ORIGINAL ARTICLE

Prospective Evaluation of a Rapid Diagnostic Test Dot EIA (Typhidot) for Typhoid Fever

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ABSTRACT

Objective: A prospective evaluation of a rapid diagnostic test Dot EIA (Typhidot) for typhoid fever.

Study design & duration: This prospective study was undertaken in a tertiary hospital from April 2007 to July 2008.

Research Methodology: The patients were divided into three groups as I, II & III with typhoid fever, typhoid suspects & non-typhoid febrile illnesses respectively. Blood culture and typhidot tests were done for the subjects included in the study. The validity of the typhidot test was evaluated by determining the sensitivity, specificity, positive and negative predictive value.

Results: The mean age in years was 28±19 (SD). Out of 150 subjects, males were more than females. The typhidot IgM yielded very high sensitivity, specificity and a negative predictive value noted in group I = 93% & 98.80% 93% respectively and in group II= 79%, 98.80%, & 83% respectively where as in group III (non-typhoid febrile controls), the IgM antibodies were not detected at all (p=0.01). Sensitivity, specificity, and negative predictive value for typhidot IgG noted in group I = 73%, 63%, & 22.4% respectively and in group II = 82%, 37%, & 68% respectively (p=0.03) and a false positive reading of 63% was noted in group III (controls).

Conclusions: Typhidot test is a valid tool in the diagnosis of typhoid fever but a reliable and valid interpretation should be based on positive IgM.

Key words: Salmonella typhi Dot- EIA (typhidot)typhoid fever.

INTRODUCTION

Typhoid fever is an important cause of morbidity in many regions of the world, with an estimated 12 to 33 million cases occurring annually.[1] Pakistan is a hyper-endemic area for typhoid fever, & according to WHO 2008 report, the incidence of typhoid fever in 5-15 years aged children was 412 per million in 2002.[2] A definitive diagnosis of typhoid fever can be made by isolation of Salmonella typhi (S.typhi) from blood or bone marrow by culture, which is regarded as "gold standard method". However, bacterial culture facilities are often unavailable, expensive, time consuming & usually negative because of prior antibiotic usage. Despite improved methods of

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bacteriologic isolation, there is a real need for rapid serologic diagnostic test for typhoid fever. The Widal test has been used for almost more than 100 years. It is widely available in developing countries, and is still regarded as a useful test in endemic areas.[3] There is, however, considerable interest in newer methods of diagnosis of typhoid fever such as latex agglutination, coagglutination, and the polymerase chain reaction.[4] The dot-enzyme immunoassay (EIA) is a relatively newer serologic test based upon the presence of specific IgG and IgM antibodies to a specific 50-kD outer membrane protein (OMP) antigen on S. typhi strains[5] and has been commercially marketed as a dot-EIA (typhidot). The test incorporates nitrocellulose strips impregnated with the OMP antigen and separately identifies IgM and IgG antibodies. Although the test has shown promising results in preliminary studies from Malaysia[6] and Philippines.[7] The interpretation of IgG response in highly endemic areas remains problematic. There is a concern that in such endemic populations pre-existing IgG antibodies to S. typhi may increase rapidly following reinfection and potentially mask concomitant IgM response. A recent, commercially available, enzyme-linked immunoassay (typhidot) is reported to circumvent these blocking antibodies by inactivating IgG antibodies, followed by an immunoassay targeting specific IgM.[8] Preliminary data using the Typhidot and Typhidot-M in combination have shown sensitivity and specificity of 95% and 86%, respectively.[9] Although the tests have shown promising results in trials from Southeast Asia, given the genetic diversity and plasticity of S. typhi strains, it is not proved if the test would be of comparable sensitivity in other regions.[3] We prospectively evaluated the efficacy of the two dot- EIA test (typhidot).

RESEARCH DESIGN AND METHODS

This prospective study undertaken in a tertiary hospital, Muhammad Medical College Hospital, Mirpurkhas, covered the period from April 2007 to July 2008. Blood culture and typhidot tests for typhoid fever & suspects were performed for the subjects admitted to the hospital. The subjects were selected who fulfilled the criteria of; ages 18-40 years, fever =14 days, clinical manifestations suggestive of typhoid fever, & no history of typhoid immunization in the recent past. Patient's history, physical examination findings, diagnostic studies, and results of blood culture and typhidot test were recorded. The blood cultures and typhidot tests were analyzed at the end of study period. Patients were divided into three groups - those with diagnosis of typhoid fever & suspects as group I and II respectively and those without typhoid as group III (controls).

Group I – 50 patients with blood culture (+) typhoid fever

Group II – 50 patients with blood culture (-) typhoid suspect

Group III – 50 patients with non-typhoid febrile illnesses.

Fifty patients with non-typhoid febrile illnesses (Group III) included; patients with pneumonia (n=7), pharyngiitis (n=5), Cholecystitis (n=5), dysentery (n=3), otitis media (n=7), malaria (n=10), urinary tract infection (n=7) and acute viral hepatitis (n=6) The validity of typhidot test was evaluated by determining the sensitivity, specificity, positive and negative predictive values in the diagnosis of culture, culture positive typhoid fever, culture negative typhoid suspects and controls

RESULTS

A total of 150 patients who fulfilled the inclusion criteria were studied, out of them 50 had positive cultures (group I) for Salmonella typhi, 50 had negative blood cultures (group II) and fifty had non-typhoid febrile illnesses (group III). The mean age in years was 28±13 for all groups I, II and III. There were more males (n=115) than females (n=35). More patients (91%) were admitted before the seventh day of illness. Sensitivity, specificity, and a negative predictive value for typhidot IgM noted in group I were 93%, 98.80% and 93% respectively, in group II these were 79%, 98.80% and 83% respectively, where as in group III (non-typhoid febrile controls), the IgM antibodies were not detected at all (p=0.01). Sensitivity, specificity, and a negative predictive value for typhidot IgG noted in group I were 73%, 63% and 22.4% respectively, In group II these were 82%, 37% and 68% respectively, where as a false positive reading of 63% was noted in group III controls (p=0.03).

DISCUSSION

The results of our study showed the sensitivity, specificity and negative predictive values of typhidot IgM in the diagnosis of typhoid fever were ranging from 93% to 98.8% in blood culture proven cases and these were slightly lower in blood culture negative typhoid suspects. IgM antibodies were not detected in the controls. Our results for typhidot IgM is well in comparison with the studies done by Choo et al.[6] However, typhidot IgG yielded more variable results with lower sensitivity, specificity, negative predictive value and high rate of false positive results among controls. This phenomenon possibly reflects the high endemicity of typhoid fever in our region where as infected cases are not recognized because of the mild presentation of the disease. Our results are comparable with the studies conducted by Sherwal et al, [10] Bhutta et al [3] and Jesudason et al, [11] which showed sensitivity and specificity of 92% & 87.5%, 80% & 77% and 92.3% & 98.8% respectively. The effectiveness of typhidot test in early stages of typhoid fever was seen in two different studies of Malaysia. [12-13] Its sensitivity and specificity was reported as 90.3% & 91.9% respectively in the first study while in the second one it showed sensitivity & specificity of 98% & 76.6% respectively.[12-13] A study conducted in Manila by Collantes et al [14] has reported sensivity & specificity of 93% & 100% in blood culture positive typhoid fever patients. One study from Pakistan of Shaikh KR et al [15] has shown sensitivity and specificity of 72.4% and 93.3% respectively, which is nearly comparable with our study. Our values of sensitivity and specificity are higher than reported by Karamat et al from Northern Pakistan. These differences may be due to several factors including the genomic diversity among S.typhi isolates in the region and differences in antigenic epitopes. Other factors responsible for reported differences in areas of high endemicity include various stages of illnesses and the rate of IgG increase in relation to (OMPs, which may interfere with identification of concomitant IgM antibodies). Most of our patients presented in the first week of their illness, whereas information on duration of illness is lacking in other studies. The relative low sensitivity of the blood culture in diagnosing typhoid fever is understandable in the wake of widespread antibiotic use in Pakistan. Although bone marrow cultures significantly increase the yield from blood cultures, but it is a invasive procedure and is difficult to obtain. It must be emphasized that although cultures are associated with a lag period of at least 48 hours

for preliminary confirmation of infection but with the recent emergence of drug resistance amongst strains of S.typhi it remains an essential investigation. The diagnostic difficulties in partially treated cases, may be reduced if the blood cultures are combined with rapid serologic tests. Our data indicates that Typhidot IgM has significant diagnostic yield. The Typhidot offers an additional advantage amongst second-line serologic diagnostic tests for typhoid fever as the test strips do not require an ELISA reader for evaluation, and only minimum training is required. Combining the Typhidot and Typhidot-M tests, may improve sensitivity but it is an expensive proposition. Given the recent call for an essential diagnostic program in developing countries, it is important that the Typhidot and Typhidot-M tests may be evaluated on a larger scale in different parts of the world with epidemiologically diverse strains of S.typhi.

Table 1: Results of IgM typhidot

	Sensitivity	specificity	NPV†
Group I (n=50)	93%	98.80%	93%
Group II (n=50)	79%	98.80%	83%

† **NPV=** negative predictive value

Table 2: Results of IgG typhidot

	Sensitivity	specificity	NPV†	False +ve
Group I (n=50)	73%	63%	22.4%	—
Group II (n=50)	82%	37%	68%	—
Group III (n=50)	—	—	—	63%

† **NPV=** negative predictive value

CONCLUSION

Typhidot IgM is a highly sensitive specific tool for the diagnosis of typhoid fever with high negative predictive value. In contrast, typhidot IgG has low sensitivity, specificity and negative predictive value. A valid conclusion can be made from a single sample, based on results of IgM titer.

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