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Original Article

Validity of Immunochromatographic Test for Antibody in Diagnosis of Typhoid Fever in Children Admitted in A Tertiary Care Hospital

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Abstract

Background: To determine the Validity of Immunochromatographic Test (ICT) in diagnosis of typhoid fever in children admitted in a tertiary care hospital. **Methods:** This cross sectional study was carried out the in Pediatric & Medicine wards of Chittagong Medical College Hospital (CMCH), Chittagong during the period July 2012 to June 2013. A total number of 150 clinically suspected cases of typhoid fever (Age >6 months to18 years) were enrolled in this study. After taking informed written consent, detailed history & clinical examination were completed. A blood culture sample was taken on the day of admission before starting antibiotic. On the 5th day onwards of appearance of fever, blood sample was taken to perform ICT. Patients received standard medical treatment of the admitting wards. **Results:** Blood C/S for Salmonella typhi was found positive in 16(10.7%) cases. Positive ICT for typhoid fever was found in 37(24.7%) cases. Among then, IgM was 18(12.0%) IgM+IgG were 8(5.3%) and IgG was 11(7.3%). ICT found true positive in 14, false positive in 23, false negative in 2 and true negative in 111 cases, where blood culture considered as gold standard. The difference was statistically significant ($p<0.05$) between two groups. Immunochromatographic Test (ICT) showed sensitivity 87.5%, specificity 82.8%, accuracy 83.3%, positive predictive value 37.8% and negative predictive value 98.2% for identification of typhoid fever. **Conclusion:** The present study has shown high sensitivity & specificity of ICT, it can be used as a useful & prospectful diagnostic tool.

Key words: Typhoid fever; Immunochromatographic Test (ICT); Blood culture.

INTRODUCTION

Typhoid fever is an acute systemic infection caused by Salmonella enterica serotype Typhi or Paratyphi. This disease is endemic in most developing countries, including South and Southeast Asia (Including Indonesia) Central America and other countries which are populous, have high urbanization and a lack of proper hygiene and sanitation (Parry et al. 2002)[1]. The worldwide incidence of typhoid fever is estimated to be approximately 16 million cases annually, of which 7 million cases occur in Southeast Asia. More than 600,000 people die due to this disease each year (Ivanoff, 1995)[2].

The isolation of S. Typhi or S. Paratyphi A from blood, bone marrow, rose spots or other sterile sites provides the most conelusive confirmation of typhoid fever. Therefore, culture should be considered as the gold standard and used for evaluating all diagnostic tests, irrespective of their level of sophistication (WHO 2003)[3]. Bacterial isolation confirms the clinical diagnosis and allows antimicrobial-susceptibility testing which can direct appropriate therapy yet it is only positive in approximately 40–60% of presumptive cases (Gilman et al. 1975 and Guerra-Caceres et al.2011)[4,5].

ICDDR, B in 2001 conducted a study at Kamalapur in Dhaka and concluded approximate incidence of typhoid fever in our population documented by positive blood culture is 3.9 episodes per year per 1000 populations.

The S. Typhi-specific antibodies from the suspected patients are detected by their ability to inhibit the binding between colored indicator particles that are coated with a monoclonal antibody (mAb) specific for the S. Typhi O9 LPS antigen, and magnetic particles that are coated with S. Typhi LPS. Typhidot (Malaysian Biodiagnostic Research SDN BHD, Kuala Lumpur, Malaysia), was developed for the detection of specific IgM and IgG against a 50-kD S. Typhi outer membrane protein (Choo et al. 1994 and Jackson et al.1995)[6,7]. Typhidot is an ELISA-based method, miniaturized into an immunodot test format.

Narayanappa et al. (2010) compared the sensitivity and specificity of Typhidot-M and Widal test with blood culture (Gold standard) for diagnosing typhoid fever in 105 children aged 1-15 years admitted with clinical suspicion of typhoid fever[8]. Of the 105 cases, blood culture was positive for S.typhi in 41 (39%) children, widal test was positive in 48 (45.7%) and Typhidot-M was positive in 78 (74.3%) cases. Sensitivity and specificity of Typhidot-M was 92.6% and 37.5% while sensitivity and specificity of Widal test was 34.1% and 42.8%, respectively. In children with fever of less than 7 days duration, Typhidot-M was positive in 97%, compared to 24.2% by widal test. Typhidot-M is a simple and sensitive test for early diagnosis of typhoid fever in children.

Pastoora et al. (2008) developed a point-of-care test for the serodiagnosis of typhoid fever in the format of an immunochromatographic lateral flow assay[9]. The sensitivity of this typhoid fever IgM flow assay for samples collected at 1st diagnosis from patients with culture-confirmed typhoid fever was determined to be 59.3%. The sensitivity ranged from 41.2% to 89.5%, depending on the duration of illness. A specificity of 97.8% was calculated based on results obtained for patients with clinical suspicion of typhoid fever that was later excluded.

Tubex™ and Typhidot®, rapid tests for typhoid fever, performed well in evaluations conducted in hospital settings among patients with culture-confirmed typhoid fever. Naheed et al. (2008) evaluated these tests in a community clinic in Bangladesh. Blood samples were obtained from 867 febrile patients for culture, Typhidot® and Tubex™ tests. Considering the 43 blood culture-confirmed cases of typhoid fever as typhoid positive and the 24 other confirmed bacteraemia cases as typhoid negative, Tubex™ was 60% sensitive and 58% specific, with 90% positive and 58% Negative Predictive Values (NPVs) Typhidot® was 67% sensitive and 54% specific, with 85% positive and 81% NPVs. When blood culture-negative patients and other bacteraemia cases together were considered typhoid negative, positive predictive values were only 14% for Tubex™ and 13% for Typhidot® increasing to only 38% and 20% when restricted to patients with 7 days of fever. The investigators conclude that the value of Tubex™ and Typhidot® tests for typhoid fever diagnosis in a community clinic in urban Bangladesh is low[10].

Typhoid remains a global public health problem, and quick accurate immunodiagnosis is needed. Rahmana et al. (2007) examined the performance of the 5-min TUBEX® O9-antibody detection kit in 243 outpatients (Mostly children and infants) in their first week of fever and 57 healthy subjects in the Bangladesh community. Based on culture results, TUBEX® was 91.2% (31/34) sensitive and 82.3% (172/209) specific in febrile subjects[11]. However, specificity was better in nonfebrile healthy subjects (89.5%, 51/57) or in febrile individuals who serologically had dengue fever (90.5%, 57/63) suggesting that some culture-negative febrile individuals could be truly typhoidal.

Typhoid fever still continues to be a major public health problem around the world. Simple, reliable, point-of-care rapid diagnostics for typhoid fever have been a long-felt need of clinicians working in endemic countries. Such tests need to be robust and suitable for use in remote areas with limited laboratory facilities and the medical staff should not require any specific technical training. The overall utility and uptake of these tests depends on their simplicity. Such tests should have limited steps and be designed to yield a simple 'positive/negative' result at preset thresholds. Ideally, the results should be available within 1 h of the initiation of the assay, so that they can be interpreted while the patient is in attendance. Evaluation of validity of ICT for antibody in diagnosis of typhoid fever will help to overcome the gap in currently available diagnostic methods. It will also provide easy and early diagnosis of typhoid fever in resource limited country like ours.

MATERIALS AND METHODS

Laboratory based descriptive cross sectional study was done during the period of July 2012 and June 2013 in Chittagong Medical College Hospital, Department of Pediatrics & Medicine. Chittagong Medical College Hospital, Department of Pediatrics & Medicine. 150 clinically suspected typhoid fever patients were included in this study. Patients were allocated a study ID number at the time of enrolment. Parents of enrolled patients were asked to give informed consent and answer a brief questionnaire about clinical signs and symptoms, antimicrobial treatment, and history of typhoid fever and vaccination. On admission, a blood culture sample was taken before receiving antibiotics. Suspected colonies were identified by serological test. Antimicrobial sensitivity was assessed by the disc diffusion methods or E-test on a Muller-Hilton agar plate according to CLSI guidelines. ICT was done from day 5 onwards of appearance of fever by using SD BIOLINE Salmonella Typhi IgG / IgM Rapid test strip. The strip is designed to simultaneously detect & differentiate IgG & IgM antibodies to Salmonella typhi in human serum, plasma on whole blood.

Interpretation of the test

Negative: The control line is only visible on the test strip. No IgG and IgM antibodies were detected. Probably not typhoid.

IgG Positive: The control and IgG line (G) are visible on the strip. This is positive for IgG antibodies. This is indicative of previous Salmonella typhi infection (In which case current fever may not due to typhoid) or relapse or reinfection.

IgM Positive: The control line and IgM line are visible on the strip. This is positive for IgM antibodies to salmonella typhi. This is indicative of acute typhoid fever.

IgG and IgM Positive: The control line, IgM line (M) and IgG line (G) are visible on the strip. This is positive for both IgM and IgG antibodies. This is indicative of acute typhoid fever.

Invalid: The control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Repeat the test using a new test device.

RESULTS

Table 1: Categorization of the study patients according to blood culture positivity for Salmonella Typhi.

Blood C/S	Number of patients	Percentage
Blood C/S for Salmonella Typhoid	16	10.7
Negative	134	89.3

Table 2 : Antibiotic Sensitivity among culture positive patients (n=16).

Sensitivity	Number of patients	Percentage
Ciprofloxacin (Intermediate sensitivity)	04	25.0
Azithromycin	16	100.0
Cefixime	11	68.8
Nalidixic acid (Intermediate sensitivity)	09	56.3
Ampicillin (Intermediate sensitivity)	03	18.8
Chloramphenicol	06	37.5
Ceftriaxone	16	100.0
Imipenam	16	100.0

Table 3 : ICT for typhoid fever (n=150).

ICT for typhoid fever	Number of patients	Percentage
Negative	113	75.3
Positive	37	24.7
*IgM	18	12.0
*IgM+IgG	8	5.3
**IgG	11	7.3

* Indicates acute Infection
**Indicates past Infection

Table 4 : Pre Admission Antibiotic in culture positive patients (n=16).

Antibiotic in culture positive	Number of patients	Percentage
Received	07	43.8
Not received	09	56.2

Table 5 : Comparison between ICT with blood culture for typhoid fever (n=150).

ICT	Blood Culture				Chi value	p value
	Positive (n=16)		Negative (n=134)			
	n	%	n	%		
Positive	14	87.5	23	17.16	38.05	0.001[s]
Negative	02	12.5	111	82.83		

S=significant, p value reached from chi square test

Table 6 : Sensitivity, specificity, accuracy, positive and negative predictive values of ICT evaluation for prediction of typhoid fever (n=150).

Validity test	Percentage
Sensitivity	88 (87.5)
Specificity	83 (82.8
Accuracy	83 (83.3)
Positive Predictive Value (PPV)	38 (37.8)
Negative Predictive Value (NPV)	98 (98.2)
TP=True Positive	FN=False Negative
TN=True Negative	FP=False Positive
PPV=Positive Predictive Value	
NPV= Positive Predictive Value	

DISCUSSION

In this present study it was observed that blood C/S for Salmonella typhi was found in 10.7% cases. A study in a superspeciality children hospital at New Delhi done by Manchanda et al. (2006) showed that a total of 56 S. typhi and 5 S. paratyphi isolates were obtained from 673 blood culture cases that comprised 8.3% of culture positivity which is comparable with the current study[12]. Krishnan et al. (2009) found in their study that 70% and 30% of the isolates were Salmonella enterica serovar typhi and Paratyphi A, respectively[13]. Abdoel et al. (2007) found 42.5% culture-positive patients in their study[14]. The final diagnosis of typhoid was based on a positive blood culture in 118 (65.9%) patients and on clinical symptoms and signs consistent with typhoid or paratyphoid fever in 61 (34.1%) patients found by Hatta et al. (2002). Prior antibiotic intake might have influenced yield of culture positive patients in this current study[15].

In this current series it was observed that Azithromycin, Ceftriaxone and Imipenam were 100% sensitive followed by Cefixime 68.8%, Nalidixic acid (Intermediately sensitive) 56.3%, Chloramphenicol 37.5%, Ciprofolxacin (Intermediately sensitive) 25.0% and Ampicillin (Intermediately sensitive) 18.8%. Krishnan et al. (2009) found that Salmonella enterica serovar

typhi and paratyphi A, were highly sensitive to choloramphenicol (86.0%) ampicillin (84%) and cotrimoxazole (88%). Highest sensitivity was seen for cephalosporins, followed by quinolones[13]. In another study, Mathura et al. (2005) showed sensitivity to ceftriaxone was 100%. There have been some reports of the re-emergence of the sensitivity of S. typhi to chloramphenicol and other first line drugs[16]. Bhatia and others reported that highest (96%) sensitivity of Salmonella typhi to chloramphenicol in India (Bhatia, Mathur and Arora 2007)[17]. Another study by Yashavanth and Vidyalakshmi found that the re- emergence of chloramphenicol (97.4%) sensitivity among the strain of S. typhi pathogens in Mangalore in 2007 (Yashavanth and Vidyalakshmi 2010)[18]. The above findings are comparable with the current study indicating more sensitivity of newer antimicrobial drugs.

In this study it was observed that almost a half (47.3%) of the patients received antibiotic during pre admission period and more than a half (52.7%) of the patients didn't received any antibiotic before hospital admission.
In this series it was observed in culture positive patients that 7(43.8%) received antibiotic and 09(56.2%) didn't receive any antibiotic during pre admission period.

In this present series it was observed that negative ICT for typhoid fever was found in 75.3% cases and positive ICT for typhoid fever was found in 24.7% cases, IgM (Acute Infection) was 12.0%, IgM+IgG (Acute Infection) were 5.3% and IgG (Past Infection) was 7.3%. Kawano et al. (2007) found Typhidot-IgM and IgG in 54.7%, and 70.7% cases respectively[19]. Sultana (2012) has done a study in Mymensingh Medical College, Mymensingh between July, 2010 and June, 2011 including 200 individuals of different age and sex. Of them, 150 were clinically suspected cases of typhoid fever and ICT positivity was found as 71.9% having IgM antibody, 21.05% having both IgM and IgG antibody and only 7.01% having IgG antibody, which closely resembles the current study[20].
In this current study it was observed that positive ICT was found in 37 cases, out of which 14 (True positive) were blood culture positive and 23 (False positive) were blood culture negative. On the other hand a total of 113 cases with negative ICT was found, out of which 2 (False negative) were blood culture positive and 111 (True negative) were blood culture negative. The difference was statistically significant ($p<0.05$) between two groups.

In this present study it was observed that the validity of Immunochromatographic Test (ICT) for typhoid fever having sensitivity 87.5%, specificity 82.8%, accuracy 83.3%, positive predictive value 37.8% and negative predictive value 98.2%.

ICT has been studied in many countries and they found significantly higher sensitivity and specificity (Jesudason, Esther and Mathai 2002; Pastoor et al. 2008; Anusha, Ganesh and Lalitha 2011)[9,21,22]. An evaluation of ICT (Typhidot) in India was found to be 100% sensitive and 80% specific compared to blood culture as gold standard (Jesudason, Esther and Mathai 2002)[22]. Considering the 43 blood culture-confirmed cases of typhoid fever as typhoid positive and the 24 other confirmed bacteremia cases as typhoid negative, ICT (Typhidot) was 67% sensitive and 54% specific, with 85% positive and 81% NPVs. Gopalakrishnan et al. (2002) evaluated the Typhidot kit and the sensitivity and specificity were found to be 82.0% and 78.0% respectively. It had a PPV and NPV of 57.7% and 90.1% with an efficiency of the test to be 72.9%[23].

CONCLUSION

The immunochromatographic Test (ICT) has been compared with the blood culture known as gold standard for diagnosis of typhoid fever. Blood culture has less sensitivity, requires well-equipped laboratory with highly skilled personnel, adequate amount of blood and at least three days for the interpretation of result & where prior intake of antibiotic affects the result. The studied ICT, on the contrary has considerable sensitivity, is feasible at bed side by any trained health personnel and requires much less amount of blood & time for interpreting results. Considering these points, ICT is likely to be more convenient to be applied in the remote areas deprived of satisfactory medical lab facilities.

DISCLOSURE

All the authors declared no competing interest.

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