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Original Article

Comparison of Results Obtained by Semi-quantitative Slide Agglutination and Tube Widal Tests in the Diagnosis of Suspected Typhoid Fever Cases

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ABSTRACT

The diagnosis of enteric fever currently depends upon the isolation of Salmonella from a patient, most commonly by blood culture. This facility is not available in many areas where the disease is endemic. The Widal agglutination test which demonstrates the presence of somatic (O) and flagellar (H) agglutinins to Salmonella in the patient's serum using O and H antigen suspension, is one of the most utilized diagnostic tests for typhoid fever in developing countries. In the recent past, a rapid slide test was developed which is now the most commonly used technique in local laboratories because of its convenience. The semi-quantitative slide test provides an approximation to the tube test titre. The present study was an attempt to compare the results of semi-quantitative slide agglutination with that of the quantitative tube method to ascertain any significant difference between the two methods. Methods: Acute phase serum samples from a total of 45 clinically suspected typhoid fever cases were collected over a period of 3 months and subjected to semi-quantitative rapid slide and single tube Widal tests using commercially available Salmonella antigen test kit (Span Diagnostics Limited). Results and conclusion: From the 45 samples studied, 29 (64.4%) were seropositive by semi-quantitative slide method and 19 (42.2%) by tube method with antibody titres > 1/80 for O and > 1/160 for H antigens. Present study shows considerable difference ($P < 0.05$) in the results obtained by the two methods of Widal test, emphasizing the need for confirmation by the quantitative tube Widal test for all suspected cases of typhoid fever.

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1. Introduction

Typhoid fever is a life threatening systemic infection that continues to be a major public health problem with significant morbidity and mortality in the developing world. The World Health Organization (WHO) estimate for annual global incidence of typhoid fever is about 21 million cases with more than 13 million cases in Asia and above 600,000 deaths worldwide annually.^{1,2}

The definitive diagnosis of the disease requires the isolation of Salmonella typhi from the blood, faeces, urine or other body fluids. In developing nations like India, facilities for isolation and culture

are often not available especially in smaller hospitals.² Delayed results obtained with blood culture (2 to 3 days), its limited diagnostic utility in the early phase of illness and decreased isolation rates due to the widespread practice of early antibiotic administration have limited its practical use in the diagnosis of typhoid fever.^{3,4,5}

The Widal test, named after Georges Fernand Isidore Widal (1896), 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.⁶ This serological test measures the agglutinating antibodies in patients serum, against the lipopolysaccharide somatic (O) and protein (H) antigens of Salmonella typhi.

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Considered next in value to the blood culture, the macroscopic Widal test is simple, practical and reliable. When used in conjunction with the blood culture we have a laboratory procedure which will seldom if ever mislead. In later years, a rapid slide test was developed which is now the most commonly used technique in local laboratories because of its convenience.^{6,7,8.}

This study aimed at comparing the results of Semi-quantitative slide agglutination and Quantitative Tube Widal tests in order to determine any statistically significant differences in the results obtained by the two methods.

2. Materials and methods

Serum samples from a total of 45 cases of clinically suspected typhoid fever were collected and subjected to both Semi-quantitative rapid slide agglutination and the quantitative tube Widal tests in the microbiology laboratory of a tertiary care hospital.

Commercially available stained Salmonella antigen kit containing *S.typhi* 'O' and *S.typhi* 'H' suspensions (Span Diagnostics Ltd) were used for the tests.

All samples were obtained in the acute phase of the illness. 5 ml of blood collected under aseptic precautions, in a clean dry tube was allowed to clot and centrifuged. Fresh serum thus obtained was used for the test. Test procedures were performed according to manufacturer's instructions in the kit manual. Appropriate control tubes with saline and antigen suspensions were included to rule out autoagglutination of the reagent. Antibody titre was taken as the highest dilution of serum showing distinct agglutination. Titres greater than 1 in 80 for 'O' and 1 in 160 for 'H' antigens were taken as cut-off titres for seropositivity.

3. Results

Samples showing positive agglutination with the semi-quantitative slide method were titrated approximately to the corresponding tube test titres for O and H antigens. (Table1). The antibody titres for O and H as obtained by the tube method are shown in Table 2.

Out of the total 45 serum samples tested by both the methods of Widal test, 29 (64.4%) were found to be seropositive with O > 1/80 and H > 1/160 by the semi-quantitative slide method and 19 (42.2%) were seropositive with O > 1/80 and H > 1/160 by the quantitative tube method of testing.(Table 3).

Statistical analysis: Chi-square test was applied and found the results obtained by the two methods of Widal testing to be statistically significant. ($\chi^2=4.46$, df=1, P=0.03461).

Table: 1 Approximated antibody titres of serum samples by the semi-quantitative slide agglutination test

ANTIGENS	Serum volumes & the corresponding tube test titres					
	0.08 ml (1/20)	0.04 ml (1/40)	0.02 ml (1/80)	0.01 ml (1/160)	0.005 (1/320)	0.025 ml (1/640)
O	4	6	8	11	7	3
H	2	2	7	14	9	6

Table: 2 Antibody titres of serum samples by the Tube Widal test

ANTIGENS	Antibody titre					
	1/20	1/40	1/80	1/160	1/320	1/640
O	3	6	5	7	4	3
H	1	5	4	9	7	3

Table: 3 Total serum samples positive for both O and H (O > 1/80 ; H > 1/160)

Test	Positive (%)	Negative (%)	Total (%)	Seropositivity
Semi-quantitative Slide Widal	29 (64.4)	16 (35.5)	45 (100)	64.4%
Quantitative Tube wida	19 (42.2)	26 (57.8)	45 (100)	42.2%

4. Discussion

The Widal test is one of the most utilized tests for typhoid fever in India with tube Widal test being largely replaced by a semi-quantitative slide test for its rapidity and convenience.

This study shows a difference of 22.2% (P<0.05) in the results obtained by the two methods with the rapid slide agglutination method giving increased seropositivity.

Thelma E. Tupasi et al in their study from various developing countries endemic to typhoid fever, found the sensitivity and specificity of tube Widal ranging from 61-90% and 88-100% respectively and that of slide agglutination method to be 53-80% (sensitivity) and 57-90% (specificity).⁵

In a study by Ayse Willke et al from Turkey, O & H antibodies were found by slide agglutination in 25% of healthy adults. Such high rates of seropositivity could be attributed to the widespread presence of salmonella infections in the community, cross-reactivity of serotype typhi antigens with other salmonella infections and the longevity of these antibodies in the serum.⁹

Keddy H Karen et al in their study concluded that the semi-quantitative slide agglutination test had shorter turnaround times than the Widal tube test & poor specificity & hence should not be used as diagnostic tool.⁶

John Wain et al state that the quantitative slide agglutination test used instead of standard Widal test should always be interpreted with reference to clinical data.¹

Slide test depends on visualisation of clumps. Weakly reactive agglutinations may require adequate light source and trained eye which could explain higher false positive/erratic results.¹⁰

Single-tube Widal test was applied in our study. Originally Widal test was recommended using paired sera, 1-2 weeks apart & demonstrating four-fold or greater rise of antibody titre.¹¹ 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. Even though the sensitivity of single tube Widal agglutination test is not very high cut-off titre is taken in the diagnosis of typhoid fever in developing countries such as India.¹²

The "gold standard" for the diagnosis of typhoid fever is the isolation of bacteria from blood or bone marrow; however the widespread and uncontrolled use of antibiotics leads to negative results. 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, other than the Widal test for the appropriate diagnosis of typhoid fever.

The Widal test interpretation in endemic areas is difficult since majority of the normal healthy individuals also carry detectable antibodies. In our study we found that in such cases, the high titres (>1/80 for Salmonella typhi "O" and >1/320 for salmonella typhi "H" antigens) in the Widal test performed on single acute-phase sera must be considered as significant and diagnostic.

4. Conclusion

In conclusion, the Widal test is an easy, inexpensive and relatively non-invasive test that can be of diagnostic value in situations where blood cultures cannot be obtained. However, the semi-quantitative slide agglutination as an alternative to tube Widal test needs to be further evaluated in view of the statistically significant difference in the results obtained by the two methods in this study. Tube Widal test should be applied for all samples to clarify erratic or equivocal agglutination reaction by the more rapid slide test in diagnosis of typhoid fever.

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