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COMPARISON OF QUANTITATIVE TUBE AGGLUTINATION AND SEMIQUANTITATIVE SLIDE AGGLUTINATION TEST FOR DIAGNOSING OF THE SUSPECTED CASES OF ENTERIC FEVER

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Abstract

Introduction: Enteric fever is the major health problem of developing country like India, with a notable morbidity and mortality. Isolation of Salmonella the causative agent from Blood is the standard laboratory method for diagnosis, but it is not available at PHC level. So, rapid and affordable diagnostic test like Widal tube and slide agglutination test are used. The present study was done to comparatively evaluate the Widal slide agglutination and tube agglutination test in detecting enteric fever.

Methods: A total of 500 patients with clinical presentation suggestive of enteric fever were included in the study whose venous blood was collected. All the samples were tested for the presence of anti O and anti H agglutinins against S. typhi and S. paratyphi A by semi quantitative slide and quantitative tube agglutination tests as per standard protocols. The titers of 1:80 (O agglutinins) and 1:160 (H agglutinins) were taken as the significant titer for the diagnosis of enteric fever.

Results: Out of 500 collected samples, 183 (36.6%) was positive by slide agglutination test, whereas, only 145 (29%) were positive by tube agglutination method. The slide test had a sensitivity of 97.2%, specificity of 88.1%, positive predictive value of 77% and negative predictive value of 98.7% 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 is a major public health problem in the developing countries such as India with significant morbidity and mortality where typhoid fever is very prevalent. Enteric fever includes typhoid and paratyphoid fever both are 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.. The World Health Organization (WHO) estimate for annual global incidence of typhoid fever is about 21 million cases.[1-3] Typhoid fever has a case-fatality rate of 10-30%, without effective treatment. This number has been reduced to 1- 4% in those receiving appropriate antibiotic and supportive therapy. Laboratory diagnosis plays crucial role in confirming the clinical diagnosis of typhoid and contribute to the effective management and treatment of typhoid cases.[4-6]

Isolation of the causative organism is the standard method for the laboratory diagnosis of enteric fever from the specimens especially blood, faeces, urine or other body fluids. Current gold standard method for confirming the diagnosis of typhoid fever is isolation of *Salmonella typhi* from bone marrow. However, in primary health-care facilities in the developing countries the equipments and trained laboratory personnel required of these methods are rarely found.[7] A blood culture gives positive results in 73-97% cases, when the sample has been taken in the first week of the disease prior the use of any antibiotics.[8-9] The demerits of the test are its cost and relatively long turnaround time. 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. [10] For this reason, the diagnosis of Enteric fever in developing countries are diagnosed with the help of serological tests which are simple, rapid, inexpensive and considered next in value to blood culture.[6]

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. Two types of agglutination techniques are available: the slide agglutination test and the Widal tube agglutination test.[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 and turn-around time. In local laboratories the slide test is now the most commonly used technique because of its convenience and rapid result. As the slide agglutination test results are available within minutes, So, practically the diagnosis is often formed on the basis it, tube agglutination is useful to clarify erratic or equivocal agglutination reactions obtained by the more rapid slide test. Both the above tests have their own merits and demerits.[11-12]

Therefore, the present study was done to compare the two tests, slide agglutination and tube agglutination in diagnosing the Enteric fever.

Material and Methods

The study was conducted for a period of 6 month from June 2019 to November 2019. Patients of all age group and both sexes attending the Inpatient (IPD) and out-patient (OPD) of Department of Medicine with clinical presentation suggestive of enteric fever were included in the study. Under strict aseptic precautions 3 ml venous blood was withdrawn in a well labeled plain vaccutainer tube of all the patients included in the study and sent to Microbiology department. The blood was allowed to clot and centrifuged at 3000 rpm for 15 min to separate serum. The test was performed as per the manufacturer's instructions (Span diagnostics Ltd.). The sera were subjected initially to slide agglutination method. One drop (50 µl) of undiluted test serum was placed on the four 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 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 then 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 a row of Felix tubes containing the same quantity of *S. typhi* O antigen and three rows of Dreyers' tubes containing the same quantity of *S. typhi* H, *S. paratyphi A* and *S. paratyphi B* antigens. The rack containing all the tubes was then incubated at 37°C in a water bath overnight. Macroscopic agglutination was noted and recorded on the following day after keeping the rack at room temperature. The highest dilution of serum giving visible agglutination was calculated and matched against the currently used local cut off titer as mentioned above, to confirm positivity. At the end, the results of both slide and tube agglutination test were compared

Results

A total of 500 cases, clinically suggestive of Enteric fever were included in the study. The samples were collected in microbiology department, tested first for slide agglutination followed by Tube agglutination. Out of 500 collected samples, 183 (36.6%) was positive by slide agglutination test, whereas, only 145 (29%) were positive by tube agglutination method. The slide test had a sensitivity of 97.2%, specificity of 88.1% shown in Table 1. The actual positivity 141 out of 183 positive results given by slide agglutination. The table 2 shows positive predictive value of 77% and negative predictive value of 98.7% of slide agglutination test as compared to Widal tube agglutination test.

Table 1: Sensitivity and specificity of slide agglutination test taking Widal tube agglutination test as standard (N=500).

Slide agglutination test	Widal tube agglutination test				
	Positive N (%)	Negative N (%)	Total sample test N(%)		
Positive N (%)	141 (97.2%)	42 (12%)	183 (36.6%)		
Negative N (%)	4 (2.8%)	313 (88%)	317 (63.4%)	Sensitivity=97.2%	Specificity = 88%
Total N (%)	145 (100%)	355 (100%)	500 (100%)		

Table 2: Positive predictive value and negative predictive value of slide agglutination test taking Widal tube agglutination test as standard (N = 500).

Slide agglutination test	Widal tube agglutination test				
	Positive N (%)	Negative N (%)	Total sample test N(%)		
Positive N (%)	141 (77%)	42 (22.9%)	183 (100%)		
Negative N (%)	4 (2.8%)	313 (97.2%)	317 (100%)	PPV=77%	NPV=98.2%
Total N (%)	145 (29%)	355 (71%)	500 (100%)		

Discussion

In developing countries mainly due to poor sanitary condition, Enteric fever has become the major health problem causing significant morbidity & mortality.[6] Although isolation of causative organism from blood, bone marrow or other body fluids is the mainstay for definitive diagnosis of enteric fever. 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 centers 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. [3]

Initially for Widal test using paired sera, 1-2 weeks apart & demonstrating four-fold or greater rise of antibody titre was recommended. But, 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. Therefore, 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.[13] 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 the sensitivity and specificity of slide agglutination was found to be 97.2% and 88% respectively. The similar finding was observed by Gaikwad *et al* who reported sensitivity and specificity of 100% and 84.9%.[9] Another study conducted by Jahan N *et al* also reported the similar results, whereas study done by Tupasi et al showed much lower sensitivity and specificity for Slide agglutination.[14-15] 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.[16] In the present study, slide agglutination showed positivity in 183 (36.6%), where as true positive only 141(77%) and 42 (12%) cases were falsely reported as positive. Our results were similar with the study done by done by Jahan N *et al.* If the slide agglutination test is solely relied upon for diagnosis, then a significant number of samples would have been falsely labeled as positive and clinicians would have started antimicrobial chemotherapy in otherwise healthy individuals. This could lead to serious consequences by unnecessarily pressuring the normal gut flora to develop antibiotic resistance. Hence, the use of slide agglutination test for diagnosis of enteric fever remains questionable.[9]

Conclusion

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

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