

A Study on the Evaluation of Typhidot M (IgM Enzyme-linked Immunosorbent Assay) in the Early Diagnosis of Enteric Fever in Children

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Abstract

Introduction: Enteric fever, or typhoid, is a major public health problem in developing countries like India. The causative organisms, *Salmonella typhi* and *paratyphi*, are developing multidrug resistance due to indiscriminate use of antibiotics even in undeserving cases. Thus, enteric fever needs to be diagnosed early not only to reduce morbidity but also to combat antimicrobial resistance.

Aims and Objectives: This study aims to determine the utility of Typhidot M (IgM enzyme-linked immunosorbent assay [ELISA]) in the early diagnosis of enteric fever in children and to determine its sensitivity, specificity, and positive and negative predictive value in comparison with Widal, keeping blood culture as the gold standard.

Materials and Methods: Children from 1 to 12 years ($n = 90$) with clinical suspicion of enteric fever based on a set of criteria were enrolled in the study. Blood for blood culture, Typhidot M, and Widal were drawn, and the results analyzed.

Results: Among 90 children enrolled, 11 (12.22%) had positive cultures. 24 (26.67%) had positive Widal tests while 58 (64.44%) were positive for IgM antibodies by Typhidot M test. The sensitivity, specificity, and positive and negative predictive values of Typhidot M test were found to be 100%, 40.5%, 18.96%, and 100%, respectively, while that for Widal were 90.91%, 82.28%, 41.66%, and 98.45%, respectively.

Conclusion: Typhidot M (IgM ELISA) is a simple, sensitive, and reliable test for the early diagnosis of typhoid fever in children.

Key words: Antimicrobial resistance, Blood culture, Enteric fever, Typhidot M (IgM enzyme-linked immunosorbent assay), Widal test

INTRODUCTION

Enteric fever is a severe multisystemic infectious disease, caused by *Salmonella typhi*. It poses a significant public health threat in a number of developing nations, including India. Its presentation may be mild gastrointestinal symptoms or life-threatening complications such as intestinal perforation, delirium, meningitis, pneumonia,

or disseminated intravascular coagulation. Any delay in diagnosis and instituting timely and appropriate therapy can cause significant morbidity and mortality. Delayed diagnosis is of particular concern since the irrational use of empirical broad-spectrum antibiotics has resulted in the emergence of multidrug-resistant strains, leading to increase in adverse outcomes. Hence, there is an urgent need for a fast and reliable diagnostic test that can detect enteric fever early so that appropriate therapy can be instituted at the earliest.

Blood culture is the gold standard, for the confirmation of enteric fever. Although the test is highly specific, the sensitivity is very low, with the yield about 70–75% at best. Moreover, the results are obtained only after a minimum period of 3 days. The yield is also dramatically influenced by antibiotic usage. It is also dependent on the stage of

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the disease at which the blood is drawn, as the sensitivity falls drastically beyond the 1st week. The quantity of blood required for cultures is also more. It must also be drawn with strict aseptic precautions so as to avoid the growth of commensals. Hence, there is a need for another diagnostic modality that can circumvent the shortcomings of blood culture and at the same time be highly reliable with good sensitivity and specificity.

The Widal test has been in use for a long time. It is a serological test that is easy to perform and is readily available in most primary health facilities. It is also not too expensive nor does it require intense personal training. However, it is highly inaccurate with several cross-reactions occurring with other diseases such as TB, rickettsial infections, chronic hepatitis, dengue, and malaria. Thus, its very validity is now being questioned. In addition, the antibody titer rises significantly only in the 2nd week of illness which may be too late to prevent complications.

Typhidot M enzyme-linked immunosorbent assay (ELISA) is a dot ELISA kit used for the diagnosis of enteric fever. This test is similar to the ELISA kit used for the diagnosis of HIV infection and is based on the principle of antigen-antibody reaction. It detects the presence of IgM antibodies against a distinct protein, 50 kDa antigen on the outer membrane of *Salmonella typhi* in the patients' sera. The test becomes positive within 3–4 days of infection with *Salmonella typhi*. A positive IgM indicates an acute infection. This test has been shown to have high sensitivity and specificity in a number of studies conducted in different parts of India as well as elsewhere in the world. Thus, it not only detects the disease at an earlier stage but also gives reliable results that can be applied universally.

Aims and Objectives

The aims of this study were as follows:

1. To determine the utility of Typhidot M test in the diagnosis of clinically suspected enteric fever in children.
2. To compare the results of Typhidot M test with blood culture and Widal test and to correlate them clinically.
3. To determine the specificity, sensitivity, positive predictive value, and negative predictive value of Typhidot M test and Widal test keeping blood culture as the gold standard.

Study Design

This was a hospital-based prospective cohort study.

Period of Study

The study duration was 1 year.

Study Subject

Children who were admitted at a tertiary care institute with fever of 4 days or more, with clinical suspicion of enteric fever were enrolled.

Inclusion Criteria

All children 1–12 years of age with a history of fever of 4 days or more, with one or more of the following:

1. Headache.
2. Nausea or vomiting.
3. Constipation or diarrhea.
4. Abdominal pain.
5. Coated tongue.
6. Hepatomegaly.
7. Splenomegaly.
8. Signs of toxemia.
9. Leukopenia or leukocytosis.
10. Response to antibiotic therapy for the treatment of typhoid fever.

Exclusion Criteria

The following criteria are excluded from the study:

1. Children with documented typhoid fever within the past 8 weeks.
2. Those who were immunized against typhoid fever.
3. Those with definite focus of infection, other than enteric fever.

The study was approved by the institute ethical committee.

METHODOLOGY

Children aged 1 year up to 12 years admitted to the Tertiary Institute, who fulfilled the inclusion criteria, were included in the study. At admission, the procedure and test requirements were explained to the parents and written consent obtained. A complete and detailed history was elicited from the parent to fill the predesigned pro forma. A meticulous systemic examination was conducted. Baseline investigations taken at admission include hemoglobin, total and differential leukocyte count and platelet count, and blood (enteric) culture. 5 ml of blood for culture was drawn at admission before the first dose of any antibiotic was administered under strict aseptic precautions as to obtain the maximum yield possible. Blood was inoculated so as to attain blood: Broth ratio of 1:5–1:10. The culture media were checked for growth as well as other properties such as turbidity and gas formation at 24 h after inoculation and incubation. Antibiotic sensitivity was determined using the Kirby–Bauer disc diffusion method, and results were reported after a period of 72 h. The report stated the name of the enteric group of organisms grown and the sensitivity pattern.

1 ml of venous blood for IgM ELISA was taken at admission or the next day in plain screw-capped test tubes. Blood for Widal test was drawn depending on the duration of fever at the time of presentation. If a child presented with fever of 7 days or less, Widal test was deferred until the 8th day of illness.

Meanwhile, the treatment of all suspected cases was started in the form of supportive measures and antibiotics. All children were monitored closely for the development of any complications. Response to therapy was defined as the absence of fever, feeling of well-being or improvement in the general condition of the child, return of appetite, and feeding well. Children who remained afebrile for 3 consecutive days and had regained appetite were discharged. In children, in whom all three investigations for typhoid turned out to be negative, other fever investigations were sent depending on clues in history and clinical examination.

KIT for IG-MELISATM: “Typhiwell” a microwell IgMELISA kit manufactured by AB Diagnopath Manufacturing Private Limited, I-11, DSIDC Industrial Area, Udyog Nagar, New Delhi – 110041 was used for this study. It is an ELISA for the detection of IgM antibodies specific to Salmonella typhi in human serum/plasma.

Interpretation

Negative: 0–0.49; it indicates IgM antibody is not present in the sample.

Positive: Equal or more than 0.50; it indicates the presence of IgM antibody against Salmonella typhi

Statistical Analysis

Data analysis was done with the help of computer using the open source software R, using the statistical software SPSS 16.0 Using this software, sensitivity, specificity, positive predictive value, and negative predictive value were calculated. *P* value was calculated using Chi-square test. Concept of *P* value: *P* value: 0.00–0.01 ≥ highly significant, *P* value: 0.011–0.05 ≥ significant, and *P* value: 0.051–1.00 ≥ not significant.

RESULTS

Total number of cases enrolled based on clinical suspicion of enteric fever = 90

Number of cases with blood culture positive for Salmonella typhi = 11

Number of cases with positive Widal test = 24

Number of cases with positive Typhidot IgM ELISA = 58.

Case Characteristics

Age

Cases were divided on the basis of age group, with maximum cases between 3 and 6 years [Table 1].

Gender distribution of cases

In this study, nearly 59% of the cases were male while 41% were female [Figure 1].

Duration of illness

Nearly half the cases presented with fever of ≤7 days duration, while the remaining half presented with fever of more than 7 days [Figure 2].

Clinical presentation

The most common presentation among children enrolled in this study, apart from fever, was nausea/vomiting. This was followed by hepatomegaly, toxic look, and coated tongue. The least common presentation was diarrhea [Figure 3].

Antibiotic administration before admission

Almost four-fifths of the study subjects had exposure to antibiotics before admission in our hospital [Figure 4].

Laboratory diagnosis

Overall, the yield of blood culture was found to be very low (12.22%). It may be due to the rampant usage of empirical antibiotics taken before the confirmation of the diagnosis [Table 2].

Table 1: Age distribution of cases

Age (in years)	Number of cases (%)
≤3	23 (25.55)
3–6	28 (31.11)
6–9	24 (26.66)
>9	15 (16.66)
Total	90

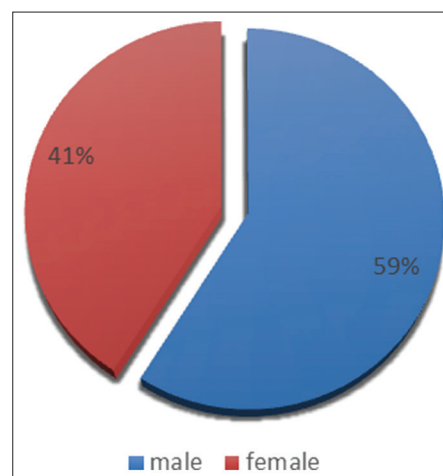


Figure 1: Gender distribution of cases

Comparison of Typhidot M - ELISA with the gold standard investigation - blood culture

Thus, Typhidot was found to have high sensitivity and negative predictive values. However, the specificity and positive predictive value were found to be below. This may be because of the low sensitivity of blood culture in this study [Tables 3a and b].

**Comparison of Widal with the gold standard investigation - blood culture [Table 4a and b]
Relation between duration of fever and investigation**

Among fevers of ≤ 7 days, Typhidot M detects the maximum number of cases. Typhidot M is able to detect 29 of 46 cases (63.04%) as compared to Widal which could detect only 7 of the 46 cases (15.21%), which was found to be statistically significant ($P = 0.03$), thus proving beneficial in the early diagnosis of typhoid fever. When compared to both blood culture and Widal, it is found

that Typhidot M detects more number of cases which is statistically significant ($P < 0.001$) [Table 5].

DISCUSSION

Typhoid is a major health problem in our country. There are various methods of diagnosis of enteric fever; however, the need for the hour is a quick and reliable test that can diagnose typhoid early so that appropriate antibiotic therapy can be instituted on time. This is necessary not only to prevent complications but also to prevent antibiotic resistance. The rampant misuse of empirical antibiotics has resulted in the rise of multidrug-resistant Salmonella typhi as well as nalidixic acid-resistant Salmonella typhi. Hence, early diagnosis is of immense importance.

This study was aimed at evaluating Typhidot M (IgM ELISA) as a possible candidate for the ideal diagnostic test for early diagnosis of typhoid fever. A total of 90 children were enrolled in this study based on clinical suspicion of enteric fever. Investigations used to confirm the diagnosis included blood (enteric) culture, Widal, and Typhidot M ELISA.

Table 2: Investigations for enteric fever

Investigations	Number of cases (%)
Enteric culture	11 (12.26)
Widal	24 (26.67)
Typhoid IgM ELISA	38 (64.44)

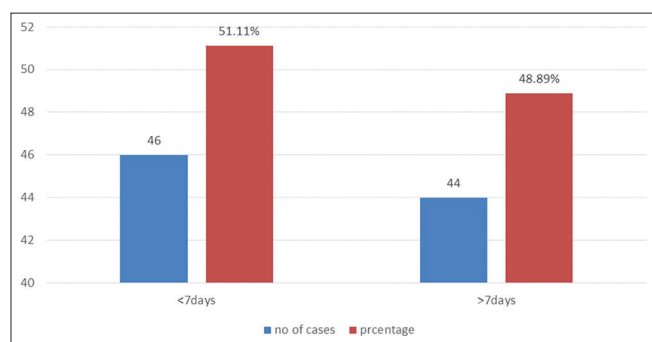


Figure 2: Duration of illness

Age

In this study, the age distribution was found to be nearly uniform across all age groups.

Antibiotic Administration and Rampant Misuse

In our study, nearly 80% of the children had at least one dose of an antibiotic before presenting to our hospital. In most cases, the antibiotics were administered even before any investigations for the confirmation of the diagnosis could be sent. This adversely affected the outcomes of our study. More importantly, such blatant misuse of antibiotics is adding to the intimidating burden of antibiotic resistance in the country and the world.

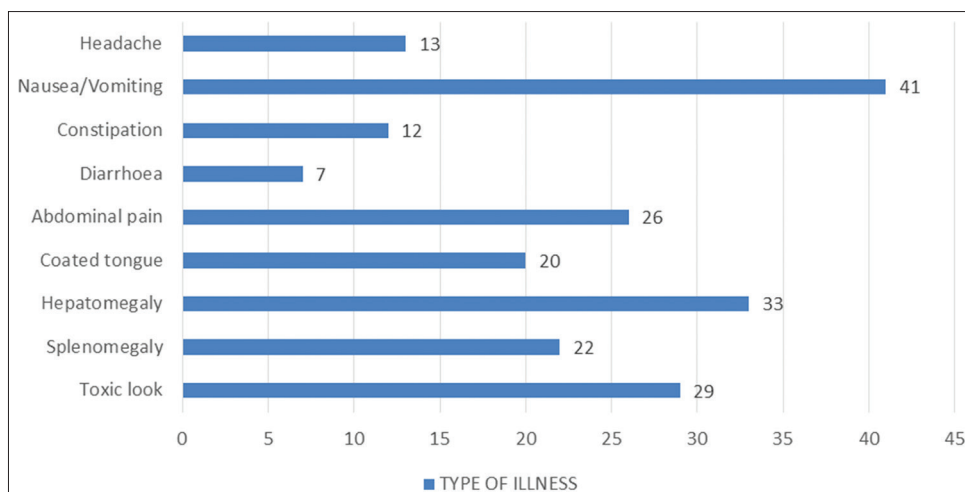


Figure 3: Clinical presentation

Table 3a: Comparison of Typhidot M - (IgM ELISA) with the gold standard investigation - blood culture

Typhidot M (IgM ELISA)	Blood culture positive	Blood culture negative
Positive	11	47
Negative	0	32

Table 3b

Sensitivity	90.91%
Specificity	82.28%
Positive predictive value	41.66%
Negative predictive value	98.48%

Table 4a: Comparison of Widal with the gold standard investigation - blood culture

Widal test	Blood culture positive	Blood culture negative
Positive	10	47
Negative	1	65

Table 4b

Sensitivity	90.91%
Specificity	82.28%
Positive predictive value	41.66%
Negative predictive value	98.48%

Blood Culture Growth

In this study, it was found that the yield of blood culture was very low, probably due to the high percentage of test subjects who had been administered antibiotics before admission here. The yield was only 12.22% which was much lower than that observed in other similar studies. Narayanappa *et al.* obtained a blood culture yield of 39.05%, while the yield was up to 68% for Sherwal *et al.*

Blood Culture Positivity

Comparison of the yield of previous studies with the present study

Study	Yield (%)
Narayanappa <i>et al.</i> ^[2]	39
Sherwal <i>et al.</i> ^[3]	68
Krishna <i>et al.</i> ^[4]	26.88
Khan <i>et al.</i> ^[5]	53.90
Begum <i>et al.</i> ^[6]	14
Anila <i>et al.</i> ^[7]	55.55
Present study	12.22

The low yield of blood cultures seen in almost all the studies reflects the poor sensitivity of this investigation. However, the test is highly specific and is believed to be the gold standard in diagnosis of typhoid. Lately, bone marrow cultures have replaced blood cultures as the gold

Table 5: Relation between duration of fever and investigation

Illness days of fever	Blood culture	Typhidot	Widal
≤7 days (46)	7	29	7
≥7 days (44)	4	29	17

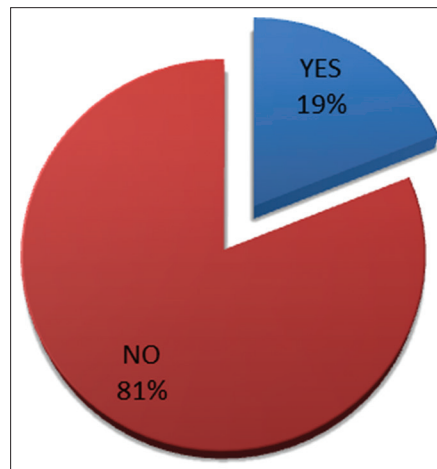


Figure 4: Antibiotic administration before admission

standard investigation for enteric fever. Blood cultures are highly susceptible to prior use of antibiotics with the culture turning negative after even a single dose of antibiotics. In our present study, almost four-fifths of the study population had been exposed to prior antibiotics. This could be the cause behind the dismal yield of blood cultures.

Another factor in the yield of cultures is the time of presentation in relation to the clinical course of the disease, i.e. duration of fever at the time of presentation. Cultures give maximum results when taken before 7 days of fever. In our study, nearly half of the study population presented with more than 7 days of fever. This may also have contributed to the low yields.

The Utility of Typhidot M - ELISA in the Diagnosis of Typhoid Fever

In this study, as in all other studies, the percentage of total study subjects in whom enteric fever was detected through Typhidot M - ELISA^[8,9] was high. Among clinically suspected cases of enteric fever, Typhidot M ELISA picked up much more number of cases as compared to both Widal and blood culture.

Comparison of Typhidot M ELISA Positivity in this Study with Other Studies

Study	Typhidot M positivity (%)
Narayanappa <i>et al.</i>	74.3
Sherwal <i>et al.</i>	79.0
Khan <i>et al.</i>	71
Begum <i>et al.</i>	73
Present study	64.5

Typhidot M ELISA detects antibodies against the 50 kDa outer membrane protein of *Salmonella typhi*. These antibodies appear early in the course of the disease. Other antibodies, especially antibodies to the O and H antigens, do not appear till late in the course of the disease. Moreover, for meaningful interpretation of Widal, rising titers need to be demonstrated while it is not so in the case of Typhidot M ELISA. Thus, Typhidot M ELISA is a simple test that gives a high yield in the detection of enteric fever cases.

Widal Test and its Utility

In our study, Widal was positive in only 26.67% of cases. This is very low when compared to other studies. Narayanappa *et al.* obtained yield of 45.7% with Widal while Sherwal *et al.* demonstrated 57.0% positivity with Widal.

Comparison of Widal Positivity in Our Study with Other Studies

Study	Widal positivity (%)
Narayanappa <i>et al.</i>	45.7
Sherwal <i>et al.</i>	57
Present study	26.67

The causes for the poor results with Widal are uncertain. However, even in other studies,^[10] the yield is not high enough for its routine use as a screening or confirmatory diagnostic test.

Typhidot Versus Blood Culture

Keeping blood culture as the gold standard, the sensitivity, specificity, and positive and negative predictive values were calculated in several studies.^[11] Our study also revealed a very high sensitivity and negative predictive value for Typhidot M ELISA test. However, the specificity and positive predictive values were quite low when compared to other studies. This may be because of the low rate of culture positivity, against which the comparisons were made.

Comparison of the Sensitivity, Specificity, and Positive and Negative Predictive Values of Typhidot M ELISA in Different Studies

Study	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Narayanappa <i>et al.</i>	92.6	37.5	-	-
Sherwal <i>et al.</i>	92	87.5	-	-
Krishna <i>et al.</i>	100	95.5	89.2	100
Khan <i>et al.</i>	71	43	59.7	56.5
Begum <i>et al.</i>	92.85	90	76.47	97.29
Anila <i>et al.</i>	100	63	-	-
Present study	100	40.5	18.96	100

PPV: Positive predictive value, NPV: Negative predictive value

All these studies showed a high sensitivity of Typhidot test, thus justifying its routine use in clinical practice as a sensitive and rapid tool in the diagnosis of typhoid fever. The studies also showed that this test had a high negative predictive value, thus preventing overdiagnosis and

overenthusiastic treatment that contributes to the burden of antibiotic resistance.

Comparison of the Sensitivity and Specificity of Widal Test in Various Studies

Study	Sensitivity (%)	Specificity (%)
Narayanappa <i>et al.</i>	34.1	42.8
Sherwal <i>et al.</i>	74	83
Anila <i>et al.</i>	82	65
Present study	90.91	82.28

Early Diagnosis of Typhoid Fever

In our study, emphasis was given to the early detection of typhoid fever cases so as to

Study	Sensitivity (%)	Specificity (%)
Narayanappa <i>et al.</i>	34.1	42.8
Sherwal <i>et al.</i>	74	83
Anila <i>et al.</i>	82	65
Present study	90.91	82.28

prevent complications and decrease the morbidity and hospital stay. In this regard, the investigations were compared as to which test gives maximum yield in the initial period of the disease, i.e. within the first 7 days of fever onset. It was found that Typhidot M ELISA detects 63.04% of cases while Widal is able to detect typhoid in only 15.21% of early fevers. Narayanappa *et al.*, 20 obtained 97% positivity in the early cases of typhoid fever with Typhidot M ELISA as compared to only 24.2% with Widal.

The poor yield of Widal in the initial days of fever is expected because of low titers of O and H antibodies in the 1st week of illness. As the sensitivity of blood cultures is also low, Typhidot M is the only and the ideal test that can detect typhoid in the 1st week of illness itself.

CONCLUSION

1. Typhidot-M (IgM ELISA) has immense utility in the early diagnosis of typhoid fever in children.
2. When compared to blood culture and Widal, this test gives a high yield and is able to detect more number of cases early in the course of the disease.
3. It has high sensitivity and negative predictive value, keeping blood culture as the gold standard. However, the specificity and the positive predictive values are not very high.
4. Typhidot M (IgM ELISA) is a simple, sensitive, and reliable test for the early diagnosis of typhoid fever in children.

Limitations of the Study

1. The yield of blood culture was very low which resulted in the low specificity of the IgM ELISA test.

2. Paratyphoid infection cannot be detected by this kit.
3. Cold storage is required for the test agents.

Recommendations

Further studies with large number of children, including infants, need to be conducted to determine the utility of the test and to make it a routine in clinical practice so as to avoid the overdiagnosis of enteric fever and prevent antibiotic misuse.

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