Original Article

Evaluation of false negativity of the Widal test among culture proven typhoid fever cases

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Abstract

Background: The Widal test is the most common, specific and quick diagnostic method available in the world for diagnosis of typhoid fever; however, false negativity is one of the obstructive features of the test. The aim of this study was to evaluate the associated factors with Widal test negativity in an endemic area.

Methods: Widal test negativity was retrospectively analyzed among culture-proven typhoid fever cases. The potential features including age, gender, previous antibiotic usage, duration of symptoms, leucopoenia, hematocrit value, and erythrocyte sedimentation rate (ESR) were evaluated for association with Widal test negativity.

Results: A total of 166 culture-proven typhoid fever cases (93 or 56.0% males) were included in the study. The mean age ± SD was 23.3 ± 10.6 years. Mean time of interval between first symptom and test performance time was 10.6 ± 7.8 days. The Widal test (STO and/or STH) was found positive in 75 cases (45.2%). The statistical analyses revealed that none of these variables were significant for false negativity of the Widal test. Age was found to be a possible factor for a false negative Widal test (p=0.06).

Conclusion: Of existing compatible clinical findings, age should be considered in cases of Widal test negativity.

Key Words: typhoid fever, Widal test, diagnosis

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Introduction

The Widal test has been used for more than 100 years as an important part of the diagnosis of typhoid fever [1-4]. It is a tube dilution test, which measures agglutinating antibodies against the lipopolysaccharide O and protein flagellar H antigens of *S. typhi*. The value of the test in the diagnosis of typhoid fever cases has long been discussed [5,6]. The definitive diagnosis of typhoid fever is based on the isolation of *S.* typhi from blood, stool, urine or other body fluids [2,7-9]. In many endemic areas, bacterial culture facilities are often unavailable and the Widal test is the only specific and quick diagnostic tool available. In various percentages of typhoid fever cases, the Widal test does not detect antibodies even in blood culture-confirmed cases [8,10].

An insufficient number of studies have been conducted on the factors related to false negative results in the Widal test. In practice, these factors may be useful for determining the treatment of suspicious cases. The aim of this study is to evaluate associated factors with false-negative Widal tests with culture-proven typhoid fever cases in an endemic area.

Methods

Dicle University Hospital, in Diyarbakir city center, is a 1,050-bed referral center for southeast Turkey. This hospital is the largest tertiary care health center for five provinces in the southeast region (about 2.5 million people). In a retrospective approach, data including epidemiological characteristics, manifest symptoms, physical signs, history of antibiotic usage before the admission (effective for *S.* typhi strains) and laboratory findings (Widal test results included) for culture-proven typhoid fever cases were collected using a standardized data collection form. The case files of all patients aged 15 years and older with culture-proven typhoid fever who were admitted to the hospital between 1998 and 2004 were retrieved from the hospital's medical record library. Widal test results against O (*Salmonella* typhi O) and/or H (*Salmonella* typhi H) sera were included in the study among culture-proven typhoid fever cases.

Further clinical details for these subjects will be reported elsewhere.

All participants in the study were in-patients. The first step of the serological examination was slide agglutination screening for *Salmonella* enterica serotype Typhi O and H. The positive sera were serially diluted in tubes with 08.5% NaCl from 1/20 to 1/1,280, and antigens (H and O) were added. The tubes were incubated at 37°C for 2 hours and then at room temperature overnight and examined for agglutination. The Widal test was performed when the patient was admitted to the hospital. The Widal test result was accepted as positive if the agglutinin titer was found to be ≥ 160 [8,9].

Features including age, gender, effective antibiotic usage before admission, duration of symptoms, anemia, leucopoenia (1 = ≤ 4,000 leucocyte/$mm^{3,}$ 0 = > 4000 leucocyte/mm^3), and erythrocyte sedimentation rate (ESR) were evaluated for association with Widal test negativity in these patients. The correlation between a false-negative Widal test and possible associated factors was evaluated.

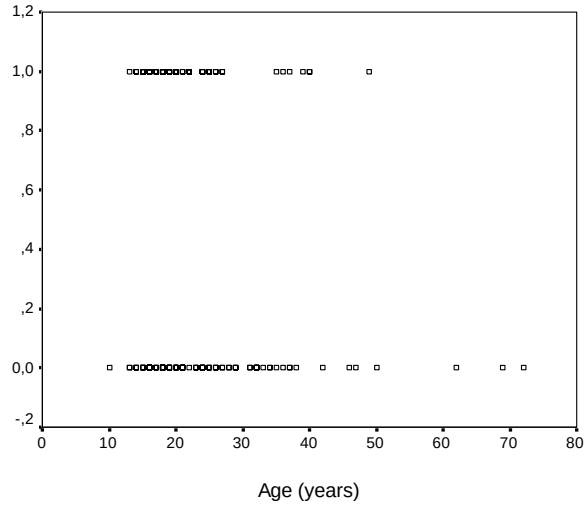
Statistical analyses

All data entry and analysis were performed using SPSS 9.05 for Windows (SPSS Inc., Chicago, IL, USA). For all univariate analyses, the chi-square test was used for binary variables. The one-Sample Kolmogorov-Smirnov Test was used for analyzing range of distribution of continuous variables. The Mann-Whitney U test was used for continuous variables if the distribution of data was not normal, and the Student's *t*-test was used for the other continuous variables. If the *P* value was found < 0.05, the difference was accepted as significant. All these variables were tested for correlation with the Widal test. At the same time, the correlation between Salmonella Typhi O (STO) and Salmonella Typhi H (STH) was evaluated for the variables.

Results

All eligible patients were included in the study. In total, 166 culture-proven typhoid fever cases were found to be suitable. The study group consisted of 93 males (56.0%) and 73 females (44.0%). The mean age (± SD) of patients was 23.3 (± 10.6) years. The mean age ± SD of sero-negative cases was 25.0 ± 11.4 (Figure 1). Mean time (± SD) interval between first symptom and test performance time was 10.6 (± 7.8) days. Widal test O titers were found to be positive in 47 cases (28.3%) and Widal test H titers were found to be positive in 44 cases (26.5%). Both Widal test O and H titers were found to be 1:160 or higher in 15 cases. In total, the Widal test (STO and/or STH) was found to be positive in 75 cases (45.2%).

Figure 1. The age distribution of typhoid fever cases according to the Widal test seropositivity and seronegativity.



The Mann-Whitney U test was used for analysing age and ESR; the Student's *t*-test was used for analysing hematocrit; and the Chi-Square test was used for analysing gender, the duration of symptoms, prior antibiotic use, and leucopenia. There was a slight relation between age and a false negative Widal test (p=0.06), but it was not statistically significant. The analyses revealed that none of these variables were significant for false negativity of the Widal test (Table 1). There was no significant correlation between STO and duration of symptoms, prior antibiotic use, hematocrit, leucopenia and ESR. There was no significant correlation between STH and age, duration of symptoms, prior antibiotic use, hematocrit, leucopenia and ESR.

Table 1. Univariate analyses of associated factors on the Widal test negativity in culture-proven typhoid fever cases.

Variables	Seropositive Widal Test				
	Proportion (%) positives	Proportion (%) negatives	OR	95% CI	P
Gender (Male)	39/75 (52.0)	54/91 (59.3)	1.14	0.87-1.50	0.34
Duration of symptoms (≥7 days)	17/36 (47.2)	30/58 (51.7)	1.10	0.72-1.68	0.67
Hematocrit (mg/dl)	36.0 ± 5.4	35.4 ± 4.6	-	-	0.29
Prior antibiotic use	19/75 (25.3)	23/91 (25.3)	1.00	0.59-1.99	0.99
Leucopoenia (< 4000/mm^3)	40/57 (70.2)	52/78 (66.7)	0.95	0.75-1.20	0.66
Age	21.4 ± 6.9	25.0 ± 11.4	-	-	0.06
ESR	31.6 ± 26.0	31.0 ± 20.8	-	-	0.50

Discussion

The Widal test is the most common test for early diagnosis of typhoid fever cases around the world. The test is very easy to perform, which makes it practical for use in the field [8,11]. The test has been used for over a century in developing countries but its sensitivity, specificity, and positive and negative predictive values, which change with geographical area, are debatable. Sharing of O and H antigens by other *Salmonella* serotypes and other members of *Enterobacteriaceae* makes the role of the Widal test even more controversial in diagnosing typhoid fever [12]. In fact, there are many false negative and false positive Widal test results in medical practice. Although the Widal test is widely used in the world to diagnose typhoid fever, insufficient studies aimed at explaining the possible mechanisms for false negativity have been completed.

In our study, the age of patients had a weak association with Widal test negativity. This result could be interesting and important for future studies. The possible reason could be related to the immune reaction capability in a young population. The humoral immunity of young people is stronger than that of older persons. Especially in endemic areas, physicians should be cautious in older patients with compatible clinical and laboratory findings of typhoid fever if their Widal test is negative.

Different approaches could be seen to explain a false negative Widal test in typhoid fever cases. There is a tendency to explain this result when samples have been obtained too early or too late in the acute phase of the disease. Some authors stated that the false negative cases have inadequate inoculum of bacterial antigen in the host to induce antibody production. In another approach, early administration of an antibiotic is one of the important reasons given for a false negative Widal test [2,5,7]. In our study, these two suggestions were found to be inaccurate. The longevity of duration of symptoms did not correlate with Widal test positivity.

In our study, there was a low sensitivity rate for the Widal test. The reason could be related to the data collection method of this study. In these cases, the Widal test was performed just at the admission of the patient to the hospital. The test performance time was not too long after the first symptoms and in many cases the test was not repeated after blood culture confirmation. In a study from Vietnam, patients were grouped and analyzed according to length of illness (less than or greater than 2 weeks) and age (children younger than 15 years old versus adults). The serum antibody responses to the LPS and flagellum antigens of serotype Typhi observed in these typhoid patients were highly variable. Serum anti-LPS IgA, IgM, and IgG antibody levels were broadly similar for adults and children. No significant differences in the responses were seen with regard to length of illness, either for children or adults. Serum anti-flagellum IgG values were generally higher for adults than for children, both for patients in the first two weeks of illness and for those who had been ill for longer than 14 days. Serum antibody levels against the flagellum antigen were higher in adult typhoid patients with a long history of illness (two weeks) than in those who had been ill for less than two weeks. No significant difference in this response was found for children with different lengths of illness [13]. In our study, the patients were mostly young adults. Seropositivity was found to be higher in younger patients than in the older group. In our study, the antibody responses to LPS (STO) were found to be higher in the younger population.

The earliest serological response in acute typhoid fever is commonly a rise in the titer of the O antibody, with an elevation of the H-antibody titer developing more slowly but persisting longer than that of the O-antibody cut-off titer [2,14]. However, a false-negative Widal test rate was not significantly higher in the cases that were treated with effective antibiotic before admission. The Vietnamese study mentioned earlier [13] showed that a long history of illness could be important for the level of anti-flagellar antibodies in adults.

Some technical difficulty or errors in the performance of the test could be an important reason for false-negativity in Widal test [8]. Although our study lacked an external quality control program, errors in the performance of the test should be minimal because of the high level of our laboratory workers' experience and test repetition. The variability in the preparation of commercial antigens is another important factor affecting false negativity. Unfortunately, it is very difficult to assess these variables in this type of study.

We used a retrospective approach and this could also be a limitation for our study. A well-designed prospective study could be helpful for further interpretations. Difficulties diagnosing typhoid fever cases and the evaluation of reasons for false negative results will require further diagnostic confirmation. To avoid such difficulties in this study, we chose blood culture-positive patients. Previous typhoid vaccination may contribute to elevated agglutinins in the non-infected population. However, vaccination is not a factor in the population that we studied. There is no

national program of typhoid vaccination in Turkey, and the typhoid vaccine is not generally available, so it is extremely rare in the study area.

In conclusion, a false negative Widal test result could be associated with age. For more useful interpretation of Widal test results, age should be considered as a correlated factor for a false negative Widal test.

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