

## Interpretation of the Widal Test in Infected Children

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### Abstract

Typhoid fever is endemic in Iran. Isolation of *S. typhi* is a gold standard for diagnosis. Laboratory diagnosis of *S. typhi* infection relies on serological tests such as the Widal test. This study describes seroprevalence of TO and TH antibody in nonfebrile healthy and febrile with non-typhoid illness. For detection of sensitivity and specificity of Widal test in typhoid fever diagnosing Widal test was performed on serum specimen of the culture-positive cases of typhoid fever in children aged between 1 and 14 years in Tehran, capital of Iran.

A cross-sectional study was carried out. Widal tests were performed on 40 healthy nonfebrile children; 40 patients with non typhoidal febrile illness and 58 cases with bacteriologically documented typhoid fever specificity, sensitivity, positive and negative predictive values and the efficacy of the test were determined.

Agglutinin titres  $\leq 1:40$  were considered normal for TO and TH at 96.25% and 93.75% confidence levels, respectively. Titres above these levels, TO  $>1:40$  and TH  $>1:40$ , were considered to be abnormal. 25% of patients showed no response to either agglutinin (TH and TO); and 44.8% of cases shown no response for TO agglutinin. TO  $>1:320$  was not seen in any cases but TH  $>1:320$  was detected in 20.6% of cases. *Salmonella typhi* TO and H agglutinin titers  $> 1/40$  were considered to be significant with 75.86% sensitivity and 93.75% specificity, respectively. The positive and negative predictive values were 89.79% and 84.26%, respectively.

This study suggests that seroprevalence studies in healthy children can help as validate use for particular serological cut-off point.

**Keywords:** Typhoid fever; Widal test, Iran

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### Introduction

Typhoid fever is endemic in Iran like other developing countries, where there is a high incidence in children (1-9). In the setting of clinical illness consistent with typhoid fever, the gold standard for diagnosis is isolation of *S. typhi* from blood, bone marrow, stool, urine or any other body fluid (10-13). However, in countries such as Iran, isolation of the organism is often jeopardized by lack of facilities or inadequate and/or improper antibiotic use prior to culture (14-16). For these reasons, laboratory diagnosis of *S. typhi* infection relies heavily on serological tests such as the widal test (17-28). In the setting of endemic disease, interpretation of the widal test hinges on knowledge of the seroprevalence of positive antibody titres among healthy members of the population (22-28). In previous studies from different parts of the world, the baseline titres of TO and TH have shown wide variations range between regions with different prevalence (28-31). This study was undertaken to determine the seroprevalence of TO and TH antibody in Rasool akram hospital, to examine the antibody titer among febrile children with non-typhoid illness, to measure the sensitivity and specificity of different antibody titres among the culture-positive cases of typhoid fever, and to compare the value of using a single antigen (TO or TH) with using both in the diagnosis of typhoid fever.

### Materials and Methods

Widal tests was performed on sera specimens from three groups of children aged 1 to 14 years. Group 1 (n=40) were children with no history of fever, 3 months prior to collection of the specimen in surgery ward of Rasool akram hospital, group 2 (n=40) were children with non-typhoidal-febrile illness (mumps, urinary tract infections or chlymidial respiratory infection and group 3 (n=58) were children with bacteriologically documented typhoid fever (blood, stool, bone marrow, or other sterile site cultures with growth of *S. typhi*). The Widal test was performed using standard tube agglutination test (Febrile antigen, Pastuer Insitiue, Iran) containing TO and TH antigen standardized by rapid agglutination, as described by the manufacturer. Specificity, sensitivity, positive and negative predictive values and the efficacy of the test were determined.

### Results

From 58 cases with typhoid fever, there 56.5% were female. TO agglutinin titres were 1:40 or less in 100% and 92.5% of healthy children, nonfebrile children and non-typhoidal febrile children, respectively. Similarly, TH agglutinin titres were 1:40 or less in 100% and 87.5%, in that order.

In non-typhoidal febrile children, in 1 case titres exceeded to TO =1:320, TH to 1:640, but in 2 cases TH titres rose to 1:80 with TO titer less than 1:40 (Table 1). Based on the above data, the titres  $\leq$  1:40 were considered normal for TO and TH at 96.25% and 93.75% confidence levels, respectively. Titres above TO>1:40 and TH>1:40, were considered to be abnormal.

Among the bacteriologically proven cases of typhoid fever, there were 26 (44.8%) with TO $\leq$ 1:40 and TH>1:40. In 4 cases (6.8%) there were TO>1:40 and TH<1:40. There were 14 (25%) cases in which TH and TO antibody was less than 1:40 (Table 1). TO titer

>1:320 not detected in this group but TH >1:320 were seen in 12 cases (20.6%). From 58 cases with bacteriologically documented typhoid fever 23 (39.6%) aged 5-10, in which 21 cases had positive titers; but in 17 cases which aged less than 5 years all of them had positive titers (Table 2). Therefore false negative results was high in this groups. On the basis of this cut-off value, and considering both the agglutinins equally important, sensitivity and specificity of the test were 75.86% and 93.75%, respectively. Similarly, the positive and negative predictive values were 89.79% and 84.26%, respectively.

**Table 1 :** Agglutinin level against TO,TH in proven cases typhoid fever children

|    | Widal Titer | <1:40 | 1.40 | 1.80 | 1.160 | 1.320 | 1.640 | 1.1280 | 1.3200 |
|----|-------------|-------|------|------|-------|-------|-------|--------|--------|
| TO | Number      | 40    | 4    | 2    | 4     | 8     | 0     | 0      | 0      |
|    | %           | 68.9  | 6.8  | 3.4  | 6.8   | 13.7  | 0     | 0      | 0      |
| TH | Number      | 18    | 1    | 5    | 3     | 19    | 9     | 3      | 0      |
|    | %           | 31.03 | 1.7  | 8.6  | 5.1   | 32.7  | 15.5  | 5.1    | 0      |

**Table 2:** Number and percentage of age in Typhoid fever

| Age   | number | percentage |
|-------|--------|------------|
| <1    | 3      | 5.1        |
| 1-4   | 14     | 24.1       |
| 5-9   | 23     | 39.6       |
| 10-14 | 18     | 31         |
| Total | 58     | 100        |

## Discussion

The Widal test is easy, inexpensive, and relatively noninvasive (17-28). It can be diagnostic value when blood cultures are not available or practical (10-13). The results must be interpreted cautiously because of the low sensitivity of the test (17-28). The Widal test done on convalescent-phase serum gave more-reliable results with higher specificity and sensitivity. Knowledge about baseline titres of O and H antibodies in a population is necessary for accurate interpretation of results of the Widal test (22-28). Widal test in diagnosis of typhoid fever in adults >18 years of age in Turkey (17) was done. Using a cut-off  $\geq$  1/200 for the O antigen test performed on acute-phase serum gave a sensitivity of 52% and a specificity of 88% with a positive predictive value (PPV) of 76% and a negative predictive value (NPV) of 71%. This increased to 90% sensitivity and specificity with a PPV of 88% and an NPV of 93% when the convalescent-phase serum was tested. The diagnostic titers of TO>40 and TH>1:40 adopted for this series proved to be significant up to 96.25% and 93.75% confidence levels, respectively, and this was associated with 89.79%, 84.26% positive and negative predictive value. The sensitivity and specificity of the test was

75.76% and 93.75%, respectively. These titers are, however, low when compared with findings in a Bangladesh (23) and Rhodesian population (21). In respect of TO titers in our study, these are low compared with Malaysian (29) and Hong Kong populations (22). Except in Bangladesh (23), it should be noted that none of these other studies present the ages of patients or controls. The incidence of a false negative Widal test among the bacteriologically proven cases of this series was very high (24%) when compared with 12% in Bangladesh (23), 6.9% in Malaysia (29) and 1% in Hong Kong (22). These false negative Widal test results lead to a low sensitivity (75.86%) and negative predictive value (84.26%) for the test. 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, etc. None of our patients was hypoproteinaemic and differentiation of strains was not possible. The hypothesis of prior antibiotic therapy is the most likely explanation in our situation since the inadequate and improper used of antibiotics is very common. One more related issue is the question of the diagnostic value of the individual agglutinins (H or O) of *S. typhi*. In this study, 44.8% (26) of the proven typhoid cases showed no antibody response to the somatic antigen (TO<1/40), even though the antibody response against flagellar antigen (TH) was raised to 1/40 or more (2 cases: 1/320; 9 cases: 1/640; 3 cases: 1/1280). Conversely, a rise in somatic antibody (TH >1/40) alone was triggered in only 6.8% of the proven typhoid cases. These findings are of paramount significance to clinicians that must often rely solely upon the results of the Widal test in making the diagnosis of typhoid fever, and among that there is a common belief that H

antigen is not useful for this purpose. Our study suggests that the Widal test is of considerable importance in the diagnosis of typhoid fever, and both the agglutinins, somatic and flagellar, are equally important for that purpose. Furthermore, as treatment based solely on a Widal test is almost the rule rather than the exception, up-to-date data of the baseline antibody titres for typhoidal agglutinins of any particular population or area should be available. Seroprevalence studies in healthy children can help to validate the use of a particular serological cut-off point.

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