Diagnosis of Enteric Fever by Widal and Two Dot-enzyme Immunoassay: Utility and Difficulties

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Enteric fever is an important cause of mortality and morbidity in developing and under developed countries. The present study was undertaken to detect antibodies to salmonella using serological tests i.e. Two dot-enzyme immunoassay (Typhidot) and Widal test and correlation of the results. Consecutive 100 blood samples collected from clinically suspected cases of enteric fever were processed using standard methods. The samples were subjected to blood culture, widal test & typhidot test. Blood culture was positive in 7(7%), Widal test positive in 20(20%) and Typhidot in 28(28%). The sensitivity, specificity, PPV and NPV for Typhidot and Widal when compared with blood culture as gold standard were 100%, 77.41%, 25%, 100% and 85.71%, 84.95%, 30%,98.75% respectively. Typhidot test being rapid, inexpensive, easy to perform and highly sensitive test, has an advantage over conventional methods like blood culture and widal test in diagnosis of enteric fever caused by salmonella typhi. Typhidot is also more useful in detection of enteric fever in the 1st and 2nd week of infection.

Key words: Enteric fever, Widal test, Typhidot test.

Typhoid fever or Enteric fever is widely recognised as a major problem in developing countries. It is a systemic prolonged febrile illness caused by certain Salmonella serotypes viz. Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B & Salmonella Sendai.¹ The incidence as estimated is approximately 16 million new cases annually, of which 13 million occur in Asia alone as estimated by World Health Organisation (WHO).² If left untreated it can lead to complications affecting various organ systems and carrier state. Due to non specific nature of symptoms, typhoid fever can clinically be confused with other febrile diseases such as dengue, malaria, rickettsiosis, leptospirosis & melioidosis. Accurate diagnosis is crucial to the management of the disease. Reliable laboratory tests are therefore

essential, so that appropriate treatment can be started as delay increases the morbidity & adds to the cost of the treatment.³

Typhidot a dot enzyme immunoassay is a relatively newer serologic test based upon the presence of specific IgM & IgG antibodies to 50kDa outer membrane protein (OMP) antigen on *Salmonella typhi* strains.⁴ The present study was undertaken to know the incidence of typhoid fever and to compare the routinely used serological method like widal test with newer rapid Typhidot test.

MATERIALS AND METHODS

A prospective study was done to evaluate the utility of widal test and a rapid test Typhidot to diagnose Enteric fever and analyse the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the tests in 100 clinically suspected cases of enteric fever.

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The study was undertaken at the department of Microbiology, Karnataka Institute of Medical Sciences, Hubli over a period of one year from December 2007 to November 2008.

Source of sample

5-10ml blood was collected from clinically suspected cases of enteric fever attending KIMS, hospital Hubli. The collected sample was divided & used for Widal test and Typhidot test. Patients with history of fever of more than 2-3 days duration and with a clinical diagnosis of enteric fever were included in study. Blood culture was done as per standard methods.⁵

Typhidot is a Dot Enzyme immuno assay kit for rapid detection of specific IgM &IgG antibodies of *Salmonella typhi* from Malaysian Bio-diagnostics Research, KualaLumpur, Malaysia. It is the first known qualitative antibody detection test designed for rapid diagnosis of typhoid fever to detect presence of IgM & IgG antibodies made against a specific antigen on the outer membrane of *Salmonella typhi*. It is detected by incubating nitrocellulose strips dotted with specific antigen protein with patient's sera & control sera.

To visualize the antigen-antibody complex, the strips are simultaneously incubated with peroxidase-conjugated antihuman IgM &IgG. Upon addition of the chromogenic substrate, the results can be read visually. Positive reading is indicated by the blue colour as intense or as more intense than that of the positive control (Fig 1). Total assay time is one hour.

The test results are interpreted as follows; IgM positive only is acute typhoid fever, IgM and IgG positive is acute typhoid fever (in the middle stage of infection), IgG positive could be previous infection or relapse or re infection (interpretation needs to be made with clinical findings) and IgM and IgG negative means infection is probably not typhoid.

Widal test was performed on serum specimen. The test was performed using standard tube agglutination test containing TO, TH and AH antigens standardized by rapid agglutination, as described by the manufacturer. The kit was procured from (Typhocheck) Tulip Diagnostics (P) Ltd.

Statistical Methods

Descriptive statistical analysis has been

J PURE APPL MICROBIO, 7(3), SEPTEMBER 2013.

carried out in the present study. Diagnostic tests viz. Sensitivity, Specificity, Positive Predictive Value & Negative Predictive Value has been applied.

RESULTS

Overall in 100 cases, 58(58%) were males and 42(42%) were females. Majority of the patients were < 10 years to 21-30 years.

32 (32%) were positive for typhidot IgM or IgG or both out of 100 suspected cases of typhoid fever. Out of these 32 cases, 12 (37.5%) cases were positive for IgM alone, a majority i.e. 16 (50%) were both typhidot IgM and IgG positive and 4 (12.5%) were positive for IgG alone. As per manufacturer's instructions a positive IgG alone was considered as negative for acute typhoid fever. Therefore typhidot positive cases in the present study were 28(28%). 20(20%) had significant titres for widal test.

Blood cultures were positive in 10 cases of which 4(4%) were Salmonella typhi, 3(3%) were salmonella paratyphi A, 2(2%) were Enterobacter species and 1(1%) was Klebsiella pneumonia. Typhidot and widal were negative in cases with Enterobacter and Klebsiella species.

Typhidot was found to have a higher sensitivity i.e. 100% when compared to widal test 85.71%, however the specificity of Widal test was marginally higher i.e. 84.95% compared to typhidot test i.e. 77.41%. The NPV and PPV were comparable (Table 1).

Out of 44 cases wherein the samples were collected in 1st week of illness, 9(20.45%) cases were positive by widal and a higher percentage i.e. 14 (31.81%) by typhidot test. In 2nd week of illness out of 22 cases, 5(22.73%) were positive by widal and 6(27.27%) by typhidot. Positive rates of two tests did not differ after 3rd week of illness (Table 2).

DISCUSSION

In the wake of emerging multidrug resistance strains of bacteria causing typhoid fever, the infection is known to be associated with significant morbidity & mortality, with 6,00,000 deaths each year.⁶The diagnosis remains a challenge as the disease often does not show a specific clinical picture and mimics other febrile illness prevalent in that region.

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Widal	85.71%	84.95%	30%	98.75%
Typhidot	100.0%	77.41%	25%	100%

Table 1. Table showing comparison of Widal & Typhidot with culture as gold standard

Table 2. Table showing Widal and Typhidot positivity with duration of fever

Duration of fever in weeks	No. of cases	Widal positive cases	Typhidot positive cases.
1	44(44%)	9(20.45%)	14(31.81%)
2	22(22%)	5(22.73%)	6(27.27%)
3	18(18%)	4(22.22%)	4(22.22%)
5	11(11%)	2(18.18%)	4(36.36%)
7	5(5%)	0	0



Fig. 1. Reaction wells showing control and test strip, well 1a and 1b showing control strips 2a 2b, 3a 3b, 4a 4b.....are test samples

The diagnosis of enteric fever has been relied on the isolation of Salmonella from blood, faeces and bone marrow. The isolation rate varies from 30-70% with the rate in bone marrow aspirate culture being the highest.¹

The Widal test which is also relied on is time consuming and requires paired sera for interpretation. It is only moderately positive and cross reactions with other Salmonella strains have been reported.^{7,8} Results are also affected by the frequency of agglutinins in the population, effect of antibiotics and antibody response to enteric fever.⁹ History of vaccination & autoimmune diseases also give false positive results.¹ False negative results are also seen in early treatment, hidden organism in the bone and joints and with relapse of typhoid fever.¹⁰ The nonspecific nature of symptoms and the limitations of blood culture make enteric fever difficult to diagnose, therefore there is a continuing search for improved diagnostic procedure to diagnose typhoid fever. Serological test has many added advantages when compared with blood culture in terms of sensitivity & turnaround time of reports.

Typhidot test is easy and rapid serological test to perform and read. The turnaround time for this test is about one hour, whereas for other tests like blood culture and widal tests, results are available at least after one day of collection of sample.

On comparison of the sensitivity, specificity, PPV and NPV of typhidot and widal (Table 1) in comparison with blood culture, it can be said that in our study typhidot was better test than widal test in terms of sensitivity however specificity of widal test was marginally higher. 100% negative predictive value of typhidot test will be useful in ruling out typhoid fever so that other causes of fever could be explored.

The sensitivity and specificity in different studies ranges from 54.7% to 100% and 52% to 98.8% respectively. ^{14,6,11-13} These differences could possibly be due to several factors including the genomic diversity among S. typhi isolates in the region and differences in the antigenic epitopes. ¹⁴

A study by Bhutta ZA et al has shown a higher specificity for widal i.e. 81% as compared to typhidot which was 77%.² In another study by Gopalkrishnan V et al the specificity for widal test

J PURE APPL MICROBIO, 7(3), SEPTEMBER 2013.

was 76.6% and 68.1% for typhidot test.¹

The findings of these studies regarding specificity of widal test being higher than typhidot test was in par with our study.

Typhidot has other advantages; it is a rapid, simple, sensitive and inexpensive test and detects both IgM and IgG. It can clearly state whether the current infection is an acute or past infection. Detection of cases was also better with typhidot compared to widal in the 1st and 2nd week of illness.

On the other hand, widal test needs paired sera to demonstrate the rising titres for accurate results. The result of a single widal test needs to be carefully correlated with clinical findings to arrive at a final diagnosis. It is also important that the test be interpreted against the prevalent endemic titre of the population in question. Besides, widal does not identify IgM and IgG separately which makes the distinction between acute and past infections difficult, thus casting a shadow of doubt regarding the accuracy of test results by clinicians.

In the first week of illness typhidot test was positive in more number of cases (Table II) i.e. 14(31.81%) whereas widal test was positive in 9(20.45%) likewise 6(27.27%) cases were positive for typhidot test and 5(22.73%) cases positive for widal test in the 2nd week of illness. As the weeks of illness advanced there was not much difference in the results of the two tests. The results suggest that typhidot is more superior in the initial 2 weeks of illness. This is probably due to the fact that typhidot detects both IgM and IgG. However we did not get any reports in the literature to support this finding. Further studies could provide conclusive data.

Like any other test even typhidot test have few drawbacks, it is indirect evidence of infection and cannot replace blood culture which is gold standard for diagnosis, antibiotic sensitivity cannot be determined and quantification of the titres cannot be done. Typhidot detects antibodies to salmonella typhi only and is therefore not useful to diagnose Salmonella paratyphi A and B.

Blood culture is routinely employed investigation for the diagnosis of typhoid fever. Although isolation remains the gold standard for diagnosis, it has its limitations & is difficult to perform where adequate microbiologic facilities are

J PURE APPL MICROBIO, 7(3), SEPTEMBER 2013.

limited. Also widespread availability & use of antibiotics before sample collection makes it difficult to isolate *Salmonella* in blood cultures. Alternative method like bone marrow culture is invasive & is difficult in paediatric patients.²

CONCLUSION

No single test processes the quality of an ideal test for diagnosis of enteric fever. It is therefore necessary to find newer, better, easy alternative tests for the diagnosis of enteric fever. Typhidot test with a few limitations may be useful in early diagnosis of typhoid fever caused by S. typhi. However it cannot replace conventional tests like blood culture and widal test.

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2382

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