



Research Article

COMPARISON OF TUBE WIDAL TEST AND SLIDE WIDAL TEST IN THE DIAGNOSIS OF ENTERIC FEVER

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Abstract- Background and Objective: Typhoid fever has been remaining important public health Problem in developing countries for the past hundred years. In developing countries, the culture facilities for isolation of Salmonella are often not available at smaller set ups and the patients usually present to the clinicians during the late course of the illness during which the organism could not be isolated from blood culture. So the Widal test still remains the only serological test to diagnose Typhoid fever. This study is aimed to evaluate the diagnostic accuracy and reliability of Slide Widal test with Tube Widal test in the diagnosis of enteric fever. **Methods:** Serum samples were obtained from 300 patients, clinically suspected to have Typhoid fever. Serum samples were also obtained from 300 patients with febrile diseases other than typhoid fever to use them as controls. All samples were tested using Slide Widal test, semi-quantitatively and Tube Widal test quantitatively for the presence of O and H agglutinins for *S typhi* in the Microbiology Diagnostic Laboratory, Govt. tertiary hospital over a period of six months (June 2011–November 2011). All samples which showed positive clumps in slide Widal agglutination tests were again tested with Tube Widal test. The significant titer for O agglutinin 1:80 and for H agglutinin 1:160 were taken as positive. **Results:** Slide Widal test showed many false positive reactions (56.6%), which were negative by Tube Widal test and clinically proved to be non-enteric cases. Of 276 slide positive samples only 99 (43.4%) samples showed positive by tube Widal test. Comparison of results of both the tests were carried out statistically to analyze the sensitivity, specificity, Positive Predictive value and Negative Predictive values using tube Widal test as a standard one. **Conclusion:** Slide Widal test should not be used to confirm the serological diagnosis of enteric fever and laboratories should perform only Tube Widal test, which is conventional, more reliable test for detecting antibodies and can be done in any set up. In addition, the slide Widal test has more sensitivity (75.3%) and Negative Predictive Value (77.2%) when compared to tube Widal test (Sensitivity 32.6%; NPV 59.6%). So the slide Widal test could be used as a good initial screening test for Typhoid fever.

Keywords- Typhoid fever, Slide Widal test, Tube Widal test, Titer

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Introduction

Typhoid fever is a life threatening systemic infection and an important cause of morbidity in developing countries. It is widely prevalent in India and several Tropical Countries with rapid population growth, increased urbanization, limited safe water, infrastructure, and health systems [1]. It is also recognized that a delay in diagnosis and institution of appropriate therapy may significantly increase the risk of adverse outcome and mortality [2]. 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 [3, 4]. Although the isolation of *Salmonella typhi* on bone marrow culture or blood culture remains the gold standard for diagnosing typhoid fever, this may be problematic in endemic areas where adequate microbiologic facilities are limited. The widespread availability and use of antibiotics in the community makes it frequently difficult to isolate the organism on blood cultures and alternative methods such as bone marrow cultures are invasive and difficult to obtain routinely in pediatric patients. Even though better techniques are readily available for cultural isolation of Salmonella, serological tests are useful for rapid diagnosis. Even after continuous practice of more than many decades in endemic areas, the Widal test still remains the better alternative to other methods in diagnosing enteric fever. The Widal test is cheaper when compared to other investigations and can be done easily in any ordinary laboratory setup.

It can be of diagnostic value when blood cultures are not available or practical. In later years, a rapid slide agglutination test was developed which is now the most commonly used technique in local laboratories because of its convenience. Slide Widal test is an easy and rapid screening test. Hence the present study is done to find out the diagnostic reliability and accuracy of slide Widal test with tube Widal test in the serological diagnosis of enteric fever.

Materials and Methods

This cross sectional comparative study was conducted in the Department of Microbiology at Govt. tertiary Medical College Hospital over a period of six months from June 2011- November 2011 on 300 febrile patients, clinically suspected to have enteric fever, and 300 patients with febrile diseases other than enteric fever that have been diagnosed after both clinical examination and laboratory investigation (as 150 patients with Fever of Unknown Origin, 50 patients with respiratory tract infection, 40 patients with rheumatoid arthritis, 30 patients with dengue infection, and 30 patients with malaria) were included in this study as control group. Patients presented within first two weeks of fever were excluded from the study as the O and H agglutinins for *Salmonella typhi* will begin to raise from second week onwards. Ethical committee clearance from the institution was obtained prior to the commencement of study. An informed oral consent was obtained from each patient before drawing blood samples. A total of 600 blood

samples were collected from both groups (Patients clinically proved to have typhoid, patients with non-typhoid illness), were centrifuged and sera were separated. All serum samples were screened by slide agglutination test, qualitatively as per the manufacturer's instructions. The results were interpreted and the samples showing clumping within a minute was considered as positive reaction, were further taken up for semi-quantitative slide agglutination test and all 600 samples were then subjected to Tube agglutination test in doubling dilutions of 1:20, 1:40, 1:80, 1:160, 1:320, 1:640. All tubes were mixed well and incubated at 37°C for 24 hrs. The results were read as compact granular appearance for O agglutinin positive reaction and as cotton wooly floccules appearance for H agglutinin positive reaction. The significant titre for 'O' agglutinin was considered as 1:80 and for 'H' agglutinin, 1:160

Results

A total of 300 serum samples were tested from clinically suspected enteric fever cases. In slide Widal test, O agglutinin was negative in 44 samples; positive 1:80 for 104 samples; 1:160 for 91 samples; and 1:320 for 31 samples. H agglutinin was negative in 30 samples; positive 1:160 for 109 samples; 1:320 for 103 samples and 1:640 for 14 samples. [Table-1] In tube Widal test, O agglutinin was negative in 121 samples; positive 1:80 for 45 samples; 1:160 for 47 samples and 1:320 for 6 samples. H agglutinin was negative in 81 samples; positive 1:160 for 62 samples, 1:320 for 28 samples and 1:640 for 8 samples. [Table-2] Of the 300 hospital controls diagnosed with febrile illnesses other than typhoid, 50 serum samples gave positive agglutination result when using slide Widal test, while one sample was positive at 1:320 titre in tube Widal test [Table-4 & 5]. Higher rate of positivity was found in slide Widal test (75.3%) than tube Widal test (32.7%) [Table-3]. The H antigen showed high positive reactions than O antigen in all the titers. The titer level was higher in slide Widal than tube Widal test [Table-1 & 2]. All the results were analyzed; Sensitivity, Specificity, Positive Predictive value, and Negative Predictive values were calculated for both the tests using appropriate statistical methods (Fisher's Exact test) and significance between the two tests were calculated by Chi Square test (P < 0.001) [Table-3 & 6].

Table-1 Serum antibody titers for O and H antigens of Typhoid by Slide Widal test

SLIDE WIDAL TEST- SEMI QUANTITATIVE METHOD						
Serum Antibody titers						
ANTIGENS	1:20	1:40	1:80	1:160	1:320	1:640
O	21	23	104	91	31	0
H	09	11	10	109	103	14

Table-2 Serum antibody titers for O and H antigens of Typhoid by Tube Widal test

TUBE WIDAL TEST - QUANTITATIVE METHOD						
Serum Antibody titers						
ANTIGENS	1:20	1:40	1:80	1:160	1:320	1:640
O	79	42	45	47	06	0
H	32	29	20	62	28	8

Table-3 Comparison of results for SlideWidal test and Tube Widal test

TEST	POSITIVE (%)	NEGATIVE (%)	TOTAL (%)
SLIDE WIDAL	226 (75.3)	74 (24.7)	300 (100)
TUBE WIDAL	98 (32.7)	202 (67.3)	300 (100)
P Value	P < 0.001	P < 0.001	

Table-4 Performance of Tube Widal test among Typhoid and Non Typhoid cases

Tube Widal Test	TYPHOID CASES		NON TYPHOID CASES	
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
	98	202	1	299
TOTAL	300	300		

Table-5 Performance of Slide Widal test among Typhoid and Non Typhoid cases

Slide Widal Test	TYPHOID CASES		NON TYPHOID CASES	
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
	226	74	50	250
TOTAL	300	300		

Table-6 Sensitivity, Specificity, Positive Predictive and Negative Predictive Values of Widal Slide agglutination test in comparison with Tube Widal test (by Fisher's Exact Test)

PARAMETER	SENSITIVITY	SPECIFICITY	PPV*	NPV**
Slide Widal Test (%)	75.3	83.3	81.8	77.2
Tube WidalTest (%)	32.7	99.7	98	59.6

* Positive Predictive Value.

** Negative Predictive value

Discussion

In our Country, Widal test is used for diagnosis of Enteric fever. The Widal test is cheaper and can be done easily in any ordinary laboratory setup. It can be done when blood culture is not available. Blood culture is 40%- 60% sensitive and repeated blood sample collection makes the culture technique more invasive. From various studies observed worldwide, the sensitivity of slide Widal test ranges from 73% - 92% and the specificity 72% -82%. In similar way the sensitivity of tube Widal test varies from 61%-82% and the specificity is from 88% -100%. In most of the previous studies conducted worldwide, the slide Widal test showed high sensitivity (92%) and the tube Widal test showed high specificity (100%). In the present study also similar findings are observed. Sherwal *et al.*, (2004) and Zulfikar Ahmed Bhutta *et al.*, (1999) in their study have reported that tube Widal test had sensitivity of 57% and specificity of 83% [5]. Jim Pruckler *et al.*, (2004) have reported that sensitivity of tube Widal test is 64% and specificity is 76% [6]. Regarding the Positive Predictive Value (PPV) and Negative Predictive values (NPV) of tube Widal test from various studies, it ranges 88% -100% and 43%-61% respectively [7]. In the present study also the tube Widal test showed higher specificity (99.7%) and high Positive Predictive Value (98%) than the slide Widal test which is similar to other studies, by Oslén *et al.*, (2004) and Willke *et al.*, (2002). In the present study, the slide Widal test showed higher sensitivity (75.3%) and Negative Predictive value (77.2%) when compared to tube Widal test which is similar to Olsen *et al.*, (2004) study. Similarly, Karen H Keddy *et al.*, (2011) have reported that the semi-quantitative slide agglutination test performed the worst and had very poor specificity and low PPV and hence an unreliable test [8]. Many false positives were observed by Ayse Willke *et al.*, (2002) and Dr. Jagadish C Das (2007) in slide Widal test [9]. In contrast, slide test have been reported to be sensitive and specific by Henry Welch *et al* (1939) and IndroHandojo *et al.*, (2004) [10].

In this study, high level of positivity was seen with O and H agglutinins in the slide Widal test similar to a study by Karen H Keddy *et al.*, (2011) [8]. Also, H agglutinin showed higher level of positive results than O agglutinin similar to a study by Basaca - sevilla *et al.*, (1979) and Roohi Aftab *et al.*, (2009) [11,12].

In the present study, slide Widal test gave positive results in many non - typhoid fever cases (16.7%). There is attributed to the possibility of cross-reactivity with non-bacterial infections such as malaria, dengue, hepatitis A, and infectious mononucleosis [13,14]. The sensitivity and Specificity of both the tests increases with when the positive titer 1:80 for 'O' agglutinin and 1:160 for 'H' agglutinin. It has been argued from studies that the Positive Predictive Value (PPV) is the most important measure of a diagnostic test since it represents the proportion of patients with positive test results that are correctly diagnosed [15]. From this, it is clear that because of poor Positive Predictive Value of slide Widal test it should not be used to detect the true typhoid cases among clinically suspected cases in endemic areas [16-21].

Conclusion

It is concluded, that even today, the Widal test remains one of the best, easily

accessible, cheap and simple method for the diagnosis of typhoid fever in endemic areas where there are limited set ups for culture isolation.

The slide Widal test, performed by 90% of laboratories is convenient, simple to set up, a fast process, may be used as a good screening test. But this test has several limitations. It leads to many false positive reactions and has very poor specificity and low reliability. Patients without typhoid fever may receive unnecessary and in appropriate antimicrobial treatment which may lead to development of drug resistance. Moreover, slide agglutination test is best for antigen detection and tube agglutination test is best for antibody detection. Hence, the slide Widal test, though provides a rapid diagnosis should not be used as a diagnostic tool due to the above limitations. Slide Widal test may be adopted as an initial screening procedure and all the positive samples should be confirmed by tube Widal test further. Because of high specificity and high PPV, the tube Widal test should alone be used for confirming the serological diagnosis of Typhoid fever and not the slide Widal test.

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Abbreviations

PPV: Positive Predictive Value

NPV: Negative Predictive value

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Author Contributions:

Type of Contribution	Author one	Author two
Concept and Design of the study	✓	
Definition of Intellectual content	✓	
Literature search		✓
Clinical studies		✓
Laboratory studies	✓	✓
Data acquisition	✓	
Data analysis	✓	✓
Manuscript preparation	✓	✓
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