Clinical Profile and the Role of Rapid Serological Tests: Typhifast IgM and Enterocheck WB in the Diagnosis of Typhoid Fever

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ABSTRACT

Introduction: During identification and diagnosis, typhoid fever (TF) causes various issues such as nonspecific symptoms and nontyphoidal *Salmonella*-associated febrile diseases. Accurate identification and diagnosis are still a substantial concern. The current study was undertaken to study the clinical profile of TF and the role of Typhifast IgM and Enterocheck WB in early diagnosis.

Materials and methods: Clinically suspected TF patients (121) were included in the study. Patients with antibiotic history during the period of febrile illness were excluded. The diagnosis was confirmed with blood culture test. Widal test and two new rapid serological tests: Typhifast IgM and Enterocheck WB were performed. The outcomes were compared with blood culture-confirmed cases to derive the sensitivity and specificity of the diagnostic tests. The clinical characteristics were compared with diagnostic tests using Chi-square test.

Results: The most common presentations of TF were fever, chills, vomiting, abdominal pain, anorexia, constipation, and diarrhea. Among the 121 clinically suspected TF patients, 67 had positive blood culture tests for *Salmonella typhi*. The Typhifast IgM and Enterocheck WB showed sensitivity of 97.01% and 68.52%, respectively. Specificity was also more with Typhifast IgM (85.07%) than with Enterocheck WB (62.96%). When tests were used in parallel combination, 97.01% sensitivity was attained, while the specificity dropped to 46.30%. When used in serial combination, sensitivity of 85.07% and specificity of 85.19% were observed.

Conclusion: Stepladder fever, abnormal serum glutamic oxaloacetic transaminase (SGOT), and abnormal albumin are associated with blood culture, Typhifast IgM, and Enterocheck WB tests. Rapid serological tests might assist in accurate and early identification of TF.

Keywords: Blood culture, Enterocheck WB, *Salmonella enterica* serotype *typhi*, Typhifast IgM, Widal test.

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INTRODUCTION

Enteric fever or typhoid fever (TF) is one of the substantial life-threatening illnesses observed predominantly in the developing countries.[1] In 2010, approximately 12 million cases were encountered with clinically determined TF, and globally, around 0.13 million TF-related mortalities occur every year.[2] Probable contributing factors for disease incidence include high population density, underprivileged and impoverished areas, inadequate sanitization, unavailability of pure drinking water, and low socioeconomic status.[2] Typhoid fever is caused by *Salmonella enterica* serovars such as *Typhi* and *Paratyphi* A, B, and C.[1] The *Salmonella* species are transmitted through the consumption of food and water that are contaminated with fecal matter-excreting pathogenic bacteria. Entry of *Salmonella* through fecal–oral route invades the gastrointestinal epithelium, thereby migrating to the gut mucosa and spreads systemically throughout the body via the bloodstream.[3] Classical clinical manifestations include high temperature, which can rise up to 38–40°C, chills, abdominal discomfort, and hepatosplenomegaly.[4] Diagnosis of TF by isolation of *Salmonella* species from the bloodstream and treatment with appropriate antibiotics are the significant methods for timely management of TF.[5]

Although the disease and its complications are known from the 19th century,[6] the burden of TF remains the same. The predominant reason for such drawback indicates the lack of suitable diagnostic tools and the emergence of drug-resistant strains.[5] Blood, bone marrow, and fecal cultures are considered as the most reliable diagnostic tools.[7] Cultures provide definitive evidence of the infection and the information regarding the antibiotic sensitivity of the isolate.[8] Sensitivity of blood culture varies from 40% to 60% and bone marrow culture varies between 55% and 67%.[9] Sensitivity and specificity of the bone marrow culture are superior to fecal and blood cultures; however, due to its invasive nature, the procedure is not frequently used. Moreover, blood culture sensitivity depends on various factors such as the volume considered for culture, degree of disease progression, bacterial load in the sample, and self-treatment with antimicrobials.[10]

Contemporary research recommends the culture tests along with the serological techniques for the early diagnosis of TF. In the developing countries, the equipment required for blood culture

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are significantly deficient, especially in the nonurban areas, due to which the clinicians depend only on serological diagnostic tools. In most developing countries, Widal test is an extensively employed serological tool, which detects the antibodies against the *S. enterica ser. Typhi* "O" and "H" antigens; however, its sensitivity and specificity are significantly poor and the predictive values vary between the geographical regions. Studies on the use of Widal test have reported misdiagnosis and inappropriate treatment of TF, which limit its employment.[11] Novel rapid serological tests such as Typhifast IgM and Enterocheck WB detect IgM or IgG antibodies to several purified antigens of the pathogen. Typhifast IgM and Enterocheck WB are rapid, simple, and affordable tests; however, few studies are available in the literature that derive the efficacy of these rapid serological tests. Considering the facts and several issues raised regarding the diagnosis and treatment of TF, the current study was undertaken to evaluate the clinical profile of TF and to analyze the role of rapid serological tests—Typhifast IgM and Enterocheck WB—individually and in combination in the early detection of TF.

MATERIALS AND METHODS

The present cross-sectional study was conducted in the department of medicine, from January to December 2014. A total of 121 patients with clinical features suggestive of TF such as fever, headache, and abdominal discomfort were included in the study. Patients aged between 12 and 65 years, irrespective of the gender, with ≥5 days of fever were included in the study. Patients receiving antibiotics during the period of febrile illness were excluded from the study. Ethical clearance was obtained from Institutional Ethics Committee. Before the commencement of the study, written informed consent was obtained from the patients.

Data Collection

Patients were interviewed regarding the demographic data such as age, gender, occupation, and history of similar complaints and treatment for the same. Patients were subjected to a thorough physical examination, including pulse rate, blood pressure, respiratory rate, and other clinical signs and symptoms of TF such as fever, abdominal discomfort, and constipation. Systemic examinations such as complete blood count, liver function test, Widal test, blood culture, urine routine and microscopy, Typhifast IgM, and Enterocheck WB were also conducted.

Widal Test

Blood serum sample was serially diluted with 0.86% saline solution. Standard O and H antigens were prepared, added to the tubes, and were incubated for 60 minutes at 37°C. The tubes were centrifuged for around 5 minutes and observed for agglutination. Widal test titer ≥1:160 was considered as the optimal cutoff titer value to detect TF in our study population.[11,12]

Blood Culture

Blood culture was performed to isolate the organism according to the standard procedures followed using the BacT\ALERT 3D automated system (bioMerieux, France).[13] Any isolated bacterium was identified using Vitek II (bioMerieux, France) identification system and the presence of the same was confirmed by slide-agglutination test with particular antisera (Remel Europe Ltd., UK).

Typhifast IgM

Typhifast IgM (AB Diagnopath Manufacturing Pvt. Ltd., New Delhi) is a commercially available serological kit based on dot-enzyme-immunoassay that specifically detects the presence of IgM antibodies triggered against the somatic, flagellar, or capsular antigens of *Salmonella* subspecies *S. enterica* ser. *Typhi*.[14] Typhifast IgM uses immunochromatography phenomenon for the detection; and the assay was performed according to the manufacturer instructions.[15] Volume of about 10 μL of serum or plasma sample and 20 μL of whole blood were added to the square well I, followed by addition of one drop of chase buffer. On the well II, three drops of chase buffer were added, and plastic tap was pulled. Results were interpreted after 10 minutes and the overall assay time required was 15 minutes.

Interpretation

Appearance of color bands with any intensity at the test line as well as on the control line was represented as positive for typhoid-specific IgM antibodies. The presence of colored band only at the control line were represented as negative for typhoid-specific IgM antibodies, and it was considered invalid if the control line was absent.

Enterocheck WB

Enterocheck WB is a rapid sandwich immunoassay, which functions on the principle of immunochromatography. It specifically detects the antibodies triggered against the lipopolysaccharide of the pathogen in the human serum.[16] Detection of the pathogen by Enterocheck WB was performed using the commercially available kit (Zephyr Biomedicals, Goa, India). The test was performed by adding 5 μL of serum sample on port "A" and five drops of running buffer on port "B". The overall time required for the assay was 15 minutes.

Interpretation

In positive samples, the serum IgM forms conjugates with anti-IgM colloid gold, which flows further along the membrane to the test line establishing a pink-purple colored band. In negative samples, only the rabbit IgG complex flows along the membrane to the control line, establishing a pink-purple colored band.

Statistical Analysis

Data were analyzed using Microsoft excel spreadsheet. Parameters such as clinical manifestations of TF were compared with blood culture, Typhifast IgM, and Enterocheck WB and the comparison was performed using Chi-square test. The continuous data were expressed as mean ± standard deviation (SD). The sensitivity and specificity of the serological tests were determined individually and in combination using MedCalc software.

RESULTS

Among the study population (121), male preponderance (81) was observed and male to female ratio was 2.02:1. Mean age was 31.70 ± 15.08 years. All (121) patients presented with fever, whereas 14 patients had stepladder pattern of fever, which was once considered as the hallmark of TF. Chills and abdominal pain were observed in 62 and 38 patients, respectively. Few patients (41) presented with atypical symptoms such as altered sensorium, burning micturition, hematemesis, melena, and sore throat.

On evaluation of blood culture, 67 patients confirmed *Salmonella enterica* ser. *Typhi* isolation; of these, 37 patients tested

positive for Widal test. The sensitivity of Widal test was 55.22% and specificity was 74.04%. Positive and negative predictive values of Widal test were 72.55% and 57.14%. Among the study population, 51 patients tested positive for Widal test.

Clinical complications such as stepladder fever, serum glutamic oxalo-acetic transaminase (SGOT), and albumin were significantly associated with blood culture, Typhifast IgM, and Enterocheck WB. Abnormal erythrocyte sedimentation rate was associated with blood culture ($p < 0.015$) and Enterocheck WB ($p < 0.001$). Total protein was associated with Typhifast IgM ($p < 0.003$) and Enterocheck WB ($p < 0.001$). Chills ($p < 0.012$), myalgia ($p < 0.050$), and constipation ($p < 0.049$) were significantly associated with blood culture (Table 1). Eosinophil count ($p < 0.045$) was associated with Typhifast IgM (Table 2). History of TF ($p < 0.049$), coated tongue ($p < 0.008$), and direct bilirubin (<0.009) were associated with Enterocheck WB (Table 3).

Among the 121 patients, 67 were blood culture-positive and 82 were Typhifast IgM positive. Of the 67 blood culture-positive patients, 65 were positive for Typhifast IgM. The sensitivity and specificity of Typhifast IgM, with blood culture as the reference standard, were 97.01% and 68.52%, respectively. The positive and negative predictive values were 79.27% and 94.87%, respectively (Table 4).

Among the 67 blood culture-positive patients, 57 were positive for Enterocheck WB. The sensitivity and specificity of Enterocheck WB, with blood culture as the reference standard, were 85.07% and 62.96%, respectively. The positive and negative predictive values were 74.03% and 77.27%, respectively (Table 4).

Global sensitivity and specificity of Typhifast IgM and Enterocheck WB, when performed in series, were 85.07% and 85.19%, respectively. When performed in parallel, sensitivity was 97.01%, whereas specificity was 46.30% (Table 4).

DISCUSSION

Typhoidal *Salmonella* infections are bloodstream infections, which bear complexity in distinguishing the various other nontyphoidal *Salmonella*-associated febrile diseases; thereby, interfering in the interpretation of the serological tests.[17] Management of TF using standard drugs such as chloramphenicol, ampicillin, and trimethoprim–sulfamethoxazole changed with the emergence of multidrug-resistant strains.[18] In the current study, most of the patients were in the age-group of 21–30 years and the mean age was 31.70 years. The probable reasons behind the highest prevalence in this age-group include mobility, consumption of unhygienic food and water, and age-group illiteracy. These observations were consistent with various other studies.[19,20] Clinical manifestations that are believed as the typical presentations of TF such as slow stepladder fever pattern and toxicity are becoming rare due to the development of multidrug resistance. The nonspecific manifestations due to multidrug resistance contribute to inaccurate diagnosis and improper management.[21] This inconsistency forces clinicians to depend specifically on the serological diagnostics.

Clinical manifestation varies from mild illness with ground-level fever to severe complications such as abdominal pain, hepatosplenomegaly, and anorexia. In the current study, all clinically suspected typhoid patients presented with fever; however, the stepladder fever pattern, which was once the hallmark of TF, was observed in 11.57% patients. *Salmonella enterica* serotypes are acid-tolerant organisms, which can survive in the gastric acid and successfully establish the infection in the gut mucosa causing

Table 1: Association between blood culture test and different clinical characteristics

| Clinical characteristics | | Blood culture | | |
		Positive (n = 67)	Negative (n = 54)	p value
History of symptoms	Stepladder fever	14	0	0.0001*
	Chills	27	35	0.012*
	Cough	16	7	0.197
	Sweating	4	1	0.501
	Myalgia	10	17	0.050*
	Malaise	9	6	0.914
	Arthralgia	4	2	0.881
	Anorexia	22	11	0.185
	Abdominal pain	22	16	0.856
	Nausea and vomiting	22	25	0.186
	Diarrhea	11	3	0.116
	Constipation	13	3	0.049*
	Others	24	23	0.567
	History of typhoid	6	4	1.00
General physical examination	Pallor	11	17	0.082
	Icterus	7	7	0.885
	Coated tongue	21	11	0.248
	Rash	4	2	0.881
	Relative bradycardia	9	2	0.125
	Toxemia	13	4	0.104
	Abnormal pulse rate	21	12	0.360
	Abnormal respiratory rate	30	20	0.500
CBC	Abnormal systolic BP	15	9	0.578
	Low hemoglobin	43	39	0.456
	Abnormal platelet count	14	17	0.264
	Abnormal TC	26	31	0.063
	Abnormal neutrophil count	23	15	0.565
	Abnormal lymphocyte count	28	23	1.00
	Abnormal eosinophil count	20	11	0.328
	Abnormal monocyte count	10	12	0.425
	Abnormal ESR	59	37	0.015*
LFT	Abnormal total bilirubin	5	1	0.321
	Abnormal direct bilirubin	27	27	0.377
	Abnormal SGOT	47	27	0.038*
	Abnormal SGPT	40	38	0.304
	Abnormal AKP	30	26	0.852
	Abnormal total protein	32	19	0.227
	Abnormal albumin	39	16	0.003*

*Significant, $p < 0.05$.
CBC, complete blood count; BP, blood pressure; LFT, liver function test; TC, total count; ESR, erythrocyte sedimentation rate; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic-pyruvate transaminase; AKP, alkaline phosphatase

Table 2: Association between Typhifast IgM test and different clinical characteristics

Clinical characteristics		Typhifast IgM Positive (n = 82)	Typhifast IgM Negative (n = 39)	p value
History of symptoms	Stepladder fever	14	0	0.014*
	Chills	38	24	0.108
	Cough	16	7	1
	Sweating	5	0	0.277
	Myalgia	19	8	0.924
	Malaise	11	4	0.843
	Arthralgia	4	2	1
	Anorexia	26	7	0.170
	Abdominal pain	25	13	0.915
	Nausea and vomiting	30	17	0.589
	Diarrhea	13	1	0.0669
	Constipation	14	2	0.127
	Others	26	17	0.283
	History of typhoid fever	5	5	0.367
General physical examination	Pallor	17	11	0.496
	Icterus	9	5	1
	Coated tongue	25	7	0.214
	Rash	4	2	1
	Relative bradycardia	10	1	0.166
	Toxemia	13	4	0.583
	Abnormal pulse rate	23	10	0.952
	Abnormal respiratory rate	36	14	0.523
CBC	Abnormal systolic BP	16	8	1
	Low hemoglobin	51	31	0.090
	Abnormal platelet count	19	15	0.125
	Abnormal TC	34	23	0.107
	Abnormal neutrophil count	27	11	0.753
	Abnormal lymphocyte count	37	14	0.445
	Abnormal eosinophil count	26	5	0.045*
	Abnormal monocyte count	21	10	1
	Abnormal ESR	19	9	1
LFT	Abnormal total bilirubin	5	1	0.697
	Abnormal direct bilirubin	33	21	0.225
	Abnormal SGOT	57	17	0.011*
	Abnormal SGPT	49	29	0.172
	Abnormal AKP	35	21	0.339
	Abnormal total protein	39	7	0.003*
	Abnormal albumin	46	3	<0.001*

*Significant, p < 0.05.
CBC, complete blood count; BP, blood pressure; LFT, liver function test; TC, total count; ESR, erythrocyte sedimentation rate; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic-pyruvate transaminase; AKP, alkaline phosphatase

Table 3: Association between Enterocheck WB and different clinical characteristics

Clinical characteristics		Enterocheck Positive (n = 77)	Enterocheck Negative (n = 44)	p value
History of symptoms	Stepladder fever	13	1	0.033*
	Chills	35	27	0.134
	Cough	16	7	0.677
	Sweating	5	0	0.210
	Myalgia	15	12	0.445
	Malaise	11	4	0.584
	Arthralgia	4	2	1.000
	Anorexia	22	11	0.832
	Abdominal pain	19	19	0.056
	Nausea and vomiting	28	19	0.584
	Diarrhea	11	3	0.347
	Constipation	14	2	0.064
	Others	25	18	0.461
	History of typhoid fever	3	7	0.049*
General physical examination	Pallor	14	14	0.137
	Icterus	11	3	0.347
	Coated tongue	27	5	0.008*
	Rash	5	1	0.552
	Relative bradycardia	10	1	0.100
	Toxemia	11	6	1.000
	Abnormal pulse rate	22	11	0.832
	Abnormal respiratory rate	33	17	0.793
CBC	Abnormal systolic BP	17	7	0.560
	Low hemoglobin	50	32	0.496
	Abnormal platelet count	19	15	0.369
	Abnormal TC	37	20	0.931
	Abnormal neutrophil count	20	18	0.133
	Abnormal lymphocyte count	31	20	0.714
	Abnormal eosinophil count	23	8	0.23
	Abnormal monocyte count	10	12	0.086
	Abnormal ESR	63	10	<0.001*
LFT	Abnormal total bilirubin	5	1	0.552
	Abnormal direct bilirubin	27	27	0.009*
	Abnormal SGOT	54	20	0.012*
	Abnormal SGPT	47	31	0.398
	Abnormal AKP	35	21	0.958
	Abnormal total protein	9	17	0.001*
	Abnormal albumin	7	20	<0.001*

*Significant, p < 0.05.
CBC, complete blood count; BP, blood pressure; LFT, liver function test; TC, total count; ESR, erythrocyte sedimentation rate; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic-pyruvate transaminase; AKP, alkaline phosphatase

Table 4: Sensitivity and specificity of Typhifast IgM and Enterocheck WB, individually and combined in series and parallel

	Typhifast IgM (%)	Enterocheck WB (%)	Series (%)	Parallel (%)
Sensitivity	97.01 (89.63–99.64)	85.07 (74.26–92.60)	85.07 (74.26–92.60)	97.01 (89.63–99.64)
Specificity	68.52 (54.45–80.48)	62.96 (48.74–75.71)	85.19 (72.88–93.38)	46.30 (32.62–60.39)
PPV	79.27 (72.02–85.03)	74.03 (66.49–80.36)	87.69 (78.86–93.16)	69.15 (63.55–74.24)
NPV	94.87 (82.36–98.65)	77.27 (64.94–86.19)	82.14 (71.98–89.17)	92.59 (75.60–98.06)

PPV, positive predictive value; NPV, negative predictive value

abdominal discomfort.[17] In the current study, gastrointestinal symptoms such as diarrhea, abdominal pain, and constipation were found prominent among the study population. This suggests that the presence of fever and gastrointestinal discomfort might assist the clinicians in suspecting the presence of TF complications.

Current study found that clinical feature, such as stepladder fever, was present significantly in both Typhifast IgM and Enterocheck WB as well as in blood culture-positive patients. Although the blood culture represented significant results for abdominal complications such as constipation, both Typhifast IgM and Enterocheck WB showed no significance toward abdominal complications. Liver function tests showed that among Typhifast IgM- and Enterocheck WB-positive patients including blood culture-positive patients, the presence of abnormal SGOT and abnormal albumin values was significantly observed. These findings suggest that along with stepladder fever and abdominal pain, the impaired liver function may invariably predict the development of TF. Histopathological study of liver showed the presence of typhoid nodules, swelling, and mononuclear cell infiltrate in focal regions, and liver biopsy culture showed the presence of bacilli in the parenchyma cells.[22] Furthermore, studies reported that TF can contribute to increased levels of aminotransferase and development of jaundice, which leads to hepatic impairment.[23]

Widal test is a widely employed serological test in the developing countries, which detects the specific agglutinating antibodies that are released against the lipopolysaccharide (O) and flagella (H) antigens of the pathogen. Widal test requires increasing titer of paired samples after 10–14 days; however, in the case of TF and blood culture-confirmed TF cases such fourfold rise is not demonstrable.[7] Results of Widal tests need careful interpretation, considering the history of vaccination and *Salmonella*-associated infections. Main drawback of Widal tests is cross-reactivity for non-*Salmonella* infections such as malaria, dengue, and brucellosis in TF-endemic regions.[24] Various studies reported the poor predictive results and controversial specificity and sensitivity for Widal tests.[11,25] In the current study, Widal test titer ≥1:160 was the optimal cutoff titer value of TF. In a study by Andualem et al.,[7] the sensitivity and specificity were 71.4% and 68.44%, respectively, in Widal test-positive titer in relation to blood culture-confirmed cases; positive and negative predictive values were 5.7% and 98.9%, respectively. The above findings suggest that Widal test neither has good sensitivity nor specificity and that relying only on Widal test in the diagnosis of TF may be misleading.

Typhifast IgM is one of the commercially available kit which works on Typhidot IgM principle. Due to the lack of literature on the studies conducted on Typhifast, the current study considered Typhidot IgM for comparison, as it employs similar working principle. Sensitivity and specificity of Typhidot vary between 70–100% and 43–90%.[8,26] In a study conducted by Krishna et al.,[26] the sensitivity and specificity of the Typhidot IgM were 100% and 95.5%, respectively, in comparison to blood culture. The positive

and negative predictive values were 89.2% and 100%, respectively. The findings of the current study were in concordance with the findings of the above study.[26]

A study by Anusha et al.[16] reported the sensitivity and specificity of Enterocheck, in relation to blood culture, were 85.5% and 88.6%, respectively; and the negative and positive predictive values were 97.7% and 51.1%, respectively. Typhifast IgM appears to be a more sensitive and specific test with a high negative predictive value. Sensitivity and specificity variations may exist between the tests due to the cross-reactivity of the outer membrane protein antigens of different organisms such as *S. enterica* ser. *Typhi* and *S. enterica* ser. *Paratyphi A*.

To our knowledge, this is the first study that used a combination of serological tests for improving the diagnosis of TF. Typhifast IgM and Enterocheck were performed in series as well as in parallel for evaluating its effectiveness, surprisingly, the study discovered that sensitivity as well as the specificity was improved when executed both in series along with better positive (74.03%) and negative predictive values (77.27%), respectively. In parallel testing, sensitivity was 97%; however, specificity and the positive predictive values were relatively reduced when compared to individual and series tests. Hence, the diagnosis can be improved with the use of a combined serological testing approach.

Current study findings conclude that both the rapid serological tests, Typhifast IgM and Enterocheck WB, offer increased sensitivity and specificity than Widal test, and even better results when employed both in combination. Both render rapidity, early diagnosis, and simplicity than blood culture and can replace the most extensively used serological test—Widal test. However, the current findings should be enriched with larger sample size and longer follow-up. Moreover, in highly suspected cases of TF, clinical management should not depend on the negative results of the serological tests.

Conclusion

In the current study, clinical features such as step ladder fever, abdominal pain, vomiting, diarrhea, and constipation were associated with the diagnosis of TF. It was observed that stepladder fever, abnormal SGOT, and abnormal albumin were present significantly in both Typhifast IgM and Enterocheck WB as well as in blood culture-positive patients. Typhifast IgM and Enterocheck WB showed promising results with good sensitivity and specificity when compared with blood culture. Rapid serological diagnostic tests, either as individual tests or in combination, are necessary in the early diagnosis of TF; however, confirmation with culture techniques is mandatory.

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