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Evaluation of Immunochromatographic Test (ICT) for Sensitivity and Specificity among Children with Typhoid Fever in a Tertiary Care Hospital, Chittagong, Bangladesh

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Original Research Article

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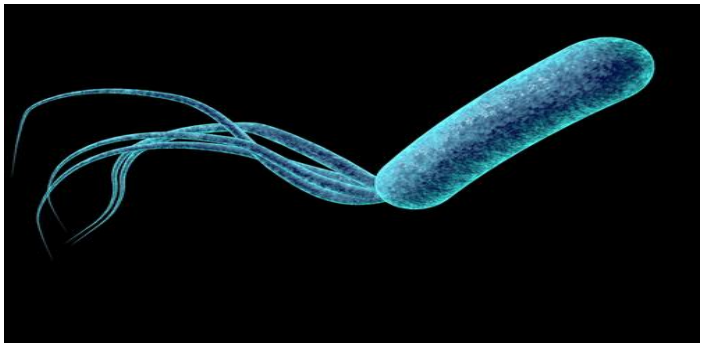
Abstract: A cross-sectional study was carried out in the Pediatric & Medicine wards of Chittagong Medical College Hospital (CMCH), Chittagong from July 2012 to June 2013. The aim of the study was to evaluate Immunochromatographic Test (ICT) for sensitivity and specificity among children with Typhoid fever. Maintaining an inclusion criteria based on the protocol, we recruited 150 suspected cases of typhoid fever (age >6 months to 18years) who were admitted in the above mentioned hospital. In the study, we found dominating age group was 1-5 years (56.7%). Males were also dominated in sex distribution. More than a half (56.0%) of the patients came from rura areal. More than one third (33.3%) patients had tongue coating, 29(19.3%) patients had palpable liver, 11(7.3%) patients had palpable spleen and 1(0.7%) patient had caecal gurgling. Negative ICT for typhoid fever was found in 113(75.3%) patients and positive ICT for typhoid fever was found in 37(24.7%) patients. IgM was 18(12.0%), IgM+IgM were 8(5.3%) and IgG was 11(7.3%) respectively. ICT was true positive in14 cases, false positive in 23 cases, false negative in 2 and true negative in 111 cases, where blood culture considered as gold standard. Performing Immunochromatographic test (ICT), we found sensitivity 87.5%, specificity 82.8%, accuracy 83.3%, positive predictive value 37.8% and negative predictive value 98.2% for diagnosis of typhoid fever. In the conclusion, we can say that Immunochroomatographic test (ICT) can facilitate the treatment of typhoid fever patients.
Keywords: Immunochroomatographic Test (ICT), Sensitivity, Specificity, Typhoid fever, Suspected case.

INTRODUCTION

Typhoid fever, along with paratyphoid fever is a type of enteric fever. It is an acute systemic infection caused by Salmonella enterica serotype Typhi or Paratyphi growing in the intestines and blood. Typhoid is spread by eating or drinking food or water contaminated with the faeces of an infected person 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 [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 [2].

The main symptom of this disease is fever with a step ladder pattern, associated with headache, malaise, anorexia, nausea, generalized aches and abdominal disturbances like discomfort, constipation or diarrhea. In severe cases intestinal bleeding and perforation can occur which may potentially be fatal. Disturbance in consciousness from apathy, delirium or coma may be present. Other symptoms, such as a coated tongue, enlargement of the liver and spleen, relative bradycardia and rose spots definitely support the diagnosis [7]. Sometimes the symptoms of typhoid fever are not typical and are misinterpreted. Other common infectious diseases, like malaria, dengue fever, pneumonia, tuberculosis or meningitis, sometimes mimic typhoid fever and may be difficult to distinguish in the early course of the disease. Early recognition and management of typhoid fever is needed to avoid the severe complications and possible fatality [3]. The isolation of S. Typhi or S. Paratyphi A from blood, bone marrow, rose spots or other sterile sites provides the most conclusive confirmation of typhoid fever.



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Therefore, culture should be considered as the gold standard and used for evaluating all diagnostic tests, irrespective of their level of sophistication [2]. 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 [7] Guerra-Caceres *et al.* [5]. The Widal test, which measures agglutinating antibodies against lipopolysaccharide (LPS; O) and flagella (H) antigens of the S. Typhi in the serum of individuals with suspected typhoid fever, was introduced more than a century ago and is still widely used [6,7]. It is simple and cheap to perform, and with the slide format, rather than tube, it takes only a few minutes. Use of the method has been hampered by a lack of standardization of reagents and inappropriate result interpretation [8,9]. The Widal test ideally requires both an acute and a convalescent-phase serum sample taken approximately 10 days apart, and a positive result is determined by a fourfold increase in antibody titer. However, antibody titers in infected patients often rise before the clinical onset, making it difficult to demonstrate the required fourfold rise between initial and subsequent samples for a confirmatory diagnosis [10]. Furthermore, in practice, the result of a single, acute-phase serum sample is often used to help patient management and, although useful in some cases, a single serum result may be confusing in others [10,11]. A single sample test is generally plagued by false-negative and false-positive results. A large number of cross-reacting antigenic determinants of typhoidal and nontyphoidal Salmonella organisms and other Enterobacteriacae are now recognized, as are several other diseases caused by non- Salmonella organisms such as malaria, dengue and TB [22, 23].

A major drawback of a rapid diagnostic and indeed of any non-culture-based method is the lack of an isolated organism and antimicrobial susceptibility result. Typhoid fever diagnostics represent a paradigm of how technology must be driven by the human and microbiological realities of the natural infection, as the infection has a unique molecular pathogenesis with a specific host response. There are a wide range of potential clinical specimens and possible technological approaches but methods must be conceived in physiological reality. Therefore, any new development of diagnostics for acute infection needs to address either the low count of bacteria in sterile sites or the cross-reactive nature of any potential antigens. Diagnosis of acute infection must take into account the relevant differential diagnosis for the setting, be informative concerning drug resistance and locally available treatment options. In considering the product profile of a new rapid test, the specifications developed by the WHO Sexually Transmitted Diagnostics Initiative for the characteristics of an ideal diagnostic test in the developing country context could equally apply to typhoid rapid diagnostic tests. 'ASSURED' tests should be affordable by those at risk of infection, sensitive and specific, user-friendly, robust, equipment-free and able to be delivered to those who need it [5].

Typhoid fever, caused by *Salmonella enterica* serotype Typhi, is a major cause of morbidity and mortality worldwide. Isolation of serotype Typhi from blood, urine, or stool is the most reliable means of confirming an infection. However, this requires laboratory equipment and technical training that are beyond the means of most primary health care facilities in the developing world. Most serotype Typhi infections are diagnosed purely on clinical grounds and treated presumptively. As a result, the diagnosis may be delayed or missed while other febrile illnesses are considered, and patients without typhoid fever may receive unnecessary and inappropriate antimicrobial therapy. Unfortunately, neither the Widal test, which remains in widespread use in the developing world, nor any of the sero-diagnostic tests that have since been developed has proven sufficiently sensitive, specific, and practical to be of value in areas where this disease is endemic [3]. Recent advances in molecular immunology have led to the identification of potentially more sensitive and specific markers in the blood and urine of patients with typhoid fever and have enabled the manufacture of practical and inexpensive kits for their detection. Bangladesh being a highly endemic zone of typhoid fever [1] and equipped with insufficient laboratory facilities need to develop a guideline to diagnose typhoid fever easily and accurately, for which ICT may offer a great help. This study is believed to evaluate the accuracy of ICT in comparison to blood

culture in the patients suffering from typhoid fever and will not only determine the patients with typhoid fever, rather will also help the health care providers to avoid unnecessary antibiotic prescriptions..

Objectives

General objective

- To evaluate Immunochromatographic Test (ICT) for the Sensitivity and Specificity among Children with Typhoid Fever.

Specific objectives

- To observe pattern of antibiotic sensitivity in culture positive typhoid fever children.
- To observe the sensitivity and specificity of different antibody titres(IgM & IgG) in ICT.

MATERIALS AND METHODS

This was a Laboratory based descriptive cross sectional study during the period of July 2012 and June 2013 in Departments of Pediatrics & Medicine, Chittagong Medical College Hospital, and Chittagong. Suspected cases of typhoid fever (age >6 months to18yrs) admitted in Pediatric & Medicine wards of CMCH were taken as study population. Clinically suspected patients of typhoid fever who were > 06 months old and who presented with ≥ 3 days of fever during the study period were eligible for enrollment. Patients were allocated a study ID number at the time of enrollment. 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 and from day 5 of onset of fever, blood samples were taken for ICT.

Blood Culture

One hundred & fifty patients of clinical Typhoid Fever cases were evaluated for the study. After admission in pediatric ward, study cases were selected according to the inclusion criteria. A written informed consent was filled up by the attendant for permission. Data was collected by a questionnaire. After a detailed history, general & relevant systemic examinations were done properly & were documented. Then under all aseptic precaution on the day of admission blood culture samples were collected (1ml. for children aged > 6months to <5years, 5ml for children aged 5 - < 15years and 8ml. for patients aged 15 – 18years) using pediatric bottle as appropriate. Bottles were incubated in the ßacT / Alert automated system for 5-7 days at a renowned well-equipped, quality-controlled clinical laboratory in Chittagong. Positive bottles were processed by preparing a smear for Gram stain and sub culturing onto sheep blood, chocolate and MacConkey agars. The sheep blood & chocolate agar was incubated in CO2 (candle jar) at 35–37c for 48hrs; the MacConkey agar in air for at 35-37c for 48hrs. 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

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. The test strip has. 3 pre-coated lines, 'G' (test line for salmonella typhi IgG).'M' (test line for Salmonella typhi IgM and 'C' (control line) on the surface of the strip. These 3 lines are not visible before applying the sample. The control line is used for procedural control which should always appear if the test procedure is performed properly and the test reagents of control line are working. A purple 'G' and 'M' lines was visible in the result window if there are enough IgG and / or IgM antibodies to Salmonella typhi in the sample. If IgG and/or IgM antibodies to Salmonella typhi are not present in sample, there is no color appears in G and /or M. When a specimen is added to the test, anti Salmonella typhi IgG and IgMs in the specimen sample reacts with Salmonella typhi proteins of colloidal gold conjugates and forms a complex of antibodies and colloidal gold conjugates. As the mixture migrates along the length of the strip by capillary action, the anti-Salmonella typhi IgG or IgM complex is captured by the relevant anti-human IgG and or anti-human IgM immobilized in two lines across the test strip and generate a colored line.

Steps of the test

01. About 01ml. of venous blood was collected under all aseptic precaution & Centrifuged to get the serum.
02. At first 04 drops (about 120µl) of assay buffer was added in a disposable test tube.
03. 01µl of specimen was the added to test tube containing assay buffer.
04. After adequate stirring, the strip was hold vertically & inserted into tube containing diluted specimen.
05. Interpretation of test result was done in 15 – 30 minutes. If the band is very faint at 15 minutes, the results were read again at 30minutes.

Selection Criteria

Inclusion Criteria

- Suspected (clinical) cases of typhoid fever admitted in Pediatrics & medicine wards of CMCH
- Probable cases of typhoid fever admitted in Pediatrics & medicine wards of CMCH
- Age limit > 6 months – 18 yrs

Exclusion Criteria

- Age >18 years & < 6 months.

- Very critically ill and haemodynamically unstable patients.
- Attendants of cases unwilling to give informed consent.

Sample Size calculation

To determine the sample size the following formula was followed

$$n = \frac{z^2 pq}{d^2}$$

N= the desired sample size, z= Standard normal deviate usually set at 1.96, p= Proportion in the population (30.8% i.e. 0.308), q= 1-p =0.692,

Examination of the microbiology laboratory records showed that 30.8% (95% CI 26.8-35.1%) consecutive patients admitted to the hospital and investigated with a blood culture and a Immunochromatographic Test (ICT) had a blood culture positive for S. typhi [1], d= Degree of accuracy which is considered as 0.05. According to this formula the targeted sample was 327.5 = 328, the duration of data collection in current study is only 6 months. So the targeted sample size could not be collected during this study duration, therefore 150 patients with suspected typhoid fever were taken in this study. Study conducted as per rule of ethical committee of CMCH and participation was voluntary with taken written consent from all the respondents.

Operational Definitions: (WHO 2003)

Confirmed case of Typhoid fever

A patient with fever (380c and above) that has lasted for at least 3 days with a laboratory confirmed positive culture.

Probable case of Typhoid fever

A patient with high fever (380c and above) that lasted for 3 days with a positive serodiagnosis or antigen/antibodydetection test but without S. Typhi isolation. Suspected Typhoid fever:

A patient with fever 380c or above with physical findings consistent with typhoid fever

- Sensitivity: Proportion of disease positive who are test positive also called true positive rate (e.g. the percentage of sick people who are correctly identified as having the condition).
- Specificity: Proportion of disease negative who are test negative (e.g. the percentage of healthy people who are correctly identified as not having the condition, sometimes called the true negative rate).
- Positive predictive value: Proportion of test positives that are truly disease positive.
- Negative predictive value: Proportion of test negatives that are truly disease negative.
- Accuracy: Proportion of all test results (positive & negative) those are correct.

All the data were collected and recorded systematically in a questionnaire and were analyzed using computer software SPSS (Statistical Package for Social Sciences). Data were presented in the form of tables and graphs. Quantitative data were presented with descriptive statistics and bivariate analysis. The level of significance of 0.05 was used for this study.

RESULTS

ICT found TP 14 cases, false positive 23, false negative 2 and true negative 111 cases, where blood culture considered as gold standard. The difference was statistically significant ($p<0.05$) between two groups. Immunochromatographic (ICT) was sensitive 87.5%, specificity 82.8%, accuracy 83.3%, positive predictive values 37.8% and negative predictive values 98.2% for identification of typhoid fever.

Table-I: Background characteristics of the study patients (n=150)

Age (years)	Number of patients	Percentage	Mean/±SD	Range
1-5	85	56.7	5.41/±3.53	1-15
6-10	52	34.7		
11-15	13	8.7		
Sex				
Male	83	55.3		
Female	67	44.7		
Residence				
Urban	66	44.0		
Rural	84	56.0		

Table-2: Distribution of the study patients according to physical examination (n=150)

Variable	Number of patients	Percentage
Tongue coating		
Present	50	33.3
Absent	100	66.7
Liver		
Palpable	29	19.3
Not Palpable	121	80.7
Spleen		
Palpable	11	7.3
Not Palpable	139	92.7
Caecal gurgling		
Present	1	0.7
Absent	149	99.3

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

	Blood Culture				Chi value	P value
ICT	Positive (n=16)		Negative (n=134)			
	n	%	n	%		
Positive	14(a)	87.5	23(b)	17.16	38.05	0.001s
Negative	02(c)	12.5	111(d)	82.83		

s=significant. P value reached from chi square te

Table-5: Sensitivity, specificity, accuracy, positive and negative predictive values of the ICT evaluation for prediction of typhoid fever (n=150)

Validity test	Percentage
Sensitivity	87.5
Specificity	82.8
Accuracy	83.3
Positive predictive value	37.8
Negative predictive value	98.2

DISCUSSION

In this current study it was observed that the mean age was found 5.41±3.53 years varied from 1 to 15 years and more than a half (56.7%) of the patients belonged to 1-5 years. Naheed *et al*. [9] showed 57.0% of their studied patients by 5 years old, which is comparable with the current study. Similarly, House *et al*. [10] found the median age was 7 years of the typhoid patients with IQR was 5 to 14 years. Choo *et al*. [24] included one patient with typhoid fever in the birth to l-year age group, three patients in the 1- to 2-year age group and 38 patients in the >2-year age group. In another study, Kawano *et al*. [15] showed the mean age was 2.5 years, which are lesser with the current study.

In this current study it was observed that positive ICT was found 37 cases, out of which 14 (True Positive) were blood culture positive and 23 (False Positive) were blood culture negative cases. On the other hand a total of 113 negative ICT was found, out of which 2 (False negative) were blood culture positive and 111 (True negative) were blood culture negative cases. The difference was statistically significant ($p<0.05$) between two groups. In this present study it was observed that the evaluation of Immunochromatographic test (ICT) for typhoid fever showed 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 [1, 15, 18]. An evaluation of ICT (Typhidot) in India was found to be 100% sensitive and 80% specific compared to blood culture as gold standard [14]. 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% PPV and 81% NPVs. Gopalakrishnan *et al*. [14] showed for the Typhidot kit, the sensitivity and specificity were found to be 82.0% and 78.0%. It had a PPV and NPV to be 57.7% and 90.1% with an efficiency of test to be 72.9%.

Limitations of the study

This study was conducted in a tertiary care hospital in Chittagong. So the study findings may not reflect the exact scenario of all around the country regarding enteric fever. The current study was conducted among 150 children, not a large study to draw a definite conclusion. In Bangladesh, there are few studies of Typhoid fever in the perspective of the objective of current study. So comparison of the study result may have limitations.

CONCLUSION AND RECOMMENDATIONS

Double Immunochromatographic Test (ICT) shows better sensitivity and specificity in diagnosis of Typhoid fever than a single test. So it can be used as a useful and prospectful diagnostic tool. ICT is easy, inexpensive and can be done in remote settings. A large scale, multi-center study over long duration can further validate it.

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