

Study of diagnostic efficacy of widal slide agglutination test against widal tube agglutination test in enteric fever

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Abstract

Introduction: Enteric fever is a major and persistent public health problem in India. The slide widal is most commonly performed rapid method to diagnose due to its convenience, but its results are required to be compare with more standardly established tube widal test for its actual diagnostic significance. This study aims to compare the result of slide widal test with tube widal test to ascertain any significant difference between two methods.

Materials and Method: The 100 pre-collected serum samples with widal test request were received for the study over the six months period. Comparison of slide widal agglutination test with tube widal agglutination was done considering tube widal as a standard.

Result: The slide widal agglutination test shows false positive result in 5.3% and 48.1% serum samples with *S.typhi* O antigen & *S.typhi* H antigen, respectively. The Positive Predictive Value of widal slide agglutination test for O antigen and H antigen is 94% and 52% respectively.

Conclusion: The slide widal agglutination test showed high false positive rate and low positive predictive value especially with *S.typhi* H antigen. Therefore, the result of slide widal agglutination needs to be confirmed by tube widal agglutination test.

Keywords: Agglutination tests, Comparison, Slide, Tube, Widal test

Introduction

Salmonella is a leading cause of community-acquired bloodstream infections in many low- and middle-income countries.^(1,2) Accurate diagnosis is crucial to the management of the disease, but typhoid fever can be clinically confused with other febrile diseases, such as dengue fever and malaria.⁽³⁾

Culture of the causative organism, *Salmonella enterica* serotype Typhi, is most definitive for diagnosis, but this is expensive, takes up to a week of work, and does not always produce appropriate results.⁽³⁾ Serological diagnosis is thus widely employed, but this is fraught with specificity problems.⁽⁴⁾ However, the universally practiced diagnostic procedure is the Widal test because other methods are either invasive, expensive and have poor utility. Moreover, the isolation of causative agent from the culture requires a good microbiological laboratory facility which makes Widal test a better alternative method of diagnosis of typhoid and paratyphoid fever, mainly in many developing countries like India.⁽⁵⁾

This study aims to compare the result of slide widal test with tube widal test to ascertain any significant difference between two methods.

Materials and Method

Pre-collected blood samples on OPD and indoor bases with widal test request from enteric fever suspected cases were received in the microbiology lab for the processing and study was done over a period of 6 months. The serum was separated using all standard precautions. All the sera first tested with widal test by slide agglutination method. The antibody titre was taken as highest dilution of serum showing distinct visible agglutination. The widal test result with the antibody titres more than or equal to 1:80 for O antigen and the titre more than or equal to 1:160 for H antigen were considered as positive. When they result positive, they were checked by tube widal test for confirmation of result. The tests were performed according to manufacturer's instructions. At the end, the result of slide and tube widal test were compared and analyzed.

Result

Total 100 serum samples with positive slide widal test were subsequently tested with tube widal test.

Table 1: Positive widal antibody titre result against different antigen

| Antigen type | <i>S.typhi</i> O antigen | <i>S.typhi</i> H antigen | <i>S.paratyphi</i> A 'H' antigen | <i>S.paratyphi</i> B 'H' antigen |
|----------------------------------|--------------------------|--------------------------|----------------------------------|----------------------------------|
| No. of Positive Slide widal test | 76 | 54 | 4 | 1 |
| No. of Positive Tube widal test | 72 | 28 | 1 | 0 |

Table 2: Comparison between slide and tube widal test

| Type of antigen | No. of slide widal test with negative tube widal | Percentage of false positive slide widal test as compared to tube widal test result |
|---------------------------|--|---|
| S.typhi O antigen | 4 | 5.3% |
| S.typhi H antigen | 26 | 48.1% |
| S.paratyphi A 'H' antigen | 3 | 75% |

Table 3: Positive Predictive value of slide widal agglutination test when compared to tube widal agglutination test as a standard

| Type of antigen | Positive Predictive value % |
|-------------------|-----------------------------|
| S.typhi O antigen | 94% |
| S.typhi H antigen | 52% |

In our study, slide widal agglutination test showed very high (48.1%) false positivity for S.typhi H antigen and very low false positivity for S.typhi O antigen, when compared to tube widal agglutination test as a standard. The slide widal agglutination test also showed high positive predictive value for S.typhi O antigen and low positive predictive value for S.typhi H antigen, when compared to tube widal agglutination test as a standard.

Discussion

The gold standard test for the diagnosis of typhoid fever is the isolation of bacteria from blood; however, the widespread and uncontrolled use of antibiotics leads to negative results. Moreover considering the poor facilities for the isolation of bacteria by culture methods in peripheral health centers and rural clinics, no other diagnostic tools is introduced thus far, other than widal test for the appropriate diagnosis of typhoid fever.⁽⁶⁾

Widal agglutination was introduced as a serologic technique to aid in diagnosis of typhoid fever. The test was based on demonstrating the presence of agglutinin (antibody) in the serum of an infected patient, against the H (flagellar) and O (somatic) antigens of *Salmonella typhi*. While the definitive diagnosis of typhoid fever depends on the isolation of *S typhi* from blood, stools, urine or other body fluids.⁽⁷⁻⁹⁾

Serological diagnosis relies classically on the demonstration of a rising titer of antibodies in paired samples 10 to 14 days apart. In typhoid fever, however, such a rise is 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 a region of endemicity, or because in some individuals the antibody response is blunted by the early administration of an antibiotic.⁽¹⁰⁾ Furthermore, patient management cannot wait for results obtained with a convalescent-phase sample. For practical purposes, a treatment decision must be made

on the basis of the results obtained with a single acute-phase sample. False-positive Widal test results have been reported for patients with non-enteric fever salmonellae infections, malaria, typhus, *C. neoformans* meningitis, immunological disorders, and chronic liver disease.⁽¹¹⁻¹⁴⁾

In our study, slide widal agglutination test showed very high (48.1%) false positivity for S.typhi H antigen and low false positivity (5.3%) for S.typhi O antigen, when compared to tube widal agglutination test as a standard. The slide widal agglutination test also showed high positive predictive value for S.typhi O antigen and low positive predictive value for S.typhi H antigen, when compared to tube widal agglutination test as a standard. The study done by Gaikwad UN et al.⁽¹⁵⁾ also showed the high false positivity and low positive predictive value by slide agglutination test and suggested to caution that the results of slide agglutination test should not be solely relied upon for diagnosis and treatment of enteric fever. The study done by Karen H Keddy⁽¹⁶⁾ showed the semiquantitative slide agglutination test performed the worst among four rapid serology tests evaluated and showed low PPV and NPV of the same.

The study done by Lavanya, V⁽⁶⁾ also showed considerable difference in the results obtained by the slide and tube Widal agglutination test, emphasizing the need for confirmation by the quantitative tube Widal test for all suspected cases of typhoid fever.

Widal test results also showed marked discrepancies using different Widal brands in study done by Wafaa MK Bakr et al.⁽¹⁷⁾ Typhidot were less specific and, in most cases, less sensitive, as shown in study done by Razel L. Kawano et al.⁽⁴⁾

Thus, though slide widal agglutination test is less specific and having low positive predictive value, it remains most commonly used rapid diagnostic test due to its advantages of easy to perform, less cumbersome less time consuming in resources restrained peripheral area in developing countries where enteric fever is endemic and blood culture facilities is not available.

Conclusion

The slide widal agglutination test showed high false positive rate and low positive predictive value especially for S.typhi H antigen. Therefore, the result of slide widal agglutination should not be solely relied upon for diagnosis and treatment of enteric fever and

needs to be confirmed by tube widal agglutination test and correlated with clinical findings.

brand to use? a study of four different widal brands: a cross sectional comparative study. *Annals of Clinical Microbiology and Antimicrobials*.2011;10:7.

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