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Research Article

Comparative evaluation of slide agglutination and Widal tube agglutination test in detecting enteric fever among patients attending a tertiary care hospital in North India

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ABSTRACT

Background: Enteric fever is a major public health problem with significant morbidity and mortality in developing countries. Although, isolation of causative organism from blood is the standard laboratory method, but due to frequent use of self-medication by patients, and its long turnaround time, it is seldom used, and enteric fever is usually diagnosed by using serological methods. Widal tube agglutination test is the standard serological test used, which is now a days replaced by slide agglutination test due to its convenience and rapidity. The present study was done to comparatively evaluate the slide agglutination and Widal tube agglutination test in detecting enteric fever.
Methods: A hospital based cross-sectional study was done from January to June 2016. A total of 117 patients with clinical presentation suggestive of enteric fever were included in the study whose venous blood was collected and serum was tested by both slide agglutination and Widal tube agglutination test.
Results: Out of 117 patients, slide agglutination test showed positive results in 39 (33.3%) samples, whereas, Widal tube agglutination test showed positive results in 23 (19.7%) samples only. The slide test had a sensitivity of 91.3%, specificity of 80.9%, positive predictive value of 53.8% and negative predictive value of 97.4% as compared to Widal tube agglutination test.
Conclusions: Due to high false positivity shown by slide test, it is suggested that serological diagnosis should not be made solely on the basis of slide test rather its results should be confirmed by using Widal tube agglutination test.

Keywords: Enteric fever, Slide agglutination test, Widal tube agglutination test, Sensitivity, Specificity

INTRODUCTION

Enteric fever which includes typhoid and paratyphoid fever is a life threatening systemic febrile illness caused by the bacterium *Salmonella enterica* subspecies *Enterica serovar Typhi* and *Salmonella enterica* subspecies *Enterica serovar Paratyphi* A, B or C respectively. It is a major public health problem with significant morbidity and mortality in the developing countries such as India where typhoid fever is very prevalent. The World Health Organization (WHO) estimate for annual global

incidence of typhoid fever is about 21 million cases of which more than 13 million cases occurring in Asia alone.[1-3] Without effective treatment, typhoid fever has a case-fatality rate of 10-30%. This number is reduced to 1-4% in those receiving appropriate therapy. Results obtained from the laboratory are important in confirming the clinical diagnosis of typhoid and contribute to the effective management and treatment of typhoid cases.[4-6]

The standard method for the laboratory diagnosis of enteric fever is the isolation of causative organism from specimens especially blood, faeces, urine or other body fluids. Isolation of *Salmonella typhi* from bone marrow is the current gold standard method for confirming a case of typhoid fever. However, this requires equipments and trained laboratory personnel seldom found in primary health-care facilities in the developing countries.[7] A blood culture gives positive results in 73-97% cases, when the sample has been taken in the early course of disease prior the use of any antibiotics.[8,9] However, in developing countries like India, sensitivity of blood cultures is lower as patients usually visit the hospital late in the course of disease and frequently they have taken antibiotics as self-medication or upon unauthorized prescription before visiting the hospital. Other demerits of the test are its cost and relatively long turnaround time.[1,10] For this reason, in developing countries typhoid cases are diagnosed with the help of serological tests (agglutination tests) which is simple, rapid, inexpensive and considered next in value to blood culture.[4]

Two types of agglutination techniques are available: the slide agglutination test and the Widal tube agglutination test. The Widal test, named after Georges Fernand Isidore Widal, has been used in the diagnosis of typhoid fever for more than a century and to date remains the only practical test available, particularly in developing endemic countries.[7] This serological test measures the agglutinating antibodies in patient's serum, against the lipopolysaccharide somatic (O) antigens of *Salmonella typhi* and protein (H) antigens of *Salmonella typhi*, *Paratyphi A* and *B*. The tube agglutination test requires greater technical work and it takes 18-24 hours to get the results. So, in later years, Welch et al introduced a slide test which is a rapid test and thus used as a screening procedure.[11] The slide test is now the most commonly used technique in local laboratories because of its convenience. Practically the diagnosis is often formed on the basis of the slide agglutination results which are available within minutes, however, tube agglutination is useful to clarify erratic or equivocal agglutination reactions obtained by the more rapid slide test.[12] Both the above tests have their own merits and demerits.

Keeping the above facts in mind, the present study was done to comparatively evaluate the slide agglutination and Widal tube agglutination test in detecting enteric fever among patients attending our tertiary care hospital of North India.

METHODS

A hospital based cross-sectional study was done during a period of 6 months from January to June 2016. The study was approved by Institutional Ethical Committee. An informed consent was taken from all patients included in the study prior to sample collection. A pre-designed questionnaire was used to extract socio-demographic information of enrolled patients.

Inclusion criteria

Patients of all age group and both sexes attending the In-patient (IPD) and out-patient (OPD) of Department of Medicine and Pediatrics with clinical presentation suggestive of enteric fever i.e. continuous high fever for ≥7 days with no obvious focus of infection, abdominal discomfort with either constipation or diarrhea, coated tongue, hepatosplenomegaly, relative bradycardia, with or without rose spots on chest, abdomen and back.

Exclusion criteria

Very severely ill patients suffering from other non-enteric diseases and those who were found to be diagnosed with other diseases such as malaria, hepatitis, dengue etc., patients on antibiotics, patients who are recently vaccinated and patients who have refused to give consent were excluded from the study.

Study subjects

Blood samples from a total of 117 cases clinically suspected of enteric fever were collected and subjected to both slide agglutination and Widal tube agglutination test in the serology laboratory of Department of Microbiology.

Sample collection

From each individual included in the study, under strict aseptic precautions 3 ml venous blood was withdrawn in a well labeled plain vaccutainer tube. The blood was allowed to clot followed by centrifugation of the tube at 3000 rpm for 15 min to separate serum.

The sera were subjected to slide agglutination method. The test was performed as per the manufacturer's instructions (Span diagnostics Ltd., India). One drop (50 µl) of undiluted test serum was placed on the circles of slide provided in the kit along with positive control serum followed by addition of one drop (50 µl) of antigens "O", "H", "AH" and "BH". The contents were mixed with separate applicator stick and the slide was rocked gently for 1 minute. If no agglutination was observed, the test was considered negative. If agglutination was visible within 1 minute the test was considered as positive and was titrated for the amount of antibodies by using the semi-quantitative slide test as per manufacturer's recommendations.

All the samples were subjected to tube Widal tube agglutination method to confirm the results of slide agglutination test. Here, 4 rows containing 6 tubes in each row were set for each serum sample to be tested. Doubling dilutions of the test serum i.e., 1:10, 1:20, 1:40, 1:80, 1:160 and 1:320 were prepared in all the rows so that each tube contained 0.4 ml of the diluted serum. To these tubes stained Salmonella antigens (Span Diagnostics Ltd, India) were subsequently added. In the first and the second rows, 0.4 ml of *Salmonella typhi*-O and H antigens, and in the third and fourth rows, 0.4 ml of Salmonella Paratyphi-AH and BH antigens were added respectively. So the final serum dilutions obtained for each antigen were 1:20, 1:40, 1:80, 1:160, 1:320 and 1:640.[13] Appropriate control tubes with saline and antigen suspensions were included to rule out auto agglutination of the reagent. The rack containing all the tubes was then incubated at 37°C in a water bath overnight. Macroscopic agglutination was noted and recorded with the help of X2 magnifying lens on the following day after keeping the rack at room temperature. Antibody titre was taken as the highest dilution of serum giving visible agglutination which was matched against the currently used local cut off titre to confirm positivity of the test. The titres of ≥80 for "O" antigen and ≥160 for "H" antigen were taken as local cut off titres (baseline or significant titres) to determine positivity of both the slide agglutination and Widal tube agglutination test.[8]

Statistical analysis

The collected data was transferred to a computer. The SPSS Data Editor Software version 20 was used for analysis of the data. Chi-square test was performed and p value ≤0.05 were considered statistically significant.

RESULTS

A total of 117 cases clinically suspected of enteric fever were enrolled in the study whose blood samples were tested serologically. The mean age of cases was 24.6 (±16.4) years which ranged from 2 to 69 years, with 68 (58.1%) males and 49 (41.9%) females. Out of 117 cases, 87 (74.4%) cases belonged to rural areas and 30 (25.6%) cases belonged to urban areas. Out of 117 cases, 35 (29.9%) belonged to out-patient department (OPD) and 82 (70.1%) belonged to in-patient department (IPD). Of these 117 cases, 84 (71.8%) were referred from Medicine department and 33 (28.2%) were referred from Pediatrics department. The blood samples from these 117 cases were first subjected to slide agglutination test. Out of 117 samples, slide agglutination test showed positive results in 39 (33.3%) samples and negative results in 78 (66.7%) samples. Positivity was for *Salmonella typhi* "O" and "H" antigens only.

Table 1: Age group, sex and residence profiles of suspected enteric fever cases and their relation with slide agglutination test reactivity (N = 117).

Characteristics		Slide agglutination test			Chi-Square (χ^2) value & *p value
		Positive, N=39 (33.3%)	Negative N=78 (66.7%)	Total N=117 (100%)	
Age group	0-9 years	14 (56.0%)	11 (44.0%)	25 (100%)	χ^2 =14.149, df=6, p=0.028
	10-19 years	11 (45.8%)	13 (54.2%)	24 (100%)	
	20-29 years	8 (27.6%)	21 (72.4%)	29 (100%)	
	30-39 years	3 (16.7%)	15 (83.3%)	18 (100%)	
	40-49 years	1 (11.1%)	8 (88.9%)	9 (100%)	
	50-59 years	1 (33.3%)	2 (66.7%)	3 (100%)	
	60-69 years	1 (11.1%)	8 (88.9%)	9 (100%)	
Sex	Male	30 (44.1%)	38 (55.9%)	68 (100%)	χ^2=8.498, df=1, p=0.004
	Female	9 (18.4%)	40 (81.6%)	49 (100%)	
Residence	Rural	34 (39.1%)	53 (60.9%)	87 (100%)	χ^2=5.043, df=1, p=0.025
	Urban	5 (16.7%)	25 (83.3%)	30 (100%)	

* p<0.05 was considered as statistically significant. df =degree of freedom. N=Number of patients

The socio-demographic profile of the 117 cases of suspected enteric fever and their relation to slide agglutination test reactivity is shown in Tables 1-4. As shown in Table 1, maximum positivity was found in age group of 0-9 years (56.0%), followed by 10-19 years (45.8%) and 50-59 years (33.3%). This finding was found to be statistically significant (p=0.028). More positive cases were found in males (44.1%) as compared to females (18.4%). Seropositivity was found to be more amongst patients belonging to rural areas (39.1%) as compared to those who belonged to urban areas (16.7%). Both these findings were found to be statistically

significant (p=0.004 and p=0.025 respectively). As shown in Table 2, maximum seropositivity was found in Illiterates (46.2%), followed by those who had primary level of education (44.4%). This finding was also found to be statistically significant (p=0.005). Table 3 showed that maximum seropositivity was found in unemployed (40.0%), followed by unskilled workers (35.0%). This finding was found to be statistically significant (p=0.001).

Maximum seropositivity was found in those patients who socio-economically belonged to lower class (55.3%) followed by those who belonged to middle class (23.1%) as shown in Table 4. This finding was also found to be highly statistically significant (p=0.002).

All the 117 samples were then subjected to Widal tube agglutination test. Out of 117 samples, Widal tube agglutination test showed positive results in 23 (19.7%) samples and negative results in 94 (80.3%) samples. Positivity was for *Salmonella typhi* "O" and "H" antigens only. Table 5 shows the comparative evaluation of slide agglutination and Widal tube agglutination test. The difference in the findings of the two tests was found to be highly statistically significantly different (p<0.001).

Table 2: Educational status of suspected enteric fever cases and their relation with slide agglutination test reactivity (N = 117).

Educational status	Slide agglutination test			Chi-square (χ^2) value & *p value
	Positive N (%)	Negative N (%)	Total N (%)	
Graduate and above	1 (7.7%)	12 (92.3%)	13 (100%)	χ^2 = 16.810, df = 5, p=0.005
High school	8 (20.5%)	31 (79.5%)	39 (100%)	
Primary	4 (44.4%)	5 (55.6%)	9 (100%)	
Pre-primary	5 (29.4%)	12 (70.6%)	17 (100%)	
Illiterate	12 (46.2%)	14 (53.8%)	26 (100%)	
Not applicable#	9 (69.2%)	4 (30.8%)	13 (100%)	
Total	39 (33.3%)	78 (66.7%)	117 (100%)	

* p<0.05 was considered as statistically significant. df =degree of freedom. N=Number of patients.
# Not applicable: Children below the age of 7 years.

Table 3: Occupational profiles of suspected enteric fever cases and their relation with slide agglutination test reactivity (N=117).

Occupational status	Slide agglutination test			Chi-square (χ^2) value & *p value
	Positive N (%)	Negative N (%)	Total N (%)	
Professionals	1 (20.0%)	4 (80.0%)	5 (100%)	χ^2 = 24.094, df=7, p = 0.001
Skilled workers	0 (0.0%)	6 (100%)	6 (100%)	
Semi-skilled workers	2 (20.0%)	8 (80.0%)	10 (100%)	
Unskilled workers	7 (35.0%)	13 (65.0%)	20 (100%)	
Unemployed	2 (40.0%)	3 (60.0%)	5 (100%)	
Housewife	4 (15.4%)	22 (84.6%)	26 (100%)	
Student	9 (33.3%)	18 (66.7%)	27 (100%)	
Not applicable#	14 (73.3%)	4 (26.7%)	18 (100%)	
Total	39 (33.3%)	78 (66.7%)	117 (100%)	

* p<0.05 was considered as statistically significant. df =degree of freedom. N= Number of patients.
# Not applicable: Patients below the age of 18 years. Those patients who were students have been included in the student group.

Table 4: Socio-economic status profiles of suspected enteric fever cases and their relation with slide agglutination test reactivity (N=117).

Socio-economic status	Slide agglutination test			Chi-Square (χ^2) value & *p value
	Positive N (%)	Negative N (%)	Total N (%)	
Upper class	3 (21.4%)	11 (78.6%)	14 (100%)	χ^2 = 12.193, df = 2, p = 0.002
Middle class	15 (23.1%)	50 (76.9%)	65 (100%)	
Lower class	21 (55.3%)	17 (44.7%)	38 (100%)	
Total	39 (33.3%)	78 (66.7%)	117 (100%)	

* p<0.05 was considered as statistically significant. df = degree of freedom. N = Number of patients.

Table 5: Sensitivity and specificity of slide agglutination test taking Widal tube agglutination test as standard (N=117).

Slide agglutination test	Widal tube agglutination test			Chi-Square (χ^2) value & *p value	Sensitivity of slide test	Specificity of slide test
	Positive N (%)	Negative N (%)	Total blood samples tested N (%)			
Positive N (%)	21 (91.3%)	18 (19.1%)	39 (33.3%)	χ^2 = 43.293, df=1, p<0.001	91.3%	80.9%
Negative N (%)	02 (8.7%)	76 (80.9%)	78 (66.7%)			
Total blood samples tested N (%)	23 (100%)	94 (100%)	117 (100%)			

* p<0.05 was considered as statistically significant. df =degree of freedom. N=Number of patients.

Table 6: Positive predictive value and negative predictive value of slide agglutination test taking Widal tube agglutination test as standard (N = 117).

Slide agglutination test	Widal tube agglutination test			Chi-Square (χ^2) value & *p value	Positive predictive value of Slide test	Negative predictive value of Slide test
	Positive N (%)	Negative N (%)	Total blood samples tested N (%)			
Positive N (%)	21 (53.8%)	18 (46.2%)	39 (100%)	χ^2 = 43.293, df=1, p<0.001	53.8%	97.4%
Negative N (%)	02 (2.6%)	76 (97.4%)	78 (100%)			
Total blood samples tested N (%)	23 (19.7%)	94 (80.3%)	117 (100%)			

* p<0.05 was considered as statistically significant. df = degree of freedom. N = Number of patients.

As shown in Table 5, the actual positivity by both slide and Widal tube test was seen in only 53.8% (21 out of 39) cases while 18 (19.1%) cases were incorrectly labeled as positive (false positive) by slide agglutination tests since they actually had titres less than the cut off levels as detected by Widal tube agglutination test, whereas, 2 (8.7%) cases, were incorrectly labeled as negative (false negative) by slide agglutination test. The slide agglutination test had a sensitivity of 91.3% and specificity of 80.9% as compared to Widal tube agglutination test shown in Table 5. The slide agglutination test had a positive predictive value (PPV) of 53.8% and negative predictive value (NPV) of 97.4% as compared to Widal tube agglutination test shown in Table 6.

DISCUSSION

Enteric fever is a major health problem causing significant morbidity & mortality in developing countries mainly due to poor sanitary condition.[4] Although, definitive diagnosis of enteric fever depends on the isolation of causative organism from blood, bone marrow or other body fluids. However, the widespread and uncontrolled use of antibiotics leads to negative results in culture. Moreover, considering the poor facilities for the isolation of bacteria by the culture methods in the peripheral health centres and rural clinics, no other diagnostic tool is introduced thus far, and the Widal test is still considered appropriate for the diagnosis of enteric fever.[1]

Originally Widal test was recommended using paired sera, 1-2 weeks apart & demonstrating four-fold or greater rise of antibody titre. However, in typhoid fever, a rise in titre between acute and convalescent sera is not always demonstrable even in blood culture confirmed cases, owing to the natural history of the infection, prior antibiotic administration or late presentation to the hospital. Patient management decisions cannot be put off for the results of convalescent phase sera and for all practical purposes, a treatment decision must be made on the basis of a single tube Widal test.[14] Hence, in our study single tube Widal agglutination test was performed. Now a day, slide agglutination test has largely replaced Widal tube test due to its inexpensive nature and rapid results especially in developing countries where the disease is endemic and needs rapid diagnosis.

In our study, positivity by slide agglutination test was found to be 39 cases (33.3%). The positivity by slide agglutination test was found to be more amongst male patients (44.1%) as compared to female patients (18.4%). This finding is similar to another study which also found high seropositivity among male patients (65.0%) as compared to female patients (35%).[4] Although both the sexes can be affected by enteric fever, but the higher frequency of infection among males may be attributed to the fact that men undergone the responsibility of working outdoor and eventually are in greater contact with the pathogens rather than the women who are more confined to indoor works.

In our study, maximum positivity was found in age group of 0-9 years (56.0%), followed by 10-19 years (45.8%). This is similar to another study which also showed that majority of the cases (21.25%) belonged to the age group of 1-5 years followed by the age group of 6-10 years (17.5%).[4] Infection can occur at any age but result of another study also showed that the prevalence of typhoid fever in children of under 5 years were much higher than other age group.[15] In our study, seropositivity was found to be more amongst patients belonging to rural areas (39.1%), those who were children aged <7 years (69.2%), followed by illiterates (46.2%) and those who were educated only up to primay school (44.4%), and those who belonged socio-economically to lower class (55.3%). All these findings could be explained on the basis of their poor knowledge about hygiene and following of poor sanitatory habits, both these are important factors responsible for prevalence of enteric fever in developing countries.

In our study, the sensitivity and specificity of slide agglutination test was found to be 91.3% and 80.9% respectively. This is found to be similar to another study which showed sensitivity and specificity of slide test to be 100% and 84.9% respectively.[8] Another study showed sensitivity and specificity of slide test to be 53-80% and 57-90% respectively.[16]

It has been found that the positive predictive value (PPV) is the most important measure of a clinical diagnostic method since it represents the proportion of patients with positive test results that are correctly diagnosed.[17] In our study, slide agglutination showed positivity in 39 (33.3%) cases, whereas, true positives were only 21 (53.8%) cases and 18 (19.1%) cases were falsely reported as positive by slide test. Hence, the PPV of slide agglutination test was found to be quite low (53.8%). Similar results of high false positivity and low PPV of slide agglutination (65.5% and 28.9% respectively) were also detected in various earlier done studies.[1,8] This clearly proves that, although the slide agglutination test is useful in screening out negative samples, the positivity expressed by it is not always helpful in diagnosing the disease correctly. The high false positivity exhibited by slide agglutination in the present study could be attributed to the endemicity of the disease in our region and possible presence of cross reacting antibodies of other bacterial and non-bacterial infections. If the slide agglutination test is solely relied upon for diagnosis, a significant number of samples would have been falsely labeled as positive and clinicians would have started antimicrobial chemotherapy. This could lead to serious consequences by unnecessarily pressuring the normal gut flora to develop antibiotic resistance and thus further increasing patient's sufferings.[8]

CONCLUSION

The slide agglutination test is an easy and inexpensive test that can be of diagnostic value in situations where blood cultures cannot be obtained for diagnosis of enteric fever. Now a day, it has replaced Widal test due to its rapidity and convenience. Slide agglutination test is actually a common practice in many resource-constrained laboratories. However, the high false positivity and low positive predictive value shown by the slide agglutination test cautions that the results of slide test should not be solely relied upon for diagnosis and treatment of enteric fever, and should always be confirmed by the Widal tube agglutination test and interpreted with reference to clinical data.

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