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Original Research Article

Diagnostic Value of Typhidot Rapid IgM Assay for Rapid Diagnosis of Enteric Fever in a Pediatric Population

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Abstract

Background: Enteric fever is an extensive public health concern in developing countries. A rapid, simple and cost-effective diagnostic test is needed for early and accurate diagnosis. This proposed study was done to evaluate sensitivity and specificity of Typhidot rapid IgM assay to find out detection of enteric fever using blood culture as gold standard.

Methods: Febrile children complain with fever of >3 days were enrolled. Blood culture by BacT/ALERT™, Widal test and Typhidot rapid IgM test were performed. Patients in whom diagnosis other than typhoid was made served as controls.

Results: Of 300 children enrolled, 134 were diagnosed clinically as enteric fever with blood cultures positive in 38% children for *S.* Typhi. Sensitivity, specificity, positive predictive value and negative predictive value of Typhidot rapid IgM test were higher than for Widal test among blood culture confirmed typhoid cases (76.9%, 87.3%, 65.5%, 92.4% and 90.3%, 97.5%, 92.1%, 97% respectively). Compared to Widal test, Typhidot rapid IgM showed better diagnostic sensitivity (67.6% vs 85.3%) and predictive values in first week of illness (52.2% vs 87.8%).

Conclusions: Typhidot rapid IgM assay is simple to perform with superior diagnostic parameters for rapid diagnosis of enteric fever in endemic areas.

Keywords: Typhoid, Blood culture, Widal test, Typhidot rapid IgM kit.

Introduction

Enteric fever, a serious GIT illness caused by *Salmonella enterica* serotype Typhi, has an annual global burden of 21.6 million cases occurs resulting for which 216000 deaths.[1] Above more than 90% of these cases are accounted by South-east Asian countries with a more incidence of disease particularly in Indian subcontinent.[2] Enteric fever may occur at any age but it is more common to be a disease mainly in children and young adults with children under 5 years of age being the worst affected.[3,4] Most cases of typhoid fever present at the outpatient department. The diagnosis is challenging as enteric fever is clinically often difficult to differentiate from another acute febrile illnesses in the

endemic areas.[5] Disease has death rate of 30% if not timely and appropriately treated.[2] Early accurate diagnosis and treatment is important to decrease disease morbidity and also for control of disease transmission. Detection of causative bacteria from blood and or bone-marrow is required for confirmation of diagnosis. Blood culture remains gold standard for diagnosis of enteric fever. Moreover, culture requires elaborate lab equipment and trained technical personnel. Moreover culture reports are often available after about 2-7 days of fever and prior antibiotic intake further lowers positive yield, thereby delaying appropriate management. Laboratories in developing countries often lack such sophisticated facilities and febrile patients presumptively diagnosed as typhoid fever are often treated with unnecessary antimicrobials, contributing to increasing rates of antimicrobial resistance in *S.* Typhi. A simple and cost-effective alternative diagnostic test is needed for rapid and efficiency diagnosis of typhoid fever, especially for developing countries where enteric fever is a major public health concern.

Several serodiagnostic lab based test on antigen/antibody detection/reaction have been developed for this purpose. Widal tube agglutination test is an age-old method used for diagnosis of enteric fever. However, Widal test has restricted diagnostic value due to its variable sensitivity and specificity particularly in endemic areas.[6] The titre of diagnostic significance depends on endemicity in the region. It is still widely used in many laboratories particularly at primary health centres and district level laboratories where facilities for culture are unavailable. Serological assays such as Rapid antigen tests and tubex tests are thus developed to surmount these limitations and provide rapid diagnosis in resource with limited lab settings.

Typhidot M, a rapid dot-enzyme immune assay (EIA), is based on inactivation of IgG antibodies and detection of specific IgM antibodies to a specific 50 kD outer membrane protein (OMP) antigen of *S.* Typhi. The antibodies appear early in the course of disease and results are available within 3 hours. Here we report, comparative evaluation of Typhidot M with Widal test in the rapid and early diagnosis of enteric fever among febrile children in the hospital.

Material and Methods

This study was done at Rama Medical College and hospital, a tertiary care referral centre at Pilkhwa, Hapur. Febrile children with aged 1-14 years who presented with history of >3 days of fever (temperature of ≥38.8° C) were enrolled. Children with prior documented history of antimicrobial intake or hospitalization were excluded from the study. Clinical findings and laboratory investigations were recorded on a standard case record form. Complete hemogram, peripheral smear and rapid antigen detection test for malarial parasites, antibody test for dengue, urine and stool routine microscopy as well as culture, liver function tests, abdominal ultrasound, lumbar puncture, chest X ray, were also done where indicated. Three tests for investigations that is Blood culture, Widal test and Typhidot M test were performed on all febrile children.

Blood Culture: About 7 ml of blood was drawn from each eligible child at the time of presentation of which 5 ml was inoculated into BacT/ALERT™ blood culture pediatric vials (bioMerieux, Marcy l'Etoile, France) and incubated in the BacT/ALERT 3 D automated microbial detection system at 37◦C for 5 days. Blood cultures were processed by standard methods and colonies were identified, suspected as *Salmonella* Typhi by standard biochemical tests [7] and confirmed by agglutination using specific O and H antisera (Denka Seiken Co.

Ltd., Tokyo, Japan). Serum was separated from the remaining 2 ml blood and kept in a sterile eppendorf tube at -700C until further use.

Widal Test: This test was performed by using tube agglutination method using standardized TO and TH antigens (CRI, Kasauli, India). Serial tube dilutions of patient sera from 1:10 to 1:640 in 0.85% physiological saline were prepared. Equal volumes of diluted serum and O and H antigens (0.4 ml each) were added separately to the tubes. The test tubes were incubated at 37°C in a water bath for 16-18 hours. Results were expressed as the inverse of the highest dilution showing agglutination. Titres ≥1:160 for TH and TO for serotype Typhi were considered significant [6]. Widal positive and negative controls were run with each batch.

Typhidot rapid IgM test: The test was performed as the guide lines of the manufacturer's instructions (Malaysia). Briefly, Typhidot rapid IgM is an indirect solid-phase immunochromatographic assay where the specific *S.* Typhi antigen is immobilized onto cellulose nitrate membrane strip. When 30 µL of test serum sample was added to the sample pad, the antibodies migrated upwards and formed an antibody-antigen complex with the immobilized antigen at the test window zone. The bound antibody-antigen complexes were subsequently detected by a dye conjugated goat anti human IgM when the chase buffer was added and it migrated downward, giving a pink-purplish colour. The control line containing rabbit antigoat IgG antibody binds with the dye conjugated goat antihuman IgM. The test was considered positive if coloured bands appeared at the control and test lines while it was negative if only the control line was visible and invalid if the control line was absent in which case the test was repeated with a new test cassette. The test results were read within 10 minutes.

The gold standard for diagnosis of enteric fever was isolation of *S. enterica* serotype Typhi in blood culture. All the enrolled febrile children were classified into three groups: 1. blood culture confirmed enteric fever; 2. clinically suspected enteric fever but negative blood

cultures; 3. febrile illnesses other than enteric fever, confirmed by laboratory investigations. The group 3 served as controls in this study.

Informed and written consent was obtained from all the patients and their attendants. The study ethical clearance protocol was approved by the Institutional Ethical committee.

Statistical Analysis: Analyses were performed using SPSS version 10.0 software. We calculated the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of Typhidot and Widal tests. For calculation, Blood culture proven enteric fever cases (group 1) were considered as true positive and febrile illnesses other than enteric fever (group 3) were considered true negative. Performance of these tests was also determined for entire cohort of clinically diagnosed enteric fever patients (group 1 and 2) in comparison with non-enteric controls (group 3). Because serologic tests detect antibody response and perform better after a period from the onset of illness, these parameters were also calculated separately for blood culture proven cases presenting with duration of fever of ≤7 days and > 7 days.

Results

A total of 300 children who presented with febrile illnesses during the one year period were enrolled. 134 were diagnosed clinically as typhoid fever. Blood cultures were positive in 52 (38%) children for *S.* Typhi (group 1). In 82 (27.3%) children diagnosis of enteric fever by other laboratory tests but

blood cultures were sterile (group 2). The remaining 166 (55.3%) children were diagnosed with febrile diseases other than enteric fever and all children had negative blood cultures for Salmonellae (group 3). The last group consisted of patients with diagnosis of dengue fever (n=57), malaria (n=29), pneumonia (n=15), urinary tract infection (n=16), pyogenic meningitis (n=8), viral hepatitis (n=10), pyogenic liver abscess (n=4), leukemia (n=8) and non-hodgkins lymphoma (n=2) and pyrexia of unknown origin (PUO, n=17). The ages of all patients ranged from 1-14 years and median age was 7 years. Majority in children

in all the 3 groups, group age of 5 to 10 years (51.3%) followed by group age of 10-14 years (29%) (Table 1). The mean age of presentation in all the three groups was 6.85±2.5 years, 7.26 ±2.98 years and 7.33±2.89 years respectively (p value-0.556). There was no difference significant in the ages of children at presentation between culture confirmed typhoid cases and other groups (p value >0.05). Male to female ratio in typhoid fever cases, confirmed and clinically suspected, was 1.36 and 0.95 respectively. High grade fever, abdominal pain and anorexia were common presenting symptoms; toxic look, coated tongue, caecal borboygmi and splenomegaly were frequent among clinically suspected enteric fever **cases (Table 2).**

Table 1: Age distribution of all febrile children

Age (years)	Group 1 (n=52)	Group 2 (n=82)	Group 3 (n=166)	Total (n=300)
2-5	8 (15.3%)	21 (25.6%)	30 (18.0%)	59 (19.6%)
5-10	32 (61.5%)	35 (42.6%)	87 (52.4%)	154 (51.3%)
10-14	12 (23.0%)	26 (32.1%)	49 (29.5%)	87 (29

(n = 52)

Table 2: Comparative evaluation of diagnostic parameters of Widal and Typhidot rapid IgM tests

	WIDAL		TYPHIDOT M	
	GROUP 1 (n=52)	GROUP 2 (n=82)	GROUP 1 (n=52)	GROUP 2 (n=82)
No. positive among typhoid fever cases (n=134)	40 (78.4%)	79 (96.3%)	47 (92.1%)	75 (91.4%)
No. positive among non-typhoid fever cases (n=166)	21(12.6%)		04 (2.4%)	
Sensitivity	76.9% (40/52) (C.I.=63.87-86.28)	88.8% (119/134) (C.I.=82.35-93.1)	90.3% (47/52) (C.I.=79.39-95.82)	91.0% (122/134) (C.I.=85-94.8)
Specificity	87.3% (145/166) (C.I.= 81.43-91.57)	87.3% (145/166) (C.I.= 81.43-91.57)	97.5% (162/166) (C.I.= 93.97-99)	97.5% (162/166) (C.I.= 93.9-99)
PPV	65.5% (40/61)	85% (119/140)	92.1% (47/51)	96.8% (122/130)
NPV	92.4% (145/157)	90% (145/160)	97% (162/167)	93.1% (162/174)

Note: Group 1- Blood culture confirmed typhoid fever; Group 2- Clinically diagnosed typhoid fever; PPV= Positive predictive value; NPV= Negative predictive value

Table 3: Diagnostic parameters of various tests with duration of fever among blood culture confirmed typhoid fever cases (n = 52)

	Widal		Typhidot M	
	≤7 d (n=34)	>7 d (n=18)	≤7 d (n=34)	>7 d (n=18)
Sensitivity	67.6% (23/34)	94.4% (17/18)	85.2% (29/34)	100% (18/18)
Specificity	87.3% (145/166)	87.3% (145/166)	97.5% (162/166)	97.5% (162/166)
PPV	52.2% (23/44)	44.7% (17/38)	87.8% (29/33)	81.8% (18/22)
NPV	92.9% (145/156)	99.3% (145/146)	97.0% (162/167)	100% (162/162)

Note: PPV=Positive predictive value; NPV=Negative predictive value; 52 typhoid fever cases considered as true positives and 166 other febrile illnesses as true negatives

Table 4: Review of Typhidot and Widal test performance in various studies.

Author Name/Year	Place	Patients	Typhidot				Widal			
			Sensitivity (%)	Specificity (%)	PPV	NPV	Sensitivity (%)	Specificity (%)	PPV	NPV
K E Choo 1994[23]	Malaysia	109	95	75	-	96	98	67	-	98
K E Choo 1999[24]	Malaysia	134	90.3	93.1	-	-	91.9	80.6	-	-
Butta et al 1999[25]	Pakistan	97	94	77	88	87	63	81	85	55
			85	89	93	77				
Jesudassan et al 2002[26]	Vellore, India	150	100	80	-	-	55	-	-	-
Jesudassan et al 2006[15]	Vellore, India	545	92.3	98.8	85.7	99.4	ND			
Gopalkrishnan et al 2002[16]	Malaysia	144	98	76.6	69.0	98.6	100	21.2	40.3	100
B L Sherwal et al 2004[10]	New Delhi, India	80	92	87.5	92	-	74	83	87.5	-
Narayanappa et al 2010[13]	Mysore, India	105	92.6	37.5	-	-	34.1	42.8	-	-
Shanta Dutta et al 2006[22]	Kolkata, India	2251	41	84	83	44	37	95	82	71.4
Farzana KB 2010[27]	Aligarh, India	145	90	100	100	92.1	40	91.4	80	64
Sanjeev H et al 2013[11]	Mangalore, India	50	100	76.5	89.1	100	78.7	58.8	78.7	58.8
Khoharo 2011[28]	Pakistan	76	96	89.5	95	-	72	87	87	-
S Krishna 201[29]	Bangalore, India	100	95.5	89.2	100	-	ND			
Present study*	**New Delhi, India**	**300**	**90.3**	**97.5**	**92.1**	**97**	**76.9**	**87.3**	**65.5**	**92.4**

Note: PPV=Positive predictive value; NPV= Negative predictive value; values in shaded areas represent data using Typhidot-M test

***By Typhidot rapid IgM test**

The diagnostic parameters of Widal test and rapid card test are depicted in Table 3. Widal was positive in 119 clinically diagnosed typhoid fever cases which included 40 (76.9%) blood culture positive children (group 1) and 79 (96.3%) children with negative blood cultures (group 2). Among control group, Widal was positive in 21 (12.6%) children and the difference between positivity among cases and controls was statistically significant (p value = 0.0001). Typhidot M test was positive in 122 children of which 47 (90.3%) were from blood culture confirmed group (group 1) and 75 (91.4%) were from clinically suspected but blood culture negative group (group 2) while among controls, Typhidot M was positive in 4 (2.4%) children (p value = 0.0001). The sensitivity, specificity, PPV and NPV of Widal test and rapid card test among blood culture confirmed typhoid cases (n=52) were 76.9%,87.3%, 65.5%, 92.4% and 90.3%, 97.5%, 92.1%, 97% respectively. The values for all the diagnostic parameters were much higher for Typhidot M test than that for Widal test. When the entire cohort of clinically diagnosed typhoid cases was analysed (group 2; n=134), the diagnostic sensitivity and PPV

of Widal test considerably improved (88.8% and 85% respectively). However, Typhidot M was still superior to Widal test for diagnosis of enteric fever with high sensitivity, specificity, positive predictive value and negative predictive value of 91.0%, 97.5%, 96.9%, 93.1% respectively. To find out the efficacy of these assays in early diagnosis of enteric fever, the diagnostic parameters were also evaluated for patients complaining with fever of ≤7 days (n=34) or >7 days (n=18) duration. Compared to Widal test, Typhidot M showed better diagnostic sensitivity (67.6% vs 85.3%) and predictive values in the first week of illness (52.2% vs 87.8%). The sensitivity and negative predictive value of both tests further increased in the second week of illness (table 4).

Discussion

Typhidot M was evaluated in 300 febrile children to assess the performance, compare with Widal test and validate against the gold standard blood culture. In other studies, culture positivity has ranged from 5.6% to 67.8%.[8-13] In the present study, blood culture positivity amongst clinically suspected enteric fever cases was 38.8% (52/134) and 53.8% (35/52) among enteric cases who presented in the first seven days of their illness. Culture yield is negatively affected by low volume of blood sampled, bacteria harboured within phagocytes and recent receipt of antimicrobial agents.[5] In this study, patients with prior antibiotic intake were excluded and we used an automated blood culture system for isolation of bacteria. Recent study found both manual and automated blood culture systems to be equally sensitive for the detection of *S.* Typhi.[14,15] The sensitivity of blood culture was comparatively better in the first seven days of illness (53.8%) in this study, thereby signifying that the time of performance of blood culture is a crucial factor in the diagnosis. In majority of studies, sensitivity of blood culture reported is not more than 40% [16-20]. Bone marrow culture is an ideal method for diagnosis with very high sensitivity of 90%,[21] however it is not suitable for routine diagnosis owing to its invasive nature and technical skills required to perform the procedure. Secondly, as most of the patients are managed as outpatients, blood culture, therefore continues to be the cornerstone for the diagnosis of enteric fever. However, as culture results are only available after 72 hours, difficulty in their follow-up is an inevitable factor. New serodiagnostic tests with better sensitivity, rapid and accurate results are the need of time particularly in resource constrained settings including primary health centres and subcentres.

Widal test is used as a diagnostic test for enteric fever for several decades. It is associated with inadequate sensitivity (58-78%), specificity (58.8-85%) and positive predictive value (69%)[10,22] (Table 4). In agreement with these studies, Widal test in the present study showed sensitivity of 77%, specificity of 87% and positive predictive value of 65.5% amongst the blood culture proven typhoid cases. As O and H agglutinins are usually detectable around after one week and in second week respectively,[10,25,27] the sensitivity and NPV improved in the second week of illness to 94.4% and 99.3% respectively. The results of our study shows that Widal test is not suitable for early and reliable diagnosis of typhoid cases in an endemic country like India where the level of agglutinins in the non-infected population vary considerably and cross reactions due to multiple other infections are considerably high; however it appears useful for excluding the diagnosis.

Typhidot M in our evaluation showed sensitivity, specificity, positive predictive value and negative predictive value of 90.3%, 97.5%, 92.1% and 97% respectively among blood culture confirmed typhoid cases which were higher than that of Widal test. Typhidot

M was also fairly sensitive (85.2%) compared to Widal test (67.6%) in the 1st week of disease approaching 100% after seven days. This could be because Typhidot M can detect IgM antibodies as early as 2nd day of the illness.[27] Only 5 cases, which were blood culture positive (fever < 7days), were negative by Typhidot M. The sensitivity and specificity of the test in our study was comparable to other studies (75-100% and 90-100% respectively) from India, Pakistan and Malaysia (Table 4). Typhidot M also showed a very high NPV of 97% as reported previously by Choo *et al*[23] (96.1%) which implies that if the test is negative, the disease can be reliably ruled out in 97% of the cases. Typhidot, which detects both IgG and

IgM, was employed in most of these studies against Typhidot M used in the present study. In recent studies from India, Typhidot M test was shown to have high diagnostic value among typhoid fever cases.[29] In contrast, in a large community-based recent study by Dutta *et al* from Kolkata, India, the sensitivity and NPV of Typhidot were found to be quite low (47% and 42% respectively).[22] When the entire cohort of 134 clinically diagnosed typhoid cases was analysed in the present study, the PPV of the test slightly increased from 90.3% (47/52) to 96.8% while other parameters remained unchanged. These additional 74 cases tested positive by Typhidot M among blood culture negative clinically diagnosed typhoid cases, apparently appearing to be false positive Typhidot results, could actually be false negative blood culture results since the reported sensitivity of single blood culture is only 40%-60%.[12,30-32] Of these 74 cases, 71 were also positive by Widal test. These seemingly false positive cases had presented with fever of >7 days when sensitivity of blood culture is known to reduce further. Narayanappa *et al* also reported similar observations speculating that as Typhidot M detects IgM antibodies, rapid diagnostic tests may be more accurate than blood culture. Jesudasan *et al* also reported successful management of clinically diagnosed typhoid cases (03) which were Typhidot positive but blood culture negative when treated as typhoid cases with ciprofloxacin.[33] Hence, the blood culture negative results in clinically diagnosed typhoid fever cases should be interpreted with caution. Widal test gave higher false positives (12.4%) than Typhidot M (2.4%) in the control group, so Typhidot M offers higher specificity (97.5%) than Widal test (87.5%). A false-positive result may be the result of past infection with serotype Typhi or another nontyphoidal *Salmonella* serotype that shares common antigens.[34]

Conclusion

Typhidot M test is simple to perform with minimal operative training and has a superior diagnostic value in terms of higher sensitivity, specificity, positive predictive value and for early diagnosis of enteric fever in the endemic areas.

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