

WIDAL AGGLUTINATION TITRE: A RAPID SEROLOGICAL DIAGNOSIS OF TYPHOID FEVER IN DEVELOPING COUNTRIES

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Background: To study the reliability of a single Widal test and to find out the diagnostic significance of 'O' and 'H' agglutinin titre in the diagnosis of typhoid fever. **Methods:** Community-based case-control study conducted from Jan 2001 to June 2007. The blood samples were collected from the medical and out door department of Sir Ganga Ram Hospitals, Lahore. The diagnostic value of an acute phase single Widal agglutination test for suspected typhoid fever was evaluated in 733 consecutive patients with fever lasting 6 or more days. **Results:** In 733 patients with fever 84 (11.45%) were positive for Widal test. A noteworthy rise 1/320 of H and/or O agglutinin titre was observed in 86 (11.3%) of patients with typhoid fever. **Conclusion:** In the absence of vaccination an elevated level of H and/or O agglutinin titre of 1: 320 is of diagnostic value for typhoid fever especially in our setting where a single sample of serum is relied on for the diagnosis of typhoid fever.

Keywords: Typhoid, Widal test

INTRODUCTION

Typhoid fever, a food- and waterborne disease caused by *Salmonella enterica* serotype typhi (*S typhi*), is a serious public health problem in developing countries that claims 600,000 lives every year.¹ Typhoid fever was introduced by typhoid cases in the households and facilitated by poor hand-washing hygiene and sharing of food from the same plate.² The association of poor hand-washing hygiene and typhoid fever was shown in Indonesia and India.^{3,4} The sign and symptoms of uncomplicated typhoid fever are non specific and an accurate diagnosis on clinical grounds alone is difficult.⁵ Although a definitive diagnosis can be made by isolation of *Salmonella typhi* from blood or bone marrow.^{6,7}

Serological diagnosis relies classically on the demonstration of a rising titre of antibodies in paired samples at an interval of 10–14 days.⁸ In typhoid fever, however, a four fold rise after 2 weeks in not always demonstrable, even in blood culture confirmed cases. This situation may occur because the acute phase sample was obtained late in the natural history of the disease, because of high levels of background antibodies in an endemic region, or because in some individuals the antibody response is blunted by the early administration of an antibiotic.⁹

For practical purpose a treatment decision must be made on the basis of results obtained with a single acute phase sample. The cut-off for a positive Widal, chosen in a particular community depends on the background level of typhoid fever (i.e., the prior probability) and the level of typhoid vaccination, which may vary with time.⁸ The result may lack sensitivity and specificity particularly in a community with endemic typhoid fever.¹⁰

In endemic areas, such as Lahore-Pakistan the bacterial culture facilities are often unavailable. The Widal test is the most simple, over utilized, specific diagnostic investigation tool available in the local Laboratories of developing countries. It is relied upon because of its convenience.

Present study tried to evaluate the value of a single acute phase Widal test for the diagnosis of typhoid fever in city of Lahore.

MATERIAL AND METHODS

Community-based case-control study conducted from Jan 2001 to June 2007. The blood samples were collected from the medical and out door department of Sir Ganga Ram Hospitals, Lahore. The diagnostic value of an acute phase single Widal agglutination test for suspected typhoid fever was evaluated in 733 consecutive patients with fever lasting 6 or more days.

Blood culture vials from outpatient facilities were transported on the day of collection to Microbiology section of Department of Pathology of Fatima Jinnah Medical College, Lahore. Blood cultures were performed and cultured organisms were identified by customary method¹¹. The Widal test was performed with standardized kits (Sanofi Diagnostics). Serum samples of patients were screened with a slide agglutination test which measures agglutinating antibodies against the lipopolysaccharide 'O' and protein flagellar 'H' antigens of *S. typhi* and Para typhi A and B. Serial dilution of sera starting at a dilution of 1:40 were made with 0.9% saline and examined for visible agglutination. Appropriate positive and negative sera were included.

Table-1: Comparison of culture with Widal test

	Widal Positive	Widal negative
Culture Positive	82	146
Culture Negative	651	567

Table-2: H and/or O agglutinin titre in patients with typhoid fever

Antigen n (%)	1:40	1:80	1:160	1:320	1:640
TO	651 (89)	351 (48)	56 (8)	18 (2)	7 (1)
TH	668 (91)	400 (55)	26 (4)	21 (3)	18 (2)
AO	701 (96)	321 (44)	14 (2)	10 (1)	8 (1)
AH	711 (97)	432 (59)	8 (1)	8 (1)	6 (1)
BO	682 (93)	202 (28)	25 (3)	20 (3)	6 (1)
BH	707 (96)	176 (24)	17 (2)	9 (1)	-

RESULTS

Comparison of culture positive/negative sensitivity with Widal positive and negative agglutination was tabulated. In 733 patients with fever, 82 (11.5%) were positive for *Salmonella typhi* on both blood culture and Widal test. On the other hand, 146 patients with Widal negative showed positive culture sensitivity. 651 cases with Widal positive indicate a negative culture sensitivity, whereas 587 patients showed negative Widal and culture sensitivity (Table-1).

In this study, 48% patients with blood culture positive typhoid fever had detectable O antibodies at a cut-off titre of 1:80 and 55% had detectable H antibodies at a cut-off titre of 1:80. A substantial H/O agglutinin titre of 1/320 was observed in 2–3% cases while only 1–2% of patients had substantial H/O agglutinin titre of 1/640. Of the sera from culture proven typhoid cases, the majority of cases showed an increased value of both H and O in 2% of patients and a positive H-antigen titre of 1:160 in 5.5%. Paratyphoid fever due to *S. paratyphoid B* gave 'O' agglutinins titre of 1:160 in only 1.0% cases. A considerable H/O agglutinin titre of 1:320 and 1:640 was also observed in 1.0% cases of Para typhi A and B (Table-2).

DISCUSSION

Typhoid fever has continued to pose considerable health problems world-wide. The proportion of paratyphoid fever cases to typhoid fever cases may change due to urbanization and increased dependency on food purchased from street vendors. Because of the difficulties in isolating *S. typhi* from blood, stool or other body fluids in developing countries, a diagnostic Widal agglutination titre of 'O' and 'H' agglutinins will be considered useful in the diagnosis of typhoid fever in our environment.¹²

There are various difficulties associated with an evaluation of the Widal test in an area where malaria, bacterial septicaemia and now dengue fever

are common causes of admission with fever. It is found that the titre of agglutinins detectable in the non infected population of different areas vary considerably.⁹ It also depends on the level of infection due to other salmonellae with cross reacting antigens^{13,14} and selection of a satisfactory gold standard for diagnosis. It is therefore critical to evaluate the Widal tests in the area in which it is to be used.

In this study we have chosen blood culture positive patients as the confirmed typhoid fever. However some patients with typhoid fever having blood culture negative were included in the study particularly in endemic areas such as Lahore, where antibiotic treatment is common. Our theme of study was supported by the study¹⁵ of a group of workers who reported that in developing countries with a high incidence of typhoid fever co agglutination test is more reliable than culture. They also found that many patients have already taken antibiotics before being seen by a physician.

Previous typhoid vaccination may contribute to elevated agglutinins in the non infected population. There is no National Programme of typhoid vaccination in Pakistan. Present study observed that a high titre of antibodies to somatic O antigen (1:160 and 1:320) is consistent with acute typhoid and high titres (1:320) are even more suggestive of this condition. Antibodies to flagellar O antigen may be found in higher titres than H. A study is in contrast to our study who found Antibodies to flagellar H antigen may be found in higher titres than O.⁷

Present study observed 20% patients with Widal positive during a period of 06 years from some areas of Lahore city. In contrast to our study, a study found 9% fever patients with *Salmonella typhi*.¹⁶ Another study found 75% bacteriologically confirmed typhoid fever due to *S. typhi* (183 cases out of 244).¹⁷

We found a noteworthy H and/or O agglutinin titre of 1/320 in 47.6% of typhoid cases and in only 3% of patients considerable H and/or O agglutinin titre of 1/640. Of the sera from culture positive typhoid cases, the majority (79.9%) showed an increase in both H and O antigens. A number of studies^{1,11} reported highly specific and positive predictive value in 1:160 makes the Widal test acceptable as a diagnostic tool. A study also found that the sera from typhoid cases which gave a significant Widal reaction, the majority (79.9%) showed increases in both H and O agglutinins.¹ Another study reported that 37(92.5%) of patients with culture-confirmed typhoid fever had 'O' agglutinin and 38(95.0%) had 'H' agglutinin.¹¹ However a group of workers reported that O agglutinins a t a titre of 1:160 or more in 5% and 'H'

agglutinins a titre of 1:160 or more in 2% of 100 individuals.¹⁴ Study stated that either 'O' or 'H' can reach the diagnostic levels during the first week of typhoid fever and can be helpful in the diagnosis of illness owing to the hyperimmune state of patients.

Our results showed that 'O' titre of agglutinins of both Paratyphi A and B were more noteworthy than 'H' agglutinins. Number of studies confirmed out results.^{17,18}

It is therefore concluded that H and/or O agglutinin titre of 1: 320 is of diagnostic value by using the technique of Widal agglutination titre. A negative Widal test in a patient with a compatible history of typhoid fever does not rule it out. The level of 'O' agglutinins was found to be more helpful than the level of 'H' antigens. The Widal test is easy, inexpensive, and relatively non-invasive. It can be of diagnostic value when blood cultures are not available or practical.

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