

Comparative study of Typhidot-M with Widal and blood culture in diagnosis of enteric fever

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ABSTRACT

Objective: To evaluate the diagnostic utility of Typhidot-M and Widal test in the early diagnosis of enteric fever (EF) in terms of sensitivity and specificity. **Methods:** The study included 270 children in the age group of 1-18 years admitted to the Department of Pediatrics from November 2012 to February 2014, with fever of 5 days or more and with clinical symptoms and signs suggestive of typhoid fever. Detailed history and clinical examination findings were recorded on a standard pro forma. Complete hemogram (hemoglobin, platelet count, and total and differential leukocyte count), Typhidot-M test, Widal tube test, and blood culture were done on day 1 of admission. For Widal test, a titer of 1 in 160 or more for “O” agglutinins and a titer of 1 in 320 or more for “H” agglutinins were considered as positive results. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. **Results:** Of 270 children included in the study, Salmonella typhi was isolated from 82 samples (30.4%) and the remaining 188 (69.6%) were blood culture negative. Widal test was positive in 107 children (39.6%) and Typhidot-M was positive in 136 (50.4%). The sensitivity was 78%, specificity was 79.3%, PPV was 59.8%, and NPV was 91.4% for Widal test. Typhidot-M test had a sensitivity of 81.7%, specificity of 84.6%, PPV of 69.8%, and NPV of 91.4%. **Conclusion:** Prompt diagnosis of EF is essential for appropriate management and it is, therefore, important to have a satisfactory test to replace conventional tests used for diagnosis. The present study compares newer test (Typhidot-M) against conventional tests such as Widal test and blood culture, and it appears to be a practical alternative to Widal test in the early detection of EF even in the resource-poor laboratories as it neither requires much laboratory equipment nor laboratory expertise to conduct the test. This test can be done within 7 days of illness, but whenever feasible confirmation with blood culture is strongly recommended, especially with the well-documented presence of multidrug-resistant strains of salmonella typhi worldwide. However, both Widal and Typhidot-M appear to correlate less satisfactorily with blood culture, and thus, there is a need for developing a test which allows accurate and early diagnosis of EF to manage a child effectively and limit its morbidity and mortality.

Key words: Blood culture, Enteric fever, Typhidot-M, Widal test

Enteric fever (EF) is a common infectious disease worldwide, especially so in developing countries such as India and other South East Asian countries. The incidence in developed countries has fallen below 10/100,000 population/year, due to improvements in standard of living, hygiene, and safe disposal of waste products, whereas it is >100/100,000 population/year in developing countries [1]. Mortality rates due to EF and its complications have fallen considerably due to availability of effective antibiotics. Still due to a higher burden of incidence, significant morbidity continues to be a problem.

EF is an infectious disease caused by salmonella typhi/paratyphi, mostly in young children. Although previous studies from Latin America and Africa suggested that salmonella typhi infection caused a mild disease in infancy and childhood [2], recent population-based studies suggest that the incidence is highest in children aged <5 years with higher rates of complications and

hospitalizations [3-6]. Clinical manifestations are varied and nonspecific, and diagnosis is confirmed by isolating the organism from blood/urine/stool/bone marrow cultures. Blood culture is positive early in the disease, and urine stool cultures are positive late in the course of disease [6-8].

While blood culture is the gold standard for diagnosing EF, it is positive in only about 30-40% of the patients (lower in patients who have already received few doses of antibiotics) [6,9-12]. Its routine availability is limited to few tertiary care centers, and its usefulness is limited due to time period (2-3 days) needed to get the report from the laboratory [13]. The diagnosis based on clinical criteria poses problems since EF mimics many common febrile illnesses without localizing signs such as acute gastroenteritis, malaria, tuberculosis, leptospirosis, and rickettsial diseases [6].

Hence, clinicians routinely depend on antigen testing methods such as Widal test and Typhidot tests to confirm the diagnosis of

EF. These tests are not gold standard and have wide variability in sensitivity and specificity and hence are not recommended by the authorities all over the world [14]. However, due to ease of availability, cost factor, and need for quickly confirming the diagnosis, these tests are widely done in our country.

Widal test implies demonstration of four-fold rise in titers of antibodies in paired blood samples 7-14 days apart which is invariably not helpful in clinical decision-making [8,15]. The test done on a single, acute phase serum sample lacks sensitivity and/or specificity in regions where EF is endemic. Cutoff titer for single test should be based on the distribution of antibody levels in "normal population" [15-17]. The titers of "O" and "H" antibodies may be falsely elevated in normal population due to cross-reacting epitopes of other Enterobacteriaceae or other tropical infections such as malaria or dengue [2,16]. On the other hand, prior antibiotic therapy may cause titers to be falsely low [15].

Typhidot is a dot blot enzyme-linked immunosorbent assay (ELISA) which detects immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies to 50 kDa outer membrane protein of salmonella typhi. The detection of IgM reveals the early phase, whereas detection of IgG and IgM suggests a middle phase of EF. The modified test Typhidot-M, in which inactivation of IgG allows access to the IgM and hence is more specific. This test is positive early in EF and has sensitivity ranging from 68% to 95% and specificity ranging from 75% to 95% in various studies [6,18]. High negative predictive value (NPV) of this test would be useful in areas of high endemicity. Thus, this test may be more appropriate than Widal test in diagnosis of EF. However, this test may be falsely negative during the second 2nd week of illness due to falling levels of IgM [19].

Molecular methods using DNA probes specific to Vi antigen gene of salmonella typhi and polymerase chain reaction (PCR)-based tests for detection of Salmonella typhi flagella gene are expensive and require highly specialized laboratory setup and hence are not yet practical in resource-poor settings [20].

Early diagnosis and appropriate antibiotic treatment (the right drug, dose, and duration) is the key to treat EF with minimal complications. The World Health Organization has issued no recommendations for typhoid rapid antibody tests [21], and studies evaluating the utility of these tests have reported wide variation regarding their sensitivity and specificity [6]. Most available studies are comparative studies of Typhidot test (both IgM and IgG) versus Widal test versus blood culture [22-25]. This study was undertaken to evaluate the diagnostic utility of Typhidot-M and Widal test in the early diagnosis of EF in terms of sensitivity and specificity by comparing them with blood culture which is the gold standard for diagnosis of EF and, as there is a paucity of literature along with conflicting results regarding their utility from our country.

METHODS

The study included 270 children in the age group of 1-18 years admitted to ESIC MC and PGIMSR, Department of Pediatrics

from November 2012 to February 2014, with fever of 5 days with signs and symptoms suggestive of typhoid fever such as anorexia, vomiting, diarrhea, toxicity, abdominal pain, constipation, headache, jaundice, obtundation, and hepatosplenomegaly [6]. Children with identified cause of fever such as respiratory illness, malaria, diarrhea, and urinary tract infection and children with documented typhoid fever within the past 8 weeks were excluded from the study.

All children included in the study were subjected to detailed history and clinical examination, and the findings were recorded on a standardized pro forma. On the day of admission, following investigations were done- complete hemogram (hemoglobin, platelet count, and total and differential leukocyte count), Typhidot-M test (Typhidot-M kit, Malaysia Bio-diagnostics Research), Widal tube test, and blood culture. For Widal test, a titer of 1 in 160 or more for "O" agglutinins and a titer of 1 in 320 or more for "H" agglutinins were considered as positive results [17].

Typhidot-M is a dot-blot ELISA for the detection of specific IgM to salmonella typhi. In this test, IgG is inactivated before carrying out the assay as for the Typhidot. The test uses a nitrocellulose membrane strip dotted with the 50 KDa specific proteins and a control antigen. 2.5 µL of patient serum and controls are pre-absorbed for at least 1 min with 90 µL of IgG inactivation reagent. 250 µL of sample diluent is then added into the reaction wells and the mixture incubated at room temperature on a rocker platform for 20 min. The strips are washed thrice for a total of 5 min, and 250 µL of antihuman IgM conjugate is added and incubated for 15 min. The strips are washed as before, and 250 µL of color development solution is added and incubated for 15 min. The reaction is stopped by washing the strips in distilled water, and the results are read. When both the dots on the test strip are as dark as or darker than their corresponding dots on the positive control strip, they are reported as positive.

Statistical analysis was done using SPSS software version 20.0. Sensitivity, specificity, positive predictive value (PPV), and NPV were calculated.

RESULTS

Of 270 children included in the study, maximum incidence was seen in the age group of 11-18 year (n = 141, 52.2%), followed by 6-10 year (n = 98, 36%) and 1-5 year (n = 31, 11.8%). Male preponderance of 1.3:1 was seen (152:118).

Salmonella typhi was isolated from 82 samples (30.4%) and the remaining 188 (69.6%) were blood culture negative. Widal test was positive in 107 children (39.6%) and Typhidot-M was positive in 136 (50.4%). The sensitivity, specificity, PPV, and NPV of Widal and Typhidot-M tests are compared in Tables 1 and 2, respectively.

DISCUSSION

In the present study, most of the children were in the age group of 5-18 years. The lower incidence in pre-school children is

in concordance with studies from South America and other parts of the world which suggest that typhoid may manifest as a mild illness in young children [1,2,26]. However, there is emerging evidence from high incidence study sites such as South Asia that the incidence of EF in pre-school children aged 2-5 years is in the same order of magnitude as that of school-aged children (5-15 years) with higher rates of complications and hospitalization [3,4,5,27].

In our study, culture positivity among clinically suspected case was 30.4%. In other studies, the culture positivity varied from 6% to 68% [22,28-30], and in majority of the studies, the culture yield was around 40% [9-12]. The major reason for this lower yield is widespread use of antibiotics in the endemic areas and the small quantities of salmonella typhi (i.e., <15 organisms/ml) typically present in blood [24]. Although blood culture is the gold standard test for diagnosis of EF, its utility in early diagnosis is limited due to lower yield, requirement of trained personnel, and the time period (2-3 days) required for reporting.

The sensitivity of Widal test was 78%, specificity was 79.3%, and PPV was 59.8% in the current study. These values are in concordance with studies published by Sherwal et al. [22] (sensitivity 74%, specificity 83%, and PPV 87.5%) and Rahman et al. [23] (sensitivity 81.8%, specificity 69.2%, and PPV 45.6%). Sharing of O & H antigen by other Salmonella serotypes and other members of *Enterobacteriaceae* makes the role of Widal test less specific, and hence, its use is controversial in diagnosis

of EF [16]. It is possible that the Widal test would have performed better if paired sera were tested to demonstrate the rising titers. Patients rarely return for outpatient follow-up once treated so that obtaining paired sera in a routine clinical setting is unlikely, and hence clinicians widely rely on “positive” Widal test done on a single serum sample.

Typhidot-M is a new, simple, rapid diagnostic test available commercially, requiring less than 30 min, and minimal training. We found that Typhidot-M test had sensitivity of 81.7%, specificity of 84.6%, PPV of 69.8%, and NPV of 91.4% ($p < 0.001$). A study by Gopalakrishnan et al. has reported similar results (sensitivity 82%, specificity 68%, PPV 57.7%, and NPV 90.1%) [9]. Comparison of sensitivity, specificity, PPV, and NPV for Typhidot test in various studies is shown in Table 3. Choo et al. have noted that the sensitivity and specificity of Typhidot and Typhidot-M were identical at 90.3% and 93.1%, respectively. In addition, they have reported that when used together, the sensitivity and NPV were higher, but at the cost of lower PPV [31]. However, this would increase the cost prohibitively and hence may not be feasible to combine these two tests in routine practice.

Sensitivity, specificity, and PPV of Typhidot-M were slightly higher than Widal test (Tables 1 and 2). Typhidot-M was found to be positive in 98% of cases who presented with <7 days fever among blood culture-positive cases. Thus, Typhidot-M appears to be useful alternative to Widal as it is more specific and useful in early diagnosis of EF.

Both Widal and Typhidot-M appear to correlate less satisfactorily with blood culture as among children with positive blood cultures, Typhidot-M was negative in 15 (28%) and Widal was negative in 18 (22%) children. Although both these tests, at best, can be used to “suspect” EF more strongly than on clinical grounds alone, it is prudent to follow them up with blood culture confirmation. With this caveat, Typhidot-M appears to have slight advantage over Widal test in that (i) results are rapidly available, (ii) there is possibility of using it earlier (1st week) in the course of the illness, and (iii) it is technically easy to perform even at the peripheral health-care facilities.

In a clinical review article, Bhutta ZA has commented that although clinical diagnosis of typhoid fever is difficult, developing simple algorithms to diagnose and triage EF in endemic areas are possible and also, has suggested that, in particular, diagnosis and triage of EF among febrile children must be included in the Integrated Management of Childhood Illness in South Asia which

Table 1: Comparison of Widal test with blood culture

Widal test	Blood culture		Total
	Positive	Negative	
Positive	64	39	107
Negative	18	149	163
Total	82	188	270

p value is<0.002. Sensitivity 78%, specificity 79.3%, positive predictive value 59.8%, and negative predictive value 91.4%

Table 2: Comparison of Typhidot-M with blood culture

Typhidot-M	Blood culture		Total
	Positive	Negative	
Positive	67	29	96
Negative	15	159	174
Total	82	188	270

p value is<0.001. Sensitivity 81.7%, specificity 84.6%, positive predictive value 69.8%, and negative predictive value 91.4%

Table 3: Comparative evaluation of Typhidot-M test in different studies

Studies	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Narayanappa et al. [19]	92.6	37.5	48.7	88.8
Sherwal et al. [22]	92	87.5	92	NA
Gopalakrishnan et al. [9]	82	68.1	57.7	90.1
Yadav et al. [24]	90	100	100	93
Beig et al. [31]	90	100	100	92.1
Bukhari et al. [25]	93	87	NA	NA
Present study	81.7	84.6	69.8	91.4

PPV: Positive predictive value, NPV: Negative predictive value, NA: Not available

currently focuses on malaria as a cause of fever without localizing signs [6]. Limitations of our study were that it was a single-center study with relatively lower rate of culture positivity in the study group.

CONCLUSION

Typhidot-M appears to be a practical alternative to Widal test in the early detection of EF even in the resource-poor laboratories as it neither requires much laboratory equipment nor laboratory expertise to conduct the test, and it is more specific also. Although this test can be done within 7 days of illness, it is advisable, whenever feasible, to confirm the diagnosis with blood culture, especially with well-documented presence of multidrug-resistant strains of salmonella typhi worldwide.

REFERENCES

- Crump JA, Mintz ED. Global trends in typhoid and paratyphoid fever. *Clin Infect Dis*. 2010;50(2):241-6.
- Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. *Bull World Health Organ*. 2004;82(5):346-53.
- Sinha A, Sazawal S, Kumar R, Sood S, Reddaiah VP, Singh B, et al. Typhoid fever in children aged less than 5 years. *Lancet*. 1999;354(9180):734-7.
- Brooks WA, Hossain A, Goswami D, Nahar K, Alam K, Ahmed N, et al. Bacteremic typhoid fever in children in an urban slum, Bangladesh. *Emerg Infect Dis*. 2005;11(2):326-9.
- Siddiqui FJ, Rabbani F, Hasan R, Nizami SQ, Bhutta ZA. Typhoid fever in children: Some epidemiological considerations from Karachi, Pakistan. *Int J Infect Dis*. 2006;10(3):215-22.
- Bhutta ZA. Current concepts in the diagnosis and treatment of typhoid fever. *BMJ*. 2006;333(7558):78-82.
- Herath HM. Early diagnosis of typhoid fever by the detection of salivary IgA. *J Clin Pathol*. 2003;56(9):694-8.
- Old DC. Salmonella. In: Collee JG, Fraser AG, Tenover BC, Tenover FC, editors. *Practical Medical Microbiology*. 14th ed. Edinburgh: Churchill Livingstone; 2006. p. 385-404.
- Gopalakrishnan V, Sekhar WY, Soo EH, Vinsent RA, Devi S. Typhoid fever in Kuala Lumpur and a comparative evaluation of two commercial diagnostic kits for the detection of antibodies to *Salmonella typhi*. *Singapore Med J*. 2002;43(7):354-8.
- Begum Z, Hossain MA, Musa AK, Shamsuzzaman AK, Mahmud MC, Ahsan MM, et al. Comparison between DOT EIA IgM and Widal Test as early diagnosis of typhoid fever. *Mymensingh Med J*. 2009;18(1):13-7.
- Kawano RL, Leano SA, Agdamag DM. Comparison of serological test kits for diagnosis of typhoid fever in the Philippines. *J Clin Microbiol*. 2007;45(1):246-7.
- Keddy KH, Sooka A, Letsoalo ME, Hoyland G, Chagnat CL, Morrissey AB, et al. Sensitivity and specificity of typhoid fever rapid antibody tests for laboratory diagnosis at two sub-Saharan African sites. *Bull World Health Organ*. 2011;89(9):640-7.
- Basnyat B, Maskey AP, Zimmerman MD, Murdoch DR. Enteric (typhoid) fever in travelers. *Clin Infect Dis*. 2005;41(10):1467-72.
- Pegues DA, Miller SI. Salmonellosis. In: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J, editors. *Harrison's Principles of Internal Medicine*. 18th ed. New York: McGraw Hill; 2012. p. 1274-81.
- Old DC, Threlfall EJ. Salmonella. In: Balows A, Duerden BI, editors. *Topley & Wilson's Microbiology and Microbial Infections*. 9th ed. London: Arnold; 1998. p. 969-98.
- Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ. Typhoid fever. *N Engl J Med*. 2002;347(22):1770-82.
- Faculty, Department of Clinical Microbiology. In: Myer's and Koshi's Manual of Diagnostic Procedures in Medical Microbiology and Immunology/Serology. Revised Edition. Vellore: Department of Clinical Microbiology, Christian Medical College and Hospital; 2001. p. 139-40.
- Jesudason M, Esther E, Mathai E. Typhidot test to detect IgG & IgM antibodies in typhoid fever. *Indian J Med Res*. 2002;116:70-2.
- Narayanappa D, Sripathi R, Jagdishkumar K, Rajani HS. Comparative study of dot enzyme immunoassay (Typhidot-M) and Widal test in the diagnosis of typhoid fever. *Indian Pediatr*. 2010;47(4):331-3.
- Rubin FA, McWhirter PD, Punjabi NH, Lane E, Sudarmono P, Pulungsih SP, et al. Use of a DNA probe to detect *Salmonella typhi* in the blood of patients with typhoid fever. *J Clin Microbiol*. 1989;27(5):1112-4.
- WHO. Background Document: The Diagnosis, Treatment and Prevention of Typhoid Fever. Geneva: World Health Organization; 2003.
- Sherwal B, Dhamija RK, Randhawa VS, Jais M, Kaintura A, Kumar M. A comparative study of Typhidot and Widal test in patients of typhoid fever. *JACM*. 2004;5:244-46.
- Rahman M, Siddique AK, Tam FC, Sharmin S, Rashid H, Iqbal A, et al. Rapid detection of early typhoid fever in endemic community children by the TUBEX O9-antibody test. *Diagn Microbiol Infect Dis*. 2007;58(3):275-81.
- Yadav K, Yadav SK, Parihar G. A comparative study of Typhidot and Widal test for rapid diagnosis of typhoid fever. *Int J Curr Microbiol Appl Sci*. 2015;4(5):34-8.
- Bukhari N, Saleem A, Khan MA, Khan SN, Jabbar A, Ahmad B, et al. Comparative clinical utility of Widal and Typhidot in the diagnosis of typhoid fever. *IJB*. 2016;9(2):92-6.
- Bhutta ZA. Salmonella. In: Kliegman RM, Stanton BF, Schor NF, St. Geme JW, editors. *Nelson Text Book of Pediatrics*. First South Asia Edition. New Delhi: Reed Elsevier India Pvt., Ltd.; 2016. p. 1382-93.
- Ochiai RL, Acosta CJ, Danovaro-Holliday MC, Baiqing D, Bhattacharya SK, Agtini MD, et al. A study of typhoid fever in five Asian countries: Disease burden and implications for controls. *Bull World Health Organ*. 2008;86(4):260-8.
- Mehul GA. Study of clinical profile of multidrug resistant Typhoid fever in children. *Indian Pediatr*. 2010;45(2):156-9.
- Retnosari S, Tumbelaka AR, Akib AP, Hadinegoro SR. Clinical and laboratory features of typhoid fever in childhood, focus on relationship between prior antibiotic administration with blood culture, Widal and Typhidot results. *Paediatr Indones*. 2001;41:149-54.
- Beig FK, Ahmad F, Ekram M, Shukla I. Typhidot M and Diazo test vis-à-vis blood culture and Widal test in the early diagnosis of typhoid fever in children in a resource poor setting. *Braz J Infect Dis*. 2010;14(6):589-93.
- Choo KE, Davis TM, Ismail A, Tuan Ibrahim TA, Ghazali WN. Rapid and reliable serological diagnosis of enteric fever: Comparative sensitivity and specificity of Typhidot and Typhidot-M tests in febrile Malaysian children. *Acta Trop*. 1999;72(2):175-83.

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