring due to other conditions such as infection with other salmonellae,\textsuperscript{4} chronic active hepatitis,\textsuperscript{13} and other immunological disorders.\textsuperscript{10}

Another related issue is the question of which agglutinin (H or O) is of greater diagnostic value. It is widely held that raised O agglutinins are of greater significance\textsuperscript{4,10} but other studies have suggested that H antibodies are more reliable.\textsuperscript{11} Our results suggest that a raised O agglutinin is of slightly greater diagnostic value than a raised H agglutinin. The data we obtained also does not support the conclusion that the titre for O antigen is the only useful value\textsuperscript{2} as a small proportion of patients with proven typhoid showed a rise only in H agglutinins.

The results obtained are also of relevance to the notion that specimens which are taken in the first week of illness are of little use in the serodiagnosis of typhoid. We found that some sera which gave a significant Widal test were collected in the first week of illness. This finding supports the conclusions of others\textsuperscript{10} that in endemic areas the H and O agglutinins could appear earlier in the course of illness. This is most probably attributable to a hyperimmune or immunologically sensitised population which is continually exposed to \textit{S. typhi} and other salmonellae.\textsuperscript{10} This observation is also of practical importance as second specimens are often not sent to the laboratory. The ability to diagnose typhoid fever on an early, single specimen is also of therapeutic value as early diagnosis is vital in typhoid; delay in starting treatment may result in complications such as perforation of, or haemorrhage from, the small bowel, which may be fatal.

We thus conclude that in endemic areas the Widal test is still of significant diagnostic value provided judicious interpretation of the test is made against a background of pertinent information, especially data which relate to agglutinin levels in normal individuals and in non-typhoidal fevers common in the region. We also point out that in endemic areas a single Widal test can be of diagnostic value in the early stage of disease and thus help in reducing morbidity and mortality from typhoid.

References

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