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Original Article

Comparative study of Dot enzyme immune assay and Widal test in the diagnosis of Typhoid fever in a tertiary care hospital in south Kerala

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Abstract

Typhoid fever is acute and often life-threatening febrile illnesses caused by systemic infection with the bacteria Salmonella enterica serotype Typhi. The disease is endemic in the Indian subcontinent including Bangladesh, South-East and the Middle East Africa, Central and South America[1] . The signs and symptoms of typhoid fever are non specific. So diagnosis relies not only on the clinical features of the disease, but also on investigative methods like culture and sensitivity, detection of agglutinating antibodies to Salmonella typhi by the Widal test. Serologic diagnostic tests for typhoid fever by immunochromatographic test (ICT) are good alternatives[2].

Aim: *Comparison of immuno dot and widal test for the detection of typhoid fever among the febrile patients of Medical college, Thiruvananthapuram.*

Materials and Methods: *A cross sectional study was conducted among febrile patients attended in Govt. Medical college Trivandrum for a period of 6months, from March to September 2015.*

Result: *Out of 433 cases 26 (6%) were positive and 407 (94%) were negative by Widal test, whereas 21 (4.85%) were positive and 413 (95.15%) were negative by immunochromatography. Sensitivity and specificity of immunochromatographic method was 80.8% and 100% respectively considering widal as the standard test.*

Conclusion: *Widal test has been used extensively used as a laboratory tool for diagnosis of typhoid fever in most laboratories, but it is laborious, time consuming and may not be positive in early stages and is to be interpreted judiciously[3]. ICT is a simple and sensitive test for early diagnosis of typhoid fever. The results can be visually interpreted and is available within one hour.*

Introduction

Typhoid fever, caused by *Salmonella Typhi*, is widely recognized as a major public health problem in many developing countries. India is the second most populous country of the world with majority inhabiting the rural areas with little access to modern diagnostic tools. It is presumed that typhoid fever is a major health problem in all those parts of the world where safe drinking water and sanitation is inadequate. It is a systemic infection and is transmitted through the faeco oral route by the consumption of contaminated water and food, particularly raw or undercooked meat, poultry, eggs and milk.

Chronic typhoid carrier status may be responsible for the endemicity and outbreaks of the disease in

the region. The O and H antigens are the major antigens used to serotype the *Salmonella*. The O antigens are Similar to the O antigens of other Enterobacteriaceae but H antigens are different in that they are diphasic. i.e, the H antigens can exist in either of two major antigenic phases. Phase1 (Specific phase) and phase 2 (non specific phase). O antigen is less immunogenic than H antigen. The titre of O antibody in serum after infection or immunisation is generally less than that of H antibody[4]. *S. typhi* produces surface antigen enveloping the O antigen, referred to as Vi antigen. Vi antigen is poorly immunogenic and induces production of low titre of antibody following infection. Vi antibody disappears in early phase of convalescence. Persistence of this antibody indicates the development of the carrier state[1].

The signs and symptoms of typhoid fever are non specific, so a definitive diagnosis of the disease depending on the clinical presentation alone is very difficult. Therefore laboratory based investigations are essential for supporting the diagnosis of typhoid fever. The gold standard for the diagnosis of typhoid fever is the isolation of *Salmonella Typhi* from appropriate samples including blood, bone marrow, urine, stool, etc.[5]It is not always available and when it is, it takes 2 to 3 days. Culture isolation of the *S. Typhi* remains the most effective diagnostic procedure in suspected typhoid fever.

Delayed and inaccurate diagnosis and treatment results in increased cost and higher rates of serious complications and deaths. Drug resistance in *S. Typhi* is a major problem for public health authorities. The emergence of antibiotic resistant strains of the bacteria is closely linked to the irrational use of antibiotic in treating human infections. Resistance to commonly used antibiotics such as chloramphenicol, ampicillin and cotrimoxazole has been reported from different parts of world including India[6.] In developing countries, facilities for isolation and culture are often not available especially in smaller hospitals. A definitive diagnosis of the disease is required for treatment and to decrease the morbidity, mortality and transmission. Other methods include detection of *S. Typhi*-specific antibodies by serological test and antigen by immunological test and identification of nucleic acid by Polymerase chain reaction[7].

The present study was designed to identify the cases of typhoid fever by employing the techniques of widal test and ICT. The ICT method has been shown to be cheap, less time-consuming, applicable for field use, easy to perform and highly sensitive and specific for detection of antibodies in patients with typhoid fever. So the ICT method was applied for the detection of *S. Typhi* specific IgM antibodies in blood samples.

Materials and Methods

A cross sectional study was conducted among febrile patients attended in Govt. Medical college Trivandrum for a period of 6months, from March to September 2015. 5ml blood samples collected under aseptic precaution and serum separated as soon as possible to avoid haemolysis. The sample were stored at 2-8^{0}C for up to 48 hrs. To maintain long term longevity of the serum, stored at -70^{0}C. The samples were subjected to immunochrom-atography and widal test.

Enterocheck-WB is a rapid, qualitative, immunoassay for the detection of IgM antibodies to *S. Typhi* in human serum/plasma or whole blood specimen. It qualitatively detects the presence of IgM class of lipopolysaccharide (LPS) specific to *S.Typhi.* It is an indirect solid-phase immunochromatographic assay. The specific *Salmonella Typhi* antigen is immobilized onto cellulose nitrate membrane strip. The conjugate pad contains two components - Anti human IgM antibody conjugated to colloidal gold and rabbit globulin conjugated to colloidal gold. As the test specimen flows through the membrane test assembly, the anti-human IgM antibody-colloidal gold conjugate complexes with the *S.Typhi* specific IgM antibodies in the specimen and travels on the membrane due to capillary action. This complex moves further on the membrane to

the test region (T) where it is immobilized by the *S. Typhi* specific LPS antigen coated on the membrane leading to formation of a pink to pink-purple coloured band. The absence of this coloured band in the test region indicates a negative test result.

For widal test antigen used was commercially available febrile antigen set kit by BEACON diagnostics. It consist of Febrile Salmonella Typhi 'O'Antigen, Febrile Salmonella typhi 'H'Antigen, Febrile Salmonella paratyphi 'A (H)'Antigen, Febrile Salmonella paratyphi 'B (H)'Antigen. Equal volumes of serial dilutions of the serum and H and O antigens are mixed and incubated at 37°C overnight. Control tubes containing the antigen and normal saline are included to check for auto agglutination. A titre of 100 or more for O antigen was considered significant and a titre in excess of 200 for H antigens was considered significant. Timing of test is important, as antibodies begin to arise during end of first week. The titre increases during second, third and fourth week after which it gradually declines. The test may be negative in early part of first week. Single test is usually of not much value. A rise in titre between two sera specimens is more meaningful than a single test. Positive results are reported only after correlating with clinical features.

Result

The study population included 433 patients attending various outpatient departments and those admitted in Medical College Hospital, Thiruvananthapuram with complaints of fever.

Table 1: Serological analysis of sample tested by widal test

Widal	Number	Percentage
Positive	26	6
Negative	407	94
Total	433	100

Table 1 shows that out of 433 cases 26 (6%) were positive and 407 (94%) were negative by widal.

Table 2: Serological analysis of sample tested for antibodies of Salmonella by immunochrom-atography

Immuno chromatography	number	Percentage
Positive	21	4.85
Negative	412	95.15
Total	433	100

Table 2 shows that out of 433 cases 21 (4.85%) were positive and 413 (95.15%) were negative by immunochromatography.

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

Test result		WIDAL		Total	sensitivity	Specificity
		Positive	Negative			
ICT	Positive	21	0	21	80.8%	100%
	Negative	5	407	412		
Total		26	407	433		

Table 3 shows that out of 26 widal positive cases, 5 were negative by immuno chromatography. Sensitivity and specificity of immuno chromatographic method was 80.8% and 100% respectively considering widal as standard. Positive predictive value was 100 and negative predictive value was 98.8.

Table 4: Age wise distribution

Age in years	Positive		Negative		Total
	Widal	ICT	widal	ICT	
	No: & %	No: & %	No: & %	No: & %	
0-10	12(12.5 %)	8 (8.3%)	84 (87.5%)	88 (91.6%)	96
11-20	3 (2.5%)	3 (2.5%)	115(97.5%)	115(97.5%)	118
21-30	3 (4%)	3 (4%)	73 (96.1%)	73(96.1%)	76
31-40	3 (4.5%)	3 (4.5%)	63 (95.5%)	63 (95.5%)	66
41-50	4 (10%)	3 (7.5%)	36 (90%)	37 (92.5%)	40
51-60	0	0	20 (100%)	20(100%)	20
>60	1 (6%)	1 (6%)	16 (94.1%)	16 (94.1%)	17
Total	26 (6%)	21(4.85)	407 (94%)	413(95.15%)	433

Table 4 shows that positivity in both widal and ICT method is maximum in the age group of 0-10 followed by age group of 41-50.

Table 5: Coinfection with leptospirosis

Typhoid positive cases		Coinfection with leptospirosis No:& %
widal test	26	5 (19.2%)
ICT	21	0 (0%)

Out of 26 widal positive cases 5 cases shows coinfection with leptospirosis. In widal test these cases gives TH titre only. These leptospira positive cases was negative for typhoid Ab by ICT method .

Table 6: Coinfection with dengue

Typhoid positive cases		Coinfection with dengue No:& %
widal test	26	1 (3.8%)
ICT	21	1 (3.8%)

Table 7: Coinfection with scrub typhus

Typhoid positive cases		Coinfection with scrub typhus No: & %
widal test	26	1(3.8%)
ICT	21	1(3.8%)

Table 6 and 7 shows 3.8% coinfection with dengue fever and scrub typhus.

Discussion

Typhoid fever is one of the most common infectious disease in developing countries including India. The disease is present especially in areas where healthcare facilities are limited and peoples are illiterate, living in unhygienic surroundings, drink raw-water from tube-wells and not habitual of hand- washing with soap after toilet. Symptoms and signs of the disease are non-specific and laboratory tests are essential for diagnosis.

In the present study, 433 patients with complaints of fever attended in various outpatient departments and those admitted in Medical college hospital, Trivandrum. were included. Out of this 26 patients were positive for widal test. 21 patients were positive by immune chromatography method. Seroprevalence rate in this study is 6% in widal test and 4.8% in ICT method. A study was conducted in the Department of Microbiology, Bhaskar Medical College, Moinabad, Rangareddy district in Andhra Pradesh showed that 8.57% were positive for widal test[8]. Study of Indu Sharma, T. L Devi and S.S Sen at Microbiology Department, Silchar Medical College and Hospital, Silchar, Assam showed 30% serum positivity for widal test [9]. Another study by Vallab Ganesh Bharadwaj, Department of Microbiology, Chennai Medical College Hospital and Research Centre, (SRM Group), Irungalur, Tiruchirapalli, Tamilnadu shows 27.3% prevalence[10].

In this study sensitivity and specificity of ICT were calculated by widal taken as standard. The sensitivity and specificity of ICT in suspected typhoid cases were found 80.8% and 100% respectively. Out of 26 widal positive samples ICT positivity can be seen in 21 cases. The cross reactive samples were ICT negative. ICT has been evaluated in many countries and they found signi-ficantly higher sensitivity and specificity[11,12,13]. An evaluation of ICT in India was found to be 100% sensitive and 80% specific compared to a blood culture as gold standard [12]. A study on rapid diagnosis of typhoid fever- a comparative study at Department of Microbiology, GGS Medical College, Faridkot, Punjab, India[14] shows sensitivity of the test as 83.8% and specificity 92.11% [14]. A similar study carried out in the southern part of India reported that ICT had a sensitivity of 92.3% and specificity of 98.8%.

Another study conducted in Department of Microbiology, Goa Medical College, Bambolim Goa, India was found to have a sensitivity of 90% and a specificity of 94.6%[3]. In agreement with my findings Sherwal *et al* 87.5% and Anggraini *et al* found 100% specificity of dot -EIA in the diagnosis of typhoid fever. Widal test has been used extensively as a laboratory tool for diagnosis of typhoid fever in most laboratories, but it is laborious, time consuming and may not be positive in early stages and is to be interpreted judiciously[3].

In this study19% coinfection seen with leptospirosis and 3.8% coinfection with dengue and scrub typhus. A study on Dengue and Typhoid Co-infection done in Government Hospital in North Delhi by Yukti Sharma shows 7.8% cases co infection with dengue. Another study by K.Mary Sushi, on Seroprevalence of Leptospirosis, Enteric fever and Dengue in patients with acute febrile illness in Tamil Nadu, India shows out of 100 cases 2% shows coinfection with leptospirosis and 1 % with Dengue [15].

Another study conducted in Department, Naval Medical Research Center, Silver Spring, Maryland shows two positive scrub typhus in acute febrile illness patients out of 167 positive typhoid cases[16].

It has been observed that the water and sewage pipelines lie close together in the slum areas of India and they are prone to leakage and cross-contamination.

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. ICT is a simple and sensitive test for early diagnosis of typhoid fever in children. The results can be visually interpreted and is available within one hour.

Conclusion

433 sera samples were analyzed for antibodies to Salmonella Typhi in this study. It was concluded that typhoid prevalence in this area was 6%. All the signs and symptoms of the disease are nonspecific and common with other acute febrile illnesses. A definitive diagnosis of the disease is required for treatment and to decrease the morbidity, mortality and transmission.

The ICT can used as the suitable method for rapid diagnosis of typhoid fever. Detection of antibody (whole blood by IgM) 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. So efforts should be made to establish antibody (IgM) detection from whole blood by ICT method at field level, especially in the endemic areas of developing countries like India, even though the standard test is the widal tube agglutination test.

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