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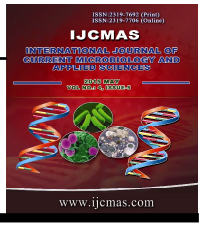

Original Research Article

A Comparative Study of typhidot and widal test for Rapid Diagnosis of Typhoid Fever

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Keywords

Typhidot test, Typhoid fever, Widal test

A B S T R A C T

Typhoid fever is a major public health problem associated with significant morbidity and mortality in many countries. There is a need for a quick and reliable diagnostic test for typhoid fever as an alternative to the Widal test. This study was aimed to evaluate typhidot *vis-à-vis* blood culture and Widal test in children. Patients aged 6 months to 12 years, having fever of more than four days duration with clinical suspicion of typhoid fever were enrolled. Patients in whom other diagnosis was made served as control. The tests under scrutiny were validated against blood culture and then all the three tests were evaluated among patients who presented in the first week of illness. Of 100 children with suspected typhoid fever, the disease was confirmed bacteriologically in 20, whereas 50 patients were considered to have typhoid fever on clinical grounds. An alternative diagnosis was made in 30 cases. The typhidot test was found to be superior to the Widal test in its diagnostic sensitivity and specificity. Typhidot is a practical alternative to widal test for the diagnosis of typhoid fever.

Introduction

Typhoid fever, caused by *Salmonella enterica serotype Typhi*, is a major cause of morbidity and mortality worldwide, causing an estimated 16.6 million new infections and 1 600,000 deaths each year.

It is endemic in the Indian subcontinent including Bangladesh, South-east and Far-east Asia, Africa and South Central America. The annual incidence of typhoid fever has been reported as more than 13 million cases in Asia (Ivanoff *et al.*, 1994).

Isolation of *Salmonella* from blood, urine or stool is the most reliable means of confirming an infection. Blood culture is regarded as the gold standard for diagnosis and carry 70–75% diagnostic yield in the first week of illness (Krishna *et al.*, 2011). However, this requires laboratory equipment and technical training that are beyond the means of most primary health care facilities in the developing world (Olsen *et al.*, 2004). In addition, easy availability and widespread use of antibiotics in the community makes it

frequently difficult to isolate the organism from blood culture and alternate methods such as bone marrow cultures may be required, which is invasive and difficult to carry out (Begum *et al.,* 2009).

Thus one has to rely on serological diagnosis, which is the mainstay of diagnosis of typhoid fever in most laboratories in developing world. Unfortunately, neither the Widal test which remains in widespread use in the developing world, nor any of the serodiagnostic tests that have since been developed, has proven sufficiently sensitive, specific and practical to be of value in areas where this disease is endemic (Olsen *et al.,* 2004).

Widal test has been used in the diagnosis of typhoid illness for long time in this country but it remains a serological test with a moderate sensitivity and specificity. Therefore, a fast, reliable, and easy to perform serodiagnostic test with a higher sensitivity and specificity than Widal test is required for rapid diagnosis and management of typhoid cases, thereby enabling clinicians to initiate an early therapy, reducing morbidity and its complications.

Typhidot is a rapid serological test for the diagnosis of typhoid fever. However, its usefulness in terms of Specificity and sensitivity as compared to Widal test has not been studied much.

This study was undertaken to systematically evaluate the utility of typhidot in diagnosis of typhoid fever in terms of sensitivity and specificity.

Material and Methods

This study was conducted in Jawahar Lal Nehru Medical College and Hospital, Ajmer. It was a hospital based prospective study, which included 100 clinically suspected enteric fever cases. All children between 6 months and 12 years of age with fever of more than four days having a clinical suspicion of typhoid fever were enrolled and admitted to the hospital. The criteria for clinical suspicion were those already used by previous workers (Bhutta *et al.,* 1994; Bhutta *et al.,* 1999; Ferdin, 1999). Detailed clinical evaluation was done and findings were recorded on a standardized format. Complete blood count, smear for malarial parasite, urine and stool routine microscopy and urine culture were also done in all cases.

Five ml of blood was inoculated into blood culture media (BHI broth) and incubated at 37 ˚C. Subcultures were done on every alternate day till the 7th day. The growth of *Salmonella* was identified as per standard protocol and confirmed by agglutination with Salmonella polyvalent "O", "O"9 and H:'d' antisera (Koneman, 1997). The Widal test was performed by slide agglutination method and it was considered positive when a titre of equal to or more than 1:160 was observed (Old, 1996).

Typhidot test is a dot ELISA kit that detects IgM and IgG antibodies against the outer membrane protein (OMP) of the *Salmonella typhi*. The typhidot test becomes positive within 2–3 days of infection and separately identifies IgM and IgG antibodies. The test is based on the presence of specific IgM and IgG antibodies to a specific 50KD OMP antigen, which is impregnated on nitrocellulose strips. The reaction tray was divided into 2 columns marked as G and M. 250 µl of sample diluent was dispensed in each well and 2.5 µl of test /control was added and then incubated for 20 minutes. The strips were washed with washing buffer thrice, 250 µl of anti human IgG and IgM

was dispensed then in each well and incubated for another 15 minutes. These were washed again, dispensed with 250 µl of colour development solution, and incubated for another 15 minutes and results were then interpreted. A positive IgM was interpreted clinically as acute typhoidal illness, while IgM and IgG positive were taken as acute typhoidal illness in middle stage of infection and IgG positive was interpreted as chronic carrier or previous infection or reinfection.

Results of blood culture, widal and typhidot test were compared in all patients for their sensitivity and specificity.

Results and Discussion

A total of 100 cases were enrolled in this study with suspected typhoid fever. The diagnosis was confirmed in 20(20/100) by isolation of *salmonella typhi* on blood culture. In 30 cases, alternative diagnosis was made and these cases constituted negative controls, consisted of 12 cases with malaria, 8 cases with viral respiratory infections, 4 with pneumonia, 4 with urinary tract infections and 2 with meningitis.

Table.1 compares the sensitivity, specificity, positive and negative predictive values for the Widal, Typhidot, and blood culture for this cohort. When only blood culture proven cases (n=20) were analyzed, Typhidot was significant superior to the widal in terms of diagnostic predictive values (Table. 2).

Typhoid fever is a systemic illness with a significant morbidity and mortality in developing countries (Sherwal *et al.,* 2004). Emergence of multidrug resistant strains of *Salmonella enterica* serotype *Typhi* has only added to the burden of the disease. Any delay in diagnosis of appropriate therapy only increases the risk of outcome (Begum *et al.,* 2009).

Blood culture has remained the gold standard for diagnosis of typhoid fever. In our study culture positivity among clinically suspected typhoid cases was 28.5 %. Culture positivity in other studies have quoted sensitivity ranging from 8.9–43% (Narayanappa *et al.,* 2009; Akoh, 1991; Jesudason and Sivakumar, 2006; Saha *et al.,* 2003). The relative low rate of isolation from blood culture had been attributed to delay in diagnosis, widespread and irrational use of antibiotics and low volume of blood obtained for cultures among children. This value is too low to satisfy the criterion of a diagnostic test, irrespective of the reasons for its low yield. Nonetheless, blood culture is the foolproof method for the diagnosis of typhoid fever and hence a substitute has to be validated against it. Furthermore, the feasibility of a test has to be taken into account.

Widal test has been used for over a century in developing countries but its diagnostic utility has been limited due to low sensitivity, specificity and positive predictive value (Sherwal *et al.,* 2004). Decreased sensitivity is due to the long latent period after which the test may become positive. Decreased specificity is due to prior infection, vaccination with TAB vaccine, cross reaction with other gram negative infections. In the present study, Widal test was positive in 68% (48/70) of the patients. Widal test was positive in 9 of the 20 blood culture positive patients and 4 of blood culture negative patients. Thus the test had sensitivity of 45% and specificity of 86%. The finding of the present study indicates a low specificity for Widal test. Similar results have been reported in other studies from endemic areas, where there may be high levels of specific and cross reacting antibodies (Sherwal *et al.,* 2004; Narayanappa *et al.,* 2009; Khoharo, 2011). Ideally a fourfold rise in antibody titre in a

paired serum is more diagnostic. Though higher sensitivity and specificity for Widal has been reported, its use in endemic areas should not be encouraged.

Typhidot test is based on detection of antibodies which appear in detectable titers as early as the second day of illness. It showed sensitivity of 90% and specificity of 100% in blood culture proved cases. The test had sensitivity of 85% and specificity of 100% in the typhoid fever cases.

Table.1 Diagnostic parameters of various tests for the entire cohort of clinically suspected typhoid fever cases (n = 70)

Diagnostic Tests	Sensitivity %	Specificity %	PPV %	NPV %
Blood Culture	28.5	100	100	37
Widal	68	86	94	57
Typhidot	85	100	100	75

Positive blood culture in cases = 20, positive blood culture in controls = 0
* Positive Widal in cases = 48, positive Widal in controls = 4
** Positive typhidot in cases = 60, positive typhidot in controls = 0

Table.2 Diagnostic parameters of various tests among blood culture positive cases (n = 20)

Diagnostic tests	Sensitivity %	Specificity %	PPV %	NPV %
Widal test	45	86	69	70
Typhidot test	90	100	100	93

* Positive Widal (cases = 9, controls = 4)
** Positive typhidot (cases = 18, controls = 0)

We do not believe that our data support the use of either the Widal or Typhidot test as a substitute for cultures in typhoid fever. It must be emphasized that although cultures are associated with a lag period of at least 48 hr for preliminary confirmation of infection, with the recent emergence of drug resistance among *S. typhi*, they remain an essential investigation.

In many circumstances, especially among partially treated cases presenting to health facilities, combining cultures with a rapid serologic test may reduce the diagnostic difficulty in typhoid fever. So combining the blood cultures with a Typhidot will significantly improve the diagnostic yield of these investigations among children who have previously received antibiotics.

The Typhidot offers an additional advantage among serologic diagnostic tests for typhoid fever in that the test strips do not require an ELISA reader for evaluation. Also, only minimal operator training is required. Nevertheless, the higher cost of the test in comparison with the Widal test, as well as cold-storage requirements for test reagents, are additional impediments in using this test in developing country.

In the present study we conclude that Typhidot is a practical alternative to Widal test in the diagnosis of Typhoid fever on account of its increased sensitivity, early detection of cases, and ease of procedure with minimal infrastructure and availability of results on the same day. However, a larger prospective study would be required

to fully evaluate the usefulness of this test in countries endemic to typhoid fever.

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