



“EVALUATING SINGLE ACUTE-PHASE WIDAL TEST FOR THE DIAGNOSIS OF TYPHOID FEVER”

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ABSTRACT (SUMMARY)

OBJECTIVE: *The study was conducted to evaluate the diagnostic utility of single acute-phase Widal test for the diagnosis of typhoid fever”.*

STUDY DESIGN: *Comparative case-control study*

PLACE & DURATION: *Department of Medicine, Muhammad Medical College Hospital Mirpurkhas from May 2007 to August 2008.*

METHODOLOGY: *This study comprised of 50 adults with a definitive diagnosis of typhoid fever, 50 adults with non-typhoid fevers, & 50 more young healthy adults in the control group. Serum samples were collected from patients and controls. The Widal tube agglutination test was done on all sera by the conventional agglutination method. Statistical analyses were performed using the computer package SPSS 10.0 for Windows.*

RESULTS: *Thirty five (70%) and fifteen (30%) of 50 typhoid fever cases had ‘TO’ and ‘TH’ agglutinin titer levels of =1:160, respectively as compared to only 3 (3%) and 1 (1%) among controls. These differences were significant (p=0.001). A ‘TO’ agglutinin titer of 1:160 had a specificity of 97%, a sensitivity of 70% and an accuracy of 90%. A ‘TH’ agglutinin titer of 1:160 had a specificity of 97%, a sensitivity of 30% and an accuracy of 83.1%. Based on the above analysis, ‘TO’ or ‘TH’ titers of 1:160 or more were indicative of typhoid fever. Similarly, when the 2 titers were considered together, either ‘TO’ or ‘TH’ titers of 1:160 or more, they were more highly significant (p=0.001).*

CONCLUSIONS: *It is concluded, that even today, Widal test remains one of the best, easily accessible, inexpensive and simple method for the diagnosis of typhoid fever.*

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INTRODUCTION:

Typhoid fever is a systemic infectious disease characterized by an acute febrile illness, the first typical manifestations of which are fever, headache, abdominal pain, relative bradycardia, splenomegaly, and leukopenia. *Salmonella.typhi (S.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^{1, 2, 3}. Pakistan is a hyper-endemic area for typhoid fever, & according to WHO 2008 report the incidence of typhoid fever in 5-15 years children was 412 per million in 2002⁴. The exact figures on the incidence and prevalence are not available for Pakistan, but the prevalence is deemed comparable to that in Southeast Asia. The reasons for high prevalence of typhoid fever pathogens in Karachi and indeed all over Pakistan include poor hygienic standards, low socioeconomic status and lack of clean water supply⁵. The signs and symptoms of typhoid fever are nonspecific, and an accurate diagnosis on clinical grounds alone is difficult. A definitive diagnosis can be made by isolation of *S.typhi* from blood or bone marrow by culture, which is regarded as “gold standard method”, but bacterial culture facilities are often unavailable, expensive, time consuming & usually negative because of prior antibiotic use^{6,7}. The sensitivity of blood culture alone is 50-70% because fewer than 15 organisms are present per millimeter of blood in patients with typhoid fever. When patients have already received antimicrobial therapy, blood cultures may be positive in only 40% of the cases¹². The Widal test has been in use for more than a century as an aid in the diagnosis of typhoid fever^{6,8}. In the original format, the Widal test required acute- and convalescent-phase serum samples taken approximately 10 days apart. More recently, the test has been adapted for use with a single, acute-phase serum sample^{9, 10}. It is a tube dilution test which measures agglutinating antibodies against the lipopolysaccharide O (TO) and protein flagellar H (TH) antigens of *S. typhi*.

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The value of the test for the diagnosis of typhoid fever has been debated for as many years as it has been available⁶. In developing countries the Widal test remains the only practical test available. Many studies, however, have produced data which have cast serious doubts on the value of Widal test. Classically, a four fold rise of antibody in paired sera is considered diagnostic of typhoid fever. However, paired sera are often difficult to obtain and specific chemotherapy has to be instituted on the basis of a single Widal test¹¹. The interpretation of a single acute phase widal test in endemic areas is difficult to interpret since majority of healthy individuals carry detectable antibodies². In view of the doubts expressed on the value of Widal test, we thought it worthwhile to reassess the utility of a single acute phase Widal test in the diagnosis of typhoid fever. The main purpose of this study was to provide local recommendations for the interpretation of Widal test results. This study also signifies the importance of single tube Widal test in the diagnosis of typhoid fever.

MATERIAL & METHOD:

The study was conducted at Department of Medicine, Muhammad Medical College Hospital Mirpurkhas from May 2007 to August 2008. All subjects were = 18 years of age. Single acute-phase sera samples for the Widal test were collected from patients and controls, which formed three groups. The data was collected on a structured proforma. The study group comprised 50 adults with a definitive diagnosis of typhoid fever as proved by the blood culture (GROUP I). The control group was comprised of 2 subgroups. There were 50 adults with non-typhoid fevers (GROUP II). The diagnosis in these patients was made on the basis of clinical evaluation and supported by laboratory investigations. There were 50 more young healthy adults in the control group (GROUP III). The Widal tube agglutination test was done on all sera by the conventional agglutination method. A 0.4 ml of two fold serially diluted patients sera (dilution from 1:20 to 1:320) in 0.9% normal saline were tested by adding an equal volume of antigen. A negative saline control was included in each batch of the test. A dilution ratio =1:160 was taken as significant titer indicative of typhoid fever. Statistical analyses were performed using the computer package SPSS for Windows. The Mann-Whitney U test was used for comparison of nonpaired samples, and the Wilcoxon sign rank test was used for comparison of paired samples. The sensitivity, specificity, positive predictive

TABLE. I:
SIGNIFICANT ANTI-TO & ANTI- TH AGGLUTININS LEVELS

	No. cases	Significant anti-TO or anti-TH titers (=1:160)	Non-significant anti-TO or anti-TH titers (<1:160)
Group I	50	35 (70%)	15(30%)
Group II	50	3(6%)	47(94%)
Group III	50	0(0%)	50(100%)

TABLE. II:
SENSITIVITY, SPECIFICITY, PPV*, NPV†, ACCURACY FOR ANTI-TO TITERS

Parameter	=1:20	1:40	1:80	1:160	1:320
Sensitivity	100	97.7	76.7	70	60
Specificity	48	74	90	97	100
PPV*	36.6	52.7	69.7	87.5	100
NPV†	100	98.7	92.1	91.5	100
accuracy	60	79.2	86.9	90	90

*positive predictive value

†negative predictive value

TABLE. III:
SENSITIVITY, SPECIFICITY, PPV*, NPV†, ACCURACY FOR ANTI-TH TITERS

Parameter	=1:20	1:40	1:80	1:160	1:320
Sensitivity	83.3	66.7	36.7	30	20
Specificity	81	94	98	97	99
PPV*	56.8	76.9	84	90	85.7
NPV†	92.4	90.4	83.7	82.5	80.5
accuracy	81.5	87.5	87.7	87.5	88.5

*positive predictive value

†negative predictive value

value (PPV), and negative predictive value (NPV) of the diagnostic tests were calculated using the following formulae: sensitivity is $a/(a+c)$, specificity is $d/(d+b)$, PPV is $a/(a+b)$, and NPV is $d/(d+c)$; in these formulae, a is test positive and true positive, b is test positive and true negative, c is test negative and true positive, and d is test negative and true negative, where true positive & true negative are the culture positive and negative respectively. A p-value of <0.05 was considered significant.

RESULTS:

The "TO" and "TH" agglutinin titer levels were assessed in a total of 150 cases comprising 50 adults with blood-culture positive typhoid fever, 50 adults with non-typhoid fevers and 50 healthy adults. The 50 non-typhoid fever patients included, pneumonia (n=7), pharyngitis (n=5), cholecystitis (n=5), dysentery (n=3), otitis media (n=7), malaria (n=10), urinary tract

infection (n=7), acute viral hepatitis (n=6) Thirty five (70%) out of 50 typhoid fever cases had a 'TO' agglutinin titer level of more than or equal to 1:160 as compared to only 3 (3%) among controls. And 15 (30%) out of 50 typhoid fever cases had a 'TH' agglutinin titer level of more than or equal to 1:160 as compared to only 1 (1%) among controls (p=0.001). Significant anti-TO & anti-TH levels are shown in table I. In order to assess the overall accuracy of the Widal test in predicting the presence or absence of typhoid fever 5 test parameters viz., sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated at each titer level. A 'TO' agglutinin titer of 1:160 had a sensitivity of 70%, a specificity of 97%, a positive predictive value of 87.5%, a negative predictive value of 91.5% and the highest overall accuracy of 90% (Table II). A 'TH' agglutinin titer of 1:160 had a sensitivity of 30%, a specificity of 97%, a

positive predictive value of 90%, a negative predictive value of 82.5% and an accuracy of 83.1% (Table III). This titer had a negligibly lower specificity and accuracy when compared with a titer of 1:80, and it had a higher positive predictive value (90% as compared to 84%). Hence, although a 'TH' agglutinin titer of 1:80 is suggestive, a titer of 1:160 was considered to be of greater significance. Similarly, when the 2 titers were considered together, either 'TO' or 'TH' titers of 1:160 or more, they were more highly significant ($p=0.001$). Based on the above analysis, a 'TO' titer in isolation, a 'TH' titer in isolation and 'TO' or 'TH' titers when considered together of greater than or equal to 1:160 were indicative of typhoid fever.

DISCUSSION:

Typhoid fever continues to be a major health problem for Pakistan. Rapid population growth, increased urbanization, inadequate human waste disposal, limited water supply and overburdened health care systems have all made disease control difficult and contribute to the endemicity^{5, 12}. The role of Widal test has been debated widely, because the sensitivity, specificity, and predictive values of this widely used test vary considerably among geographic areas¹⁴. Some centers have found Widal test helpful when it is used with locally determined cutoff points¹⁵. The present study reveals that a single Widal test is still a useful diagnostic tool in typhoid fever. A 'TO' titer in isolation, a 'TH' titer in isolation and a 'TO' or 'TH' titer when considered together, or more than or equal to 1:160, with relevant clinical findings was found to be highly suggestive of typhoid fever. At a titer more than or equal to 1:160, the sensitivity of the 'TO' titer (70%) was greater than that of the 'H' titer (30%), and the overall accuracy of the 'TO' titer was greater (90.8% compared to 83.1%). Hence, the 'TO' titer was considered to be of greater diagnostic significance. Similar observations are made by Kulkarni¹¹ et al & Willke¹ et al. Willke et al¹ has reported that TO and TH agglutinin titers of =1/200 are recommended as being considered of

diagnostic significance. In Rhodesia, very high titer of >1/480 for both TO and TH antibodies^{6, 16} has been reported of diagnostic significance. Similar results are reported from countries where typhoid is endemic¹. The diagnostic titers for the Widal test ranges between 1/40 and 1/480 in India, Singapore, Ceylon, Malaysia, and the Philippines¹. Parry et al⁶ have reported that a TO-agglutinin titer of =200 and a TH-agglutinin titer of =100 in an appropriate clinical context is likely to indicate typhoid in an area such as Vietnam, while the TO-agglutinin titer =400 or the TH-agglutinin titer =200, can diagnose the typhoid fever with reasonable confidence. According to Rab Z Z et al¹³, & Taiwo SS et al¹⁷, at a cut off titers of TO agglutinin = 80, the diagnostic sensitivity and specificity were 90% and 87.3% and for TH agglutinins =80, the diagnostic sensitivity and specificity were 90% and 88.5% respectively. Our results are highly comparable with the above mentioned studies.

CONCLUSIONS:

Our poor population cannot afford the latest expensive laboratory tests for the diagnosis of typhoid fever. So it is concluded, that even today, Widal test remains one of the best, easily accessible, inexpensive and simple method for the diagnosis of typhoid fever, if interpreted in an appropriate clinical context.

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