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Comparative Evaluation of Typhoid IgM/IgG Test and Blood Culture in Early Diagnosis of Enteric Fever

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Abstract **Original Research Article**

Context: Enteric fever is potentially life threatening systemic infection occurring in lesser developed areas of the world and continues to be a major public health problem. We have evaluated the rapid point of care Typhoid IgM/IgG test as compared to blood culture in rapid and early diagnosis of enteric fever. ***Material and methods:*** A total of 70 patients with febrile illness were evaluated and tested with Typhoid IgM/IgG test and blood culture. The patients were divided into two groups: Gp I included 40 patients with likely clinical diagnosis of enteric fever and GpII comprised 30 patients with other laboratory confirmed illnesses were taken as control. We have in addition evaluated the significance of Typhoid IgM/IgG test in blood culture negative patients. ***Results:*** The overall sensitivity, specificity, positive predictive value and negative predictive value of Typhoid IgM/IgG test considering blood culture as gold standard was 92.5%, 93.33%, 97.40% and 87.83% respectively. Among blood culture negative patients, rapid Typhoid IgM/IgG test detected 93.33% additional cases of enteric fever.
Conclusion: The rapid Typhoid IgM/IgG test offers affordability, simplicity, increased sensitivity, specificity, rapidity and early diagnosis over blood culture.
Keywords: Blood culture, Typhoid IgM/IgG test, enteric fever, rapid point of care test, *Salmonella* Typhi.

INTRODUCTION

Enteric fever is potentially life threatening systemic infection occurring in lesser-developed areas of the world and continues to be a major public health problem (WHO annual incidence 21 million- >6 lacs deaths). The cases are more likely to be seen in India, South, Central America, and Africa i.e. in areas with rapid population growth, increased urbanization, and limited safe water, infrastructure, and health systems [1]. Accurate diagnosis of typhoid fever at an early stage is important not only for early institution of therapy, but also to prevent individuals that may serve as a potential carrier (may cause acute outbreaks). The emergence of multidrug-resistant strains of *Salmonella* Typhi is known to be associated with significant morbidity and mortality.

Options for the diagnosis of typhoid fever are clinical signs and symptoms, blood culture, serological markers, antigen detection and DNA amplification. Since the clinical presentation may not be specific; much onus falls on the laboratory for the rapid, reliable, and accurate early diagnosis of typhoid fever. Blood culture and Widal test are routinely employed investigations for diagnosis. Blood culture although gold standard, the yield of culture has declined due to prior use of antibiotics. The sensitivity of urine and stool culture is much lower and they become positive after one week of infection [2].

Whereas, Widal test (cheaper and easy to perform test) requires four fold rise in convalescent phase sera for a positive test and is not reliable because of cross reactivity (leading to false-positive and false negative results) [2].

Therefore, rapid, affordable, and accurate tests to address current diagnostic deficiencies for typhoid fever are greatly needed especially, in endemic country like ours, where current diagnostic methods are proving to be of limited utility. Typhoid IgM/IgG (Reliable Pro-detect Biomedical Pvt. Ltd.) is one such rapid test based upon lateral flow chromatographic immunoassay & has higher sensitivity/specificity. It detects antibodies against outer membrane protein of *Salmonella* Typhi (highly specific). It becomes positive as early as first week of fever; the result is available within half hour. Evaluation of studies in Asian countries like Malaysia, Indonesia, Philippines, Pakistan, Bangladesh and India have shown that Typhoid is superior to the culture methods as well as Widal test [3,4].

We at the tertiary care center in North India, planned a prospective study to evaluate diagnostic accuracy of rapid point of care test in the early diagnosis of enteric fever vis-à-vis blood culture. To the best of our knowledge, there is limited data in the literature regarding significance of Typhoid IgM/IgG test in the blood culture negative cases. Here an attempt has also been made to evaluate this rapid point of care test in blood culture negative test.

SUBJECTS AND METHODS

Study design

This prospective study was aimed to compare a rapid point of care test viz Typhoid IgM/IgG test with traditional gold standard method blood culture for diagnosis of Enteric fever. Patients with clinical suspicion of enteric fever (fever ≥5 days, relative bradycardia, headache, pain abdomen) of age 15 years or more coming to department of Medicine from April, 2015 to January, 2016 were enrolled.

Settings

Patients enrolled as per inclusion criteria in the department of Medicine and tested in the department of Microbiology of a tertiary care center in North India

Patient data

The sample from these patients was processed for blood culture and Typhoid IgM/IgG test in the department of Microbiology. Data collection includes the demographic profile of the patients, results of the blood culture and rapid immunological point of care test. Total 70 patients with febrile illness were evaluated and divided into two groups. Gp I included 40 patients with likely clinical diagnosis of enteric fever and Gp II comprised 30 patients with other laboratory confirmed illnesses were taken as control.

Blood culture

Blood samples from each patient were collected taking all aseptic precautions. For all the samples phlebotomy was performed after achieving antisepsis of vein puncture site with 70% alcohol followed by 2% tincture iodine. Five to ten ml of blood was collected which was then inoculated in 50 ml brain heart infusion (BHI) broth. Blood culture bottles were incubated at 37°C aerobically for 24 hrs followed by subcultures on blood agar and MacConkey agar. Blood culture bottles which do not show signs of growth (turbidity or hemolysis) were again subcultered on 2^{nd}, 3^{rd} and 7^{th} day and were reported negative on 7^{th} day after final subculture. Isolates were identified by standard microbiological procedures including Gram's stain, colony morphology, and biochemical reactions[3,4]. These were also confirmed by automated identification system i.e. B.D. Phoenix.

Typhoid IgM/IgG test

It is a rapid test based upon lateral flow chromatographic immunoassay. The test test cassette was placed on a flat surface. One drop, about 30-40 µl, of serum was dispensed into the sample well with the help of a calibrated dropper followed by 1 drop of sample diluent. Results were read after 15 minutes. IgM/IgG antibodies if present in the sample got bind to the HO conjugates, forming a burgundy colored M band or G band, indicating a S. Typhi IgM or IgG positive test returns. Absence of any test bands suggested a negative result [5, 6].

Statistical analysis

To evaluate the diagnostic accuracy of Typhoid IgM/IgG test, various statistical parameters like sensitivity, specificity, negative predictive value and positive predictive value were calculated. The chi square test was used to assess the differences between the groups. Statistical analysis was done using software SPSS version 22 and statistical significance was considered when $p<0.05$.

RESULTS

The patients included in the study were of different age groups. Incidence was highest in age cohort 21-30 yrs and was lowest in age group 41-60 yrs. Mean age of study population was 33.18 ± 13.19 yrs. Total number of females in study population far exceeded males. There were 15 males (37.50%) and 25 females (62.50%). Mean duration of presentation to hospital in study population was 17.12 ± 23.96 days. A high number (42.5%) patients presented within 10 days and only 7.5% of patients presented after more than 1 month. This depicts the common problem of late presentation in developing countries like ours.

The late presentation of the patients can be explained from the fact that 80% of patients had taken some treatment in the form of antibiotics, NSAIDS or some nonspecific treatment before coming to this hospital. This is depicted in the Table 1. Out of the 70 cases only 10 were found to be positive for *S.* Typhi and rest were sterile. Among the culture positive cases (Gp I), 9 (90%) were also positive by Typhoid IgM/IgG. Among the 30 non enteric fever cases (Gp II), only 1 (3.33%) was positive by Typhoid IgM/IgG. The overall sensitivity, specificity, positive predictive value and negative predictive value of Typhoid IgM/IgG test considering blood culture as gold standard was 92.5%, 93.33%, 97.40% and 87.83% respectively. The data is depicted in table 2 & 3 and figure 1.

Out of 40 patients tested for enteric fever, 30 were blood culture negative, among these blood culture negative patients, 28 (93.33%) were positive for either IgM or IgG antibodies by Typhoid rapid test. Follow up of this blood culture negative but rapid Typhoid IgM/IgG test positive showed that they were clinically diagnosed as enteric fever and has other laboratory test

positive viz. total leukocyte count, differential leukocyte count and C-reactive protein. These patients also responded well to intravenous ceftriaxone treatment. The data was statistically analyzed and found to be significant with p value <0.016.

Table-1: Demographic profile of the study population

Category	Number of cases (n=40)	Percentage (%)
Age group (years)		
• 11-20	7	17.5
• 21-30	14	35
• 31-40	9	22.5
• 41-50	5	12.5
• 51-60	5	12.5
• Mean age	33.18±13.19	
Gender		
• Male	15	37.50
• Female	25	62.50
Duration of presentation		
• <10	17	42.5
• 11-20	15	37.5
• 21-30	5	12.5
• >30	3	7.5
Prior treatment history	32	80

Table-2: Comparison of blood culture and Typhoid IgM/IgG test among study groups.

Test		Group I (n=40)	Group II (n=30)
Blood culture	Positive	10	0
	Negative	30	30
Typhoid IgM/IgG	Positive	37	1
	Negative	3	29

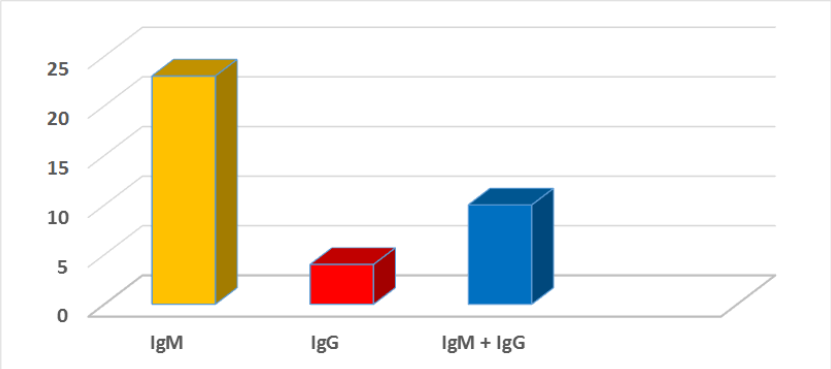

Fig-1: Type of antibodies detected among test group (n=40)

Table-3: Diagnostic accuracy of Typhoid IgM/IgG test as compared to blood culture.

Parameter	Percentage (%)
Sensitivity	92.5
Specificity	93.33
Positive predictive value	97.40
Negative predictive value	87.83

DISCUSSION

We have evaluated the commercially available rapid point of care test viz Typhoid IgM/IgG test for early diagnosis of enteric fever. The result of this commercial test and blood culture as gold standard have been analyzed prospectively. During April, 2015 to January, 2016, total 70 patients with febrile illness were evaluated and divided into two groups. Gp I included 40 patients with likely clinical diagnosis of enteric fever and Gp II comprised 30 patients with other laboratory confirmed illnesses were taken as control. Majority of patients in this study were females and 80 % were given antibiotics prior to admission. Clinical examination revealed fever in 100%, loss of appetite in 62.5%, abdominal pain in 35%, headache in 27.5%, and joint

pain in 2.5% pts. Main clinical signs were hepatomegaly (55%), relative bradycardia (50%), coated tongue (47.5%), & splenomegaly (40%).

The overall sensitivity, specificity, positive predictive value and negative predictive value of Typhoid IgM/IgG test considering blood culture as gold standard was 92.5%, 93.33%, 97.40% and 87.83% respectively. Similar results were reported by a study carried out by Jesudason *et al.*[6] at CMC Vellore i.e. 92.3% and 98.8% sensitivity and specificity respectively. Choo *et al.*[7] similarly reported sensitivity and specificity of 90.3% and 91.9% respectively. Our studies are also comparable to that of other studies done in India and abroad, who also recommend this rapid immunochromatographic test over the commonly employed Widal test and as an adjunct to blood culture which has poor sensitivity.

The significant feature of our study is that we have also evaluated the role of rapid Typhoid IgM/IgG test in blood culture negative patients. Out of 40 patients tested for enteric fever, 30 were blood culture negative, among these blood culture negative patients, 28 (93.33%) were positive for either IgM or IgG antibodies by Typhoid rapid test. Follow up of this blood culture negative but rapid Typhoid IgM/IgG test positive showed that they were clinically diagnosed as enteric fever and has other laboratory test positive viz. total leukocyte count, differential leukocyte count and C-reactive protein. These patients also responded well to intravenous ceftriaxone treatment. Similar results have seen in studies by Narayanappa *et al*. [8] and Baig *et al*.[9] with 73% and 63% additional cases reported in blood culture negative cases. This can be explained by the fact that sensitivity of blood culture is low i.e 50-70% and the sensitivity decreases after the first week of illness and antibiotic therapy [10,11]. Moreover, rapid test can detect IgM antibodies as early as two days of fever and upto two weeks of fever. However, further studies are needed to evaluate the role of Typhoid IgM/IgG test in early diagnosis of enteric fever in blood culture negative cases where molecular diagnosis can act as gold standard.

CONCLUSION

Thus, in this era of antibiotic misuse, the clinical profile of enteric fever and its traditional investigations like blood culture and Widal test have lost their reliability in making diagnosis of enteric fever. Positive rapid ICT in blood culture negative cases should always be correlated clinically for the signs of enteric fever. Typhoid IgM/IgG test is emerging as a highly sensitive & specific test for enteric fever especially for blood culture negative patients, who receive antibiotics before diagnosis.

Ethical approval

The study was conducted after getting approval from the ethical committee of the institution.

REFERENCES

1. Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ. Typhoid fever. N Engl J Med. 2002;347:1770-82.
2. Farooqui BJ, Khurshid M, Ashfaq MK, Khan MA. Comparative yield of Salmonella typhi from blood and bone marrow cultures in patients with fever of unknown origin. Journal of clinical pathology. 1991 Mar 1;44(3):258-9.
3. Threlfall EJ. Salmonella. In: Borrielo SP, Murray PR, Funke G. Topley and Wilson's Microbiology and Microbial Infections Bacteriology. Vol 2. 10th ed Ch. 54. Hodder Arnold, London: ASM press. 2005 1398-434.
4. Gupta A. Multidrug-resistant typhoid fever in children: epidemiology and therapeutic approach. The Pediatric infectious disease journal. 1994 Feb 1;13(2):134-40.
5. Sherwal BL, Dhamija RK, Randhawa VS, Jais V, Kaintura A, Kumar M. A comparative study of Typhidot M and widal test in patients of Typhoid fever. Journal of Indian Academy of Clinical Medicine. 2004;5(3).
6. Jesudason MV, Sivakumar S. Prospective evaluation of a rapid diagnostic test Typhiot® for typhoid fever. Indian Journal of Medical Research. 2006 Apr 1;123(4):513.
7. Choo KE, Davis TM, Ismail A, Ibrahim TT, 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 tropica. 1999 Mar 15;72(2):175-83.
8. 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 pediatrics. 2010 Apr 1;47(4):331-3.
9. 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. Brazilian Journal of Infectious Diseases. 2010 Dec;14(6):589-93.
10. Anatharayan R, Panikar CK. Textbook of Microbiology. Chennai:Orient longman.1999:244-9
11. Olsen SJ, Pruckler J, Bibb W, Thanh NT, Trinh TM, Minh NT, Sivapalasingam S, Gupta A, Phuong PT, Chinh NT, Chau NV. Evaluation of rapid diagnostic tests for typhoid fever. Journal of clinical microbiology. 2004 May 1;42(5):1885-9.