Original Research Article

A comparative study of Widal test and immunochromatographic assay for rapid diagnosis of typhoid fever in a tertiary care hospital

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Abstract

Background: Typhoid fever is a major public health problem associated with significant morbidity and mortality in many countries, caused by the bacterium Salmonella enterica subspecies enteric serovar typhi. Although, isolation of causative organism from blood is the standard laboratory method but due to frequent use of self-medication by patients and its long turnaround time, it is seldom used, and typhoid fever is usually diagnosed by serological methods. Widal tube agglutination test is the standard serological test used, which is now a day's replaced by slide agglutination test due to its convenience and immunochromatographic test (ICT) is also good alternative for rapid diagnosis of typhoid fever. **Aim:** The present study was done to determine the reliability of immunochromatographic test(ICT) for the early diagnosis of typhoid fever when compared to the widal test. **Material and Methods:** A hospital based cross-sectional study was done from Jan 2019 to March 2019 in a tertiary care hospital. A total of 247 patients with clinical presentation suggestive of typhoid fever were included in the study whose venous blood was collected and serum was tested by both widal slide agglutination test and immunochromatographic test. **Results:** Out of 247patients, showed immunochromatographic test positive results in 22 (8.90%) samples whereas widal slide agglutination test showed positive results in 18 (7.29%) samples only. The immunochromatographic test had a sensitivity of 100%, specificity of 98.5%, positive predictive value of 81.82% and negative predictive value of 100% as compared to widal slide agglutination test. **Conclusion:** The present study concludes that ICT can be used as the suitable method for rapid diagnosis of typhoid fever, as it is more easy, non-invasive and highly sensitive and specific method.

Key Words: Immunochromatographic test, Typhoid fever, Widal test.

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INTRODUCTION

Typhoid fever is a major public health problem associated with significant morbidity and mortality worldwide, causing an estimates between 11 to 21 million cases and 128 000 to 161 000 typhoid-related deaths occur annually worldwide.[1]

It is endemic in the Indian subcontinent including Bangladesh, South-east and Fareast Asia, Africa and South Central America.[2] Salmonella enterica serovar Typhi, is the human-specific causative agent of typhoid fever. The clinical diagnosis of enteric fever is not always accurate because of a wide range of other common fever-causing infections like malaria, dengue fever, leptospirosis, hepatitis, melioidosis and rickettsiosis endemic in India. Accurate diagnosis differentiating typhoid fever from these conditions is often difficult, both in the clinics and laboratory, but is imperative for

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effective treatment selection. [3] The standard method for the laboratory diagnosis of enteric fever is the isolation of causative organism from specimens especially blood, faeces, urine or other body fluids. Isolation of Salmonella typhi from bone marrow is the current gold standard method for confirming a case of typhoid fever. However, this requires equipments and trained laboratory personnel seldom found in primary health-care facilities in the developing countries.[4] A blood culture gives positive results in 73-97% cases, when the sample has been taken in the early course of disease prior the use of any antibiotics.[5,6] 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. Other demerits of the test are its cost and relatively long turnaround time.[7,8] The sensitivity of stool and urine cultures is much lower and they become positive after the first week of infection.[9] For this reason, in developing countries typhoid cases are diagnosed with the help of serological tests which is simple, rapid, inexpensive and considered next in value to blood culture.[10] 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.[11] Poor specificity is because of pre-existing baseline antibodies in endemic areas, cross reaction with other Gram-negative infections and non-typhoidal Salmonella and prior TAB or oral typhoid vaccination. The Widal test lacks single titer reading reliability, thus requiring a paired sera showing fourfold rise in titer, and it also requires more than 1 week for a significant titre to buildup in the blood. This makes it, though widely used, not a satisfactory and reliable diagnostic tool.[9] The rapid and early immunodiagnosis of typhoid fever can be done by the detection of anti-salmonella anti-bodies by immunochromatographic test (ICT) which does not require any specialized laboratory or highly skilled personnel and can be done in field areas also. Its usefulness has been shown to detect the anti-salmonella antibodies as early as 4 days of fever onset. False positivity of immunochromatographic tests in control population detecting anti-salmonella antibodies is very low.[12] Keeping the above facts in mind, the present study was done to comparatively evaluate the widal test and immunochromatographic test in diagnosing typhoid fever in a tertiary care hospital.

MATERIALS AND METHODS:

This prospective cross sectional study was undertaken at the Department of Microbiology in a tertiary care hospital from Jan 2019 to March 2019.A total of 247 clinically suspected typhoid fever cases were included in this study.

Sample collection and Processing

From each patient included in the study, under strict aseptic precautions 3ml venous blood was withdrawn in a well labeled plain vaccutainer tube. The blood was allowed to clot followed by centrifugation of the tube at 3000 rpm for 15 min to separate serum.13

Widal slide agglutination test

The sera were subjected to slide agglutination method. The test was performed as per the manufacturer's instructions (Beacon diagnostics Ltd.India). One drop (50 ul) of undiluted test serum was placed on the circles of slide provided in the kit along with positive and negative control serum followed by addition of one drop (50 ul) of antigens "O", "H", "AH", "BH". The contents were mixed with separate applicator stick and the slide was rocked gently for 1 minute.

Interpretation:

Granular agglutination in case of "O" and flocculating agglutination in case of "H" or "AH", or "BH" indicates positive reaction.

Immunochromatographic test

Typhoid IgM/IgG test device is a two site sandwich immunoassay based on the principle of immunochromatography on a membrane. This test is a qualitative antibody detection test with total assay time of 15 minutes. The test device comprises of two membrane assemblies, one for IgM detection and the other for IgG detection. The IgM detection test assembly has a conjugate pad of anti human IgM colloidal gold conjugate, nitrocellulose membrane predispensed with S.typhi antigen (LPS) at test line region T and a control line protein at control region C. The IgG membrane assembly has a conjugate pad of protein-A colloidal gold conjugate, nitrocellulose membrane predispensed with S.typhi antigen (LPS) at test line region T and a control line protein at control line region C. When test specimen is applied into sample well(S) of the test device, the specimen migrate by the capillary action across the nitrocellulose membrane. If antibody to S. typhi is present in the specimen, it will react to the colloidal gold conjugate and makes an immune complex. The immune complex moves on the membrane and reacts with immobilized antigen of S. typhi resulting in formation of pink/purple line at "T" region. The test contains an internal inbuilt control which should exhibit a pink or purple line at "C" region. The result is invalid if pink/purple line at "C" is invisible.[14] According to instructions given by manufacturer (Oscar Medicare Pvt.Ltd. India), about 5 µl of specimen was added using micropipette into the S+B well or fill the provided disposable plastic dropper with the specimen up to the

indicated mark on dropper and add into S+B well. Add 2 drops of buffer into S+B and wait for appearance of pink/purple lines in result window. Results were read within 15 minutes.

Interpretation

Positive result was observed as appearance of pink/purple lines at "T" and "C" region. Negative result was observed as appearance of only one pink/purple line at "C" region.

RESULT

A total of 247 patients were enrolled in the present study with clinically suspected typhoid fever.

Table 1: Serological analysis of sample tested by slide widal test

Widal test	Number	Percentage
Positive	18	7.29%
Negative	229	92.71%
Total	**247**	**100**

Table 1 shows that out of 247 patients 18 (7.29%) were positive and 229(92.71%) were negative by slide widal test.

Table 2: Serological analysis of sample tested for antibodies of Salmonella by immunochromatography test

Immunochromatography test (ICT)	Number	Percentage
Positive	22	8.90%
Negative	225	91.10%
Total	**247**	**100**

Table 2 shows that out of 247 cases 22 (8.90%) were positive and 225 (91.10%) were negative by immunochromatography test

Table 3: Comparison of widal test and immunochromatography for the diagnosis of typhoid fever

Test Result		Widal test		Total	Sensitivity	Specificity
		Positive	Negative			
ICT	Positive	18	4	22	100%	98.5%
	Negative	0	225	225		
Total		**18**	**229**	**247**		

Table 3 shows that out of 22 immunochromatography test positive cases,4 were negative by widal test. Sensitivity and specificity of immunochromatographic method was 100% and 98.5% respectively considering widal as standard. Positive predictive value (PPV) was 81.82% and negative predictive value (NPV) was 100%. Disease accuracy of immunochromatography test was 98.38%.

DISCUSSION

Typhoid fever is one of the most common infectious diseases in developing countries including India. It is an diagnostic challenge for the clinicians as well the microbiologists with a number of other tropical infections mimicking the clinical presentations.[15] In the present study, out of 247 clinically suspected patients 18 were positive by widal slide agglutination test and 22 patients were positive by immunochromatographic test. Seroprevalence rate in this study is 7.2% in widal test and 8.9% in ICT method. A study was conducted in the Department of Microbiology, in Tiruvananthapuram showed that 6% and 4.8% were positive for widal and ICT method respectively.[16] Another study by Akhtar *et. al*[15], in Aligarh showed 14 % of prevalence by widal and ICT method. In this current study it was observed that positive ICT was found in 22cases, out of which 18 (True positive) were widal test positive and 4 (False positive) were widal test negative. On the other hand a total of 225 cases with negative ICT was found, out of which no any case (False negative) was widal test positive and all i.e.225 (True negative) were widal test negative as shown in Table no.3.In our study, sensitivity and specificity of immunochromatography test were calculated by widal taken as a standard test. The sensitivity and specificity of immunochromatography test in clinically suspected typhoid fever were found 100% and 98.5% respectively. Out of 18 widal positive samples ICT positivity also seen in all (18) cases. A study on rapid diagnosis of typhoid fever-a comparative study at Department of Microbiology, Tamil nadu, shows sensitivity of ICT as 71% and specificity 81.4%.[9] A similar study carried out in Aligarh district found that sensitivity and specificity of ICT as 51.85% and 98.7% respectively.[15] Our study is in near agreement with findings of Jose *et al*,[17] where they found 90% sensitivity and 94.6% specificity of ICT when compared with widal test. It has been stated that PPV is the most important measure of a diagnostic method since it represents the proportion of patients with positive test results that are correctly diagnosed [18], in our study PPV

was 81.82% while NPV was 100%. A good NPV indicates that negative immunochromatography test result have a good indication for the absence of the disease. A study of Jose *et al*.,[17] from Goa has reported similar results with 84.9% PPV and 96.5% NPV while in contrast to this findings Sengupta *et al.,*[19] from Kolkata reported 100% PPV and 68.9% NPV. The diagnostic accuracy of immunochromatography test in our study was 98.38%. In the present study both widal and ICT methods were used for detection of antibodies to typhoid. Sensitivity, specificity and usefulness of ICT were studied. Though Widal test is performed widely it has some limitation due to poor standardization and difficulty in interpretation on a single sample.[20] Considering the practical situation of laboratory diagnosis, detection of anti-Salmonella antibodies by ICT has been found to be quite reliable, easy to perform and may be a good adjunct to clinical suspect in early days of fever.

CONCLUSION

The study concludes that ICT can be used as the suitable method for rapid diagnosis of typhoid fever. Detection of antibody from ICT method is more easy, non-invasive and highly sensitive and specific method. It is useful for small, less equipped as well as for the laboratories with fewer facilities. Since detection rate of antibody by ICT method is quite satisfactory. This test can be applied for field level use, especially in the endemic areas of developing countries like India, even though the standard test is the widal agglutination test.

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