

Original Article

Evaluation of Typhidot (IgM) for Early Diagnosis of Typhoid Fever

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Abstract

Typhoid fever still continues to be a major public health problem, particularly in many developing countries. A simple, reliable, affordable and rapid diagnostic test has been a long-felt need of the clinicians. We, therefore, prospectively evaluated the sensitivity and specificity of Typhidot (IgM), a serological test to identify IgM antibodies against *Salmonella typhi*. The study was carried out in the department of Microbiology, Mymensingh Medical College, Mymensingh between June, 2006 and July, 2007, on a total of 100 samples from clinically suspected patients to have typhoid fever. Blood culture as well as Typhidot test were performed for each of the cases. Out of 100 clinically diagnosed typhoid fever, 14 were blood culture positive for *S. typhi* and 73 were Typhidot (IgM) positive. Among 14 culture positive cases, 13 (92.85%) were Typhidot (IgM) positive. The test was also positive in 04 (20%) out of 20 febrile controls. None of the healthy controls was positive by Typhidot (IgM). The sensitivity, specificity, positive predictive value and negative predictive value of the test using blood culture as gold standard were 92.85%, 90.00%, 76.47% and 97.29% respectively for typhoid fever. Typhidot (IgM) test is rapid, easy to perform and reliable test for diagnosing typhoid fever, and useful for small, less equipped laboratories as well as for the laboratories with better facilities.

Key words: Typhoid fever, *Salmonella typhi*, Typhidot (IgM) test

Introduction

Typhoid fever is widely recognized as a major public health problem in developing countries. The disease is endemic in the Indian subcontinent including Bangladesh, South-East and Far-East Asia, the Middle East, Africa, Central and South America.^{1,2} Incidence of typhoid fever has been estimated as approximately 22 million cases with at least 200,000 deaths occurring annually.³ The disease may occur in all ages, with

the highest incidence found particularly in children.⁴ In Bangladesh, the overall incidence of typhoid fever is 390 cases per 100,000 population per year.⁵

In the wake of emerging multidrug-resistant strains of the bacteria causing typhoid fever, the disorder is known to be associated with significant morbidity and mortality.^{6,7} It is also recognized that a delay in diagnosis and institution of appropriate therapy may significantly increase the risk of adverse outcome and mortality.⁷ Therefore, reliable laboratory tests are essential to establish early diagnosis of typhoid fever, so that appropriate treatment can be applied.

The current standard diagnostic test for typhoid fever is blood

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culture, which may not be available or can not be done properly where adequate microbiological facilities are limited. In addition, easy availability and widespread use of antibiotics in the community makes it frequently difficult to isolate the organism from blood cultures and alternative methods such as bone marrow cultures may be required.⁸ However, the later tests are invasive and difficult to carry out routinely. Therefore, a simple and rapid non-culture assay for the diagnosis of typhoid fever would be of great benefit in circumstances where more sophisticated laboratory support is not in practice.

Recently, such a test has become commercially available under the proprietary name Typhidot. It is a dot-Enzyme Immunoassay (EIA), a new serologic test based upon the presence of specific IgM antibodies to a specific 50 kDa outer membrane protein (OMP) antigen on *Salmonella typhi*.⁹ The test become positive as early as in the first week of the fever, the results can interpreted visually and available within one hour.^{9,10} However, its usefulness in the early serodiagnosis of typhoid fever has not been studied in this region. So, in this study, the newly developed rapid Typhidot assay was applied for the detection of *S. typhi*-specific IgM antibodies in serum and results were compared with blood culture to see the sensitivity and specificity of Typhidot for diagnosis of typhoid fever.

Methods

This cross-sectional study was carried out in the Department of Microbiology, Mymensingh Medical College, Mymensingh, Bangladesh during one year from June, 2006 to July, 2007.

Blood samples from patients clinically suspected to have typhoid fever were collected for both culture and serological test. Blood culture was done for both typhoid fever cases and non-typhoidal febrile controls by conventional or traditional methods using the Trypticase soya broth with Sodium polyanethol sulfonate. Any isolated bacterium was identified according to the recommended standard protocol.¹¹

The Typhidot test was carried out as per manufacturer's instruction. An amount of 250 µl of the diluent was dispensed into a test strip supplied by the manufacturer, 2.5 µl of a sample was added and the mixture was then incubated at room temperature for 20 minutes on a rocker platform. The strip was washed thrice, and 250 µl of anti-human IgM was

added to the well and incubated for 15 minutes. The strip was washed as before, and 250 µl of the colour development solution was added and incubated for another 15 minutes. The reaction was stopped, the strip was washed in distilled water and the result was read. When both the dots on the test strip were as dark as or darker than their corresponding dots on the positive control strip, they were reported as positive. The absence of visible spot indicated a negative test result. If the spots were fainter than the control, that sample was also considered negative, according to the directions given in the kit. In case of discrepant appearance of the duplicate spots, the test was repeated and only if both dots were darker than control, the sample was taken as positive.

Sensitivity and specificity were calculated by the following formulae: Sensitivity=100 x a/a+c, Specificity=100 x d/ b+d; Positive predictive value=a x 100 /a+b; Negative predictive value=d x 100 /c+d, where a=True positive, b=False positive, c= False negative and d=True negative.

Results

All of the 100 clinically suspected typhoid fever cases were concurrently tested by blood culture and Typhidot (IgM) tests. Out of 100 clinically diagnosed typhoid fever cases, 14 (14%) were blood culture positive for *Salmonella typhi* and 73 (73%) were Typhidot (IgM) positive. Among 14 culture positive cases, 13 (92.85%) were Typhidot (IgM) positive. The test was also positive in 04(20%) out of 20 non-typhoidal fever controls. Of these 04 false positive cases, 02 showed infection with *S. paratyphi A* by Blood culture and rest 02 were other infections (01 Urinary tract infection and other 01 Respiratory tract infection). None of the healthy controls were positive by DOT EIA (IgM).

Table I: Results of Typhidot (IgM) in different study groups

Study group	No. of individuals	Typhidot (IgM) positive	Typhidot (IgM) negative
Group I (Blood culture-positive)	14	13(92.85%) (True positive)	01(7.14%) (False negative)
Group II (no-typhoidal fever)	20	04 (20.00%) (False positive)	16 (80.00%) (True negative)
Group III (healthy individuals)	20	00 (00%)	20 (100.0%) (True negative)

Table II: Diagnostic accuracy of Typhidot for IgM

Parameter	Accuracy
Sensitivity	92.85%
Specificity	90.00%
Positive predictive value	76.47%
Negative predictive value	97.29%

Discussion

Typhoid fever still remains a major endemic health problem in Bangladesh. Isolation of the causative agent by culture has remained the gold standard for diagnosis of typhoid fever. However, it is well recognized that facilities for culture are not readily available or limited in many areas. Although the culture method is the gold standard, it is, however, time consuming, expensive and the culture positive cases are also very less and hence the best alternative is the Typhidot (IgM) test for early diagnosis of typhoid fever.

In the present study, out of 100 clinically diagnosed typhoid fever, 14 were blood culture positive for *S. typhi*. Similar findings were also reported from Kolkata and the investigators found an isolation rate of 21.1%.¹² Two investigations from Bangladesh reported isolation rates of *S. typhi* 16.67% and 26.7%.^{13,14} In contrast from Bangladesh and India, others reported isolation rates of only 8.40% and 6.92%.^{15,16} The relative low sensitivity of the blood culture in diagnosing typhoid fever was due to the widespread and irrational use of antibiotics, and the difficulties in obtaining adequate volume of blood for cultures from children.^{17,18}

In the present study, 13 (92.85%) out of 14 culture-positive cases were positive for Typhidot (IgM). These results are consistent with the findings from Pakistan showing 43 (93.47%) Typhidot-positives out of 46 culture-positive typhoid fever cases, and in India, 35 (92.10%) out of 38 culture-positive cases were Typhidot positive.^{17,19} The only 01(7.14%) out of 14 culture-positive cases of the present study was negative in Typhidot (IgM), was a 3 years old child attended in the 2nd day of fever. The false negative Typhidot (IgM) in this case was probably due to the failure of the test to detect the antibodies or perhaps the antibodies did not yet reach the detectable level in this patient.

The false positive results found in the paratyphoid A fever cases of the present study might be due to cross-reaction between the OMP antigen of *S. typhi* and *S. paratyphi A*.²⁰ The false positive results in other 2 non-typhoidal fever cases

are supposed to be due to high level of typhoid fever endemicity in the region indicating persistence of preexisting antibody from previous exposure or may be due to recent sub-clinical infection.²⁰ Inconsistent with our finding, Bhutta and Mansurali from Pakistan found Typhidot positive only in 6 (23.07%) out of 26 and Sherwal *et al* from India also found only 3 (12.5%) out of 24 non-typhoidal febrile cases.^{17, 19}

In the present study, the Typhidot IgM was found to have high specificity of 90%. In agreement with our findings Bhutta and Mansurali¹⁷ found 89% specificity, Sherwal *et al*¹⁹ 87.5% and Anggraini *et al*⁴ found 100% specificity of Typhidot in the diagnosis of typhoid fever.^{4,17,19} In contrast, a study from Pakistan reported a much lower specificity (77%) of the test.¹⁷ This was due to a high rate of (23%) of Typhidot positivity among non-typhoidal fever patients.

After analyzing the findings of the present study, it was concluded that although blood culture is the gold standard for diagnosis of typhoid fever, Typhidot (IgM) might be a practical alternative in the country due to its high sensitivity, specificity and negative predictive value. Typhidot will be specially useful in those areas where facility for blood culture is not available.

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[Conflict of Interest: none declared]