

Widal Test in Diagnosis of Typhoid Fever in Turkey

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We studied the value of the Widal tube agglutination test for the diagnosis of typhoid fever. The subjects were all adults >18 years of age and were divided into four groups: (i) 317 healthy blood donor controls, (ii) 31 bacteriologically confirmed patients with *Salmonella enterica* serotype Typhi, (iii) 21 patients with a clinical diagnosis of typhoid fever, and (iv) 41 febrile nontyphoid patients. Blood donor controls were screened with a slide agglutination test for the *Salmonella enterica* serotype Typhi O and H antigens, and positives were then tested with the Widal test. Acute- and convalescent-phase sera from patients in groups 2, 3, and 4 were obtained 7 to 10 days apart and tested by the Widal test. Using a cutoff of $\geq 1/200$ 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. We concluded that O and H agglutinin titers of $\geq 1/200$ are of diagnostic significance. The Widal test is easy, inexpensive, and relatively noninvasive. It can be of diagnostic value when blood cultures are not available or practical. The results must be interpreted cautiously because of the low sensitivity of the test. The Widal test done on convalescent-phase serum gave more-reliable results with higher specificity and sensitivity.

Typhoid fever is a systemic infectious disease characterized by an acute illness, the first typical manifestations of which are fever, headache, abdominal pain, relative bradycardia, splenomegaly, and leukopenia (5, 19). *Salmonella enterica* subsp. *enterica* serotype Typhi is the etiological agent of typhoid fever. Typhoid fever is an important cause of morbidity in many regions of the world, with an estimated 12 to 33 million cases occurring annually (16). Cases are more likely to be seen in areas like India, South and Central America, and Africa with rapid population growth, increased urbanization, and limited safe water, infrastructure, and health systems. In recent years, cases have been reported from Eastern Europe (10). According to records of the Health Ministry in Turkey, about 20,000 cases have been seen each year and the morbidity of typhoid fever was about 30 of 100,000 cases in the years 1994 and 1995 (20).

The diagnosis of typhoid fever on clinical grounds is difficult, as the presenting symptoms are diverse and similar to those observed with other febrile illnesses. The definitive diagnosis of typhoid fever requires the isolation of *Salmonella enterica* serotype Typhi from the patient. Cultures of blood, stool, urine, rose spots, blood mononuclear cell-platelet fraction, bone marrow, and gastric and intestinal secretions can all be useful for diagnosis. Bacteria can be isolated from blood in 73 to 97% of cases before antibiotic use (19). However, in our country, since (i) patients often receive antibiotics prior to medical diagnosis, (ii) bacteria can be isolated from the blood cultures in only 40 to 60% of the cases (4, 12, 25), and (iii) culture facilities may not be available, serologic analysis becomes more important. The Widal tube agglutination test,

which is almost 100 years old, has been widely used in the serologic diagnosis of typhoid fever in Turkey. Clinicians in Turkey generally consider a titer of $\geq 1/200$ as diagnostic of typhoid fever. This is the first study done in Turkey to assess the significance of the Widal test.

This is a prospective study done from 1997 to 1999 in the Clinical Bacteriology and Infectious Diseases Department of Ibn-i Sina Hospital, which is a 1,100-bed teaching hospital of Ankara University.

Study groups. All subjects were >18 years of age. The 317 healthy controls were voluntary blood donors who gave to the blood bank of Ibn-i Sina hospital in Ankara University. Patients were grouped into three categories: 31 blood culture-positive typhoid cases (group 1), 21 cases that were blood culture negative but clinically consistent with typhoid fever (group 2), and 41 nontyphoidal febrile cases (group 3). In group 3 there were 11 cases of paratyphoid fever, 10 cases of brucellosis, 5 cases of atypical pneumonia, 3 cases of bacillary dysentery, 2 cases of urinary tract infection, 2 cases of hepatitis, 1 case of malaria, 1 case of leukemia, 1 case of Hodgkin's disease, 1 case of streptococcal pharyngitis, 1 case of systemic lupus erythematosus, 1 case of acute rheumatic fever, 1 case of cytomegalovirus infection, and 1 case of bacteremia with involvement of the central nervous system.

Isolation of bacteria. *Salmonella enterica* serotype Typhi was isolated from blood cultures by the BACTEC automated culture system (Becton Dickinson). Stool cultures were plated on eosin-methylene blue, salmonella-shigella agar, and Selenite-F broth. Lactose-nonfermenting colonies were isolated from these culture plates, and tests using various agents were performed. Serotype Typhi strains were identified as H₂S positive, motile, urease negative, non-gas forming, and agglutination positive with factor 9 antiserum (8, 19).

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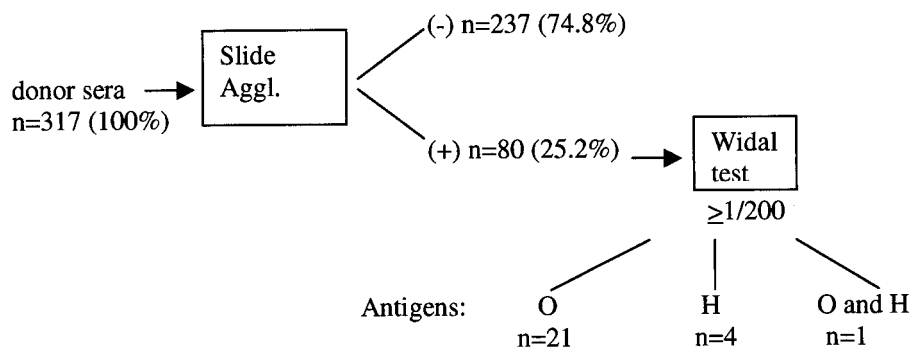


FIG. 1. Test results for donor sera.

Widal test. Donor sera were screened by slide agglutination with *Salmonella enterica* serotype Typhi O and H antigens (Difco). The positive donor sera and all patients' sera were serially diluted in tubes with 08.5% NaCl from 1/50 to 1/6,400, and antigens (H and O) were added. The tubes were incubated at 37°C for 2 h and then at room temperature overnight and examined for agglutination by an agglutinoscope. The Widal test was performed for group 1, group 2, and group 3 patients, with an acute-phase serology done when the patient was asymptomatic and a second (follow-up) serology done 7 to 10 days later.

Statistical analysis. The results of different groups were analyzed by the test performance criteria, namely, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). These were calculated by using the following formulas: sensitivity is $a/(a + c)$, specificity is $d/(d + b)$, PPV is $a/(a + b)$, and NPV is $d/(d + c)$, where a is test positive and culture positive (group 1), b is test positive and culture negative (group 3 and blood donors), c is test negative and culture positive, and d is test negative and culture negative (group 3 and blood donors) (7, 9).

Donor sera. Eighty of 317 (25.2%) healthy blood donors were positive by the slide agglutination test. Twenty-six sera had titers $\geq 1/200$ by the Widal test, with 21 samples positive for the O antigen, 4 samples positive for the H antigen, and 1 sample positive for both antigens (Fig. 1).

Group 1—blood culture-positive typhoid cases ($n = 31$). The number of samples with O and H antibody titers $\geq 1/200$ were 16 (52%) and 9 (29%), respectively, when the acute-phase sera were used. Analysis of the follow-up sera collected 7 to 10 days later increased the number of positives to 28 (90%).

Group 2—blood culture-negative typhoid cases ($n = 21$). The number of samples with O and H antibody titers $\geq 1/200$ were 10 (48%) and 3 (14%), respectively, for acute-phase sera, and these numbers increased to 17 (81%) and 12 (57%) for the follow-up serum samples.

Group 3—nontyphoid febrile cases ($n = 41$). The numbers of samples with O and H antibody titers $\geq 1/200$ for 41 patients were 5 (12%) and 2 (5%), respectively, for the acute-phase serum samples and 4 (10%) and 3 (7%), respectively, for the follow-up samples. The total number of patients whose samples were $\geq 1/200$ positive for either O or H antibodies was 8;

of these eight, five were diagnosed with paratyphoid, two with brucellosis, and one with malaria.

This study was done to assess the role of the Widal test in the diagnosis of typhoid fever. In order to use the Widal test effectively, each country should find the appropriate titer with which to diagnose typhoid fever. There have been numerous reports on the single Widal test but no consensus as to its diagnostic value in regions in which typhoid is endemic (1, 2, 11, 14, 15, 17, 21, 22).

In our study, O and H antibodies were found by slide agglutination in 25% of healthy adults. One of the reasons for this high rate of seropositivity against serotype Typhi is the widespread presence of salmonella infections in the community. The other factors for the seroepidemiologic data are the cross-reactivity of serotype Typhi antigens with other salmonella infections and the longevity of these antibodies in the serum (20).

In a study from Malaysia, 61% of healthy adults were seropositive against the H antigen and 6% were seropositive against the O antigen (23). On the other hand, in a retrospective study from Holland, the rate of seropositivity obtained when using the Widal test was found to be 1% (13).

According to test performance criteria, the optimal titer for diagnosis was determined to be 1/200. Among 317 donor sera, O and H antibody titers $\geq 1/200$ were found in 7 and 2%, respectively, of the samples. In a recent study from Vietnam, the diagnostic Widal titers were defined as 1/200 for the O antibody and 1/100 for the H antibody. In Rhodesia, the titer was $>1/480$ for both O and H antibodies (18, 24). Similar results were found in countries where typhoid is endemic. The diagnostic titers for the Widal test ranges between 1/40 and 1/480 in India, Singapore, Ceylon, Malaysia, and the Philippines (3, 7, 17, 23).

Although the titers of O or H antibodies were $\geq 1/200$ in 8% of donor sera, for only one patient (0.3%) were both antibody titers $\geq 1/200$. This shows that the two antibodies have to be considered together. Because of this false positivity, the typhoid fever cannot be diagnosed by the Widal test alone, but this test can be helpful for the diagnosis of patients with a clinical profile related to typhoid.

Among the serotype Typhi blood culture-positive patients ($n = 31$), the O antibody titer was $\geq 1/200$ for 16 patients (52%) and the H antibody titer was $>1/200$ for 9 patients (29%). In

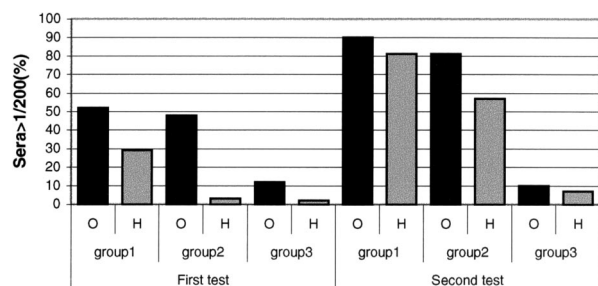


FIG. 2. Percentages of samples with O and H agglutinin titers $\geq 1/200$ in the acute (First test) and convalescent (Second test) phases.

the acute-phase sera, false negativity was 48% for the O antibody and 71% for the H antibody. In the follow-up serum specimen test which was performed 7 to 10 days later, O and H titers were $\geq 1/200$ for 90 and 81% of samples, respectively (Fig. 2).

The comparison of culture-positive typhoid fever patients (group 1) and febrile patients with etiologies of fever other than typhoid (group 3) was important to determine the reliability of the test, particularly in terms of differential diagnosis. In these groups, the O antibody titer of $\geq 1/200$ in the Widal test is 52% sensitive and 88% specific. By comparing these two groups, it can be said that for a $\geq 1/200$ titer in the Widal test, the diagnosis can be predicted in 76% of cases and can be ruled out in 71% of cases with an acuity of 72%. In comparing group 1 and group 3, the H antibody titer of $\geq 1/200$ has a 29% sensitivity, which was lower than that for O antibodies, and a 95% specificity, which was higher than that for O antibodies. This was an expected result since it was known that H antibody peaks later than O antibody in the serum.

The second Widal test for both group 1 and group 3 has a sensitivity and specificity of over 90%, a PPV of 88%, and an NPV of 93%, with 90% acuity. Similar results were reached for H antibodies. Since it is not practical to wait 7 to 10 days for diagnosis, this test must be considered a confirmatory tool.

The false seropositivity of the first test may be due to other infections including nontyphoid salmonella and collagenic and immunologic diseases. In group 3, among the eight patients with significant titers of O or H antibodies, five had paratyphoid B, two had brucellosis, and one had malaria.

The second test is also more reliable for the diagnosis of

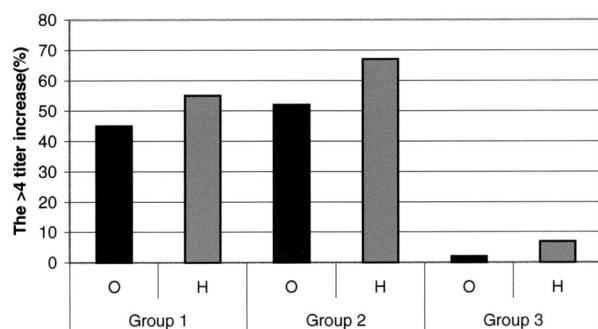


FIG. 3. Increases (≥ 4 -fold) of agglutinin titers for three patient groups.

patients in group 2. The ≥ 4 -fold increases in groups 1 and 2 are significantly higher than in group 3 and represent a greater sensitivity (Fig. 3).

Typhoid fever is still an endemic fecal-oral infection in Turkey, with a recorded number of 20,000 patients a year. In making the diagnosis, the isolation of bacteria from blood or bone marrow is the "gold standard," but widespread uncontrolled use of antibiotics leads to negative culture results. The culture facilities for isolation from bone marrow or blood are limited outside the teaching hospitals. In noneducational primary and secondary health centers, there is no other diagnostic tool yet that could substitute for the Widal test. In health centers with limited facilities, the Widal test $\geq 1/200$ titer may be helpful for its high specificity, although its sensitivity may be low.

In conclusion, (i) O and H agglutinin titers of $\geq 1/200$ are recommended as being considered of diagnostic significance, (ii) the Widal test is an easy, inexpensive, and relatively non-invasive test that can be of diagnostic value in situations where blood cultures cannot be obtained (but the results must be interpreted cautiously, as negative results do not exclude typhoid fever), and (iii) the Widal test performed 7 to 10 days after hospitalization gave the most-reliable results with high specificity, sensitivity, PPV, and NPV.

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