

Comparative Study between IgM ELISA and Widal Test for Diagnosis of Enteric Fever at a Tertiary Care Centre

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Abstract: Enteric fever is major public health problem in developing countries like India. Early and accurate diagnosis is necessary for prompt treatment. We have compared diagnostic accuracy of IgM ELISA test and Tube Widal test for early and rapid diagnosis of Enteric fever considering blood culture as gold standard test. A total 100 clinically suspected enteric fever cases were included. Blood culture, Tube Widal and IgM ELISA tests were performed. In 33 patient's alternative diagnosis was made after 48- 72 hours which was considered as control group. Among 67 clinically diagnosed enteric fever patients, only 8.9% were blood culture positive for *Salmonella* species., 58.21% tube widal positive and IgM ELISA 26.87% patients. Positivity percentage of IgM ELISA was 47% in first week of illness, 18% in second, it was significantly decreased ($p < 0.05$) to 9% in the third week whereas positivity percentage of Tube Widal test was (39.13%) in first week which was increased to 78% in second week & was 36.36% in third week. Sensitivity, specificity, Positive predictive value, and Negative predictive value of IgM ELISA 83.33%, 78.69%, 90.00%, 38.75% as compared to Tube Widal test 50.00%, 40.98%, 7.69%, 89.29% respectively considering blood culture as gold standard. Conclusion: IgM ELISA is sensitive, specific rapid and easy to perform test for early diagnosis of enteric fever.

Keywords: Widal test, IgM ELISA test, Enteric fever, Blood culture, *S.typhi*, *S.paratyphi*

1. Introduction

Enteric fever caused by *Salmonella enterica* serotype typhi and paratyphi A, B is a major public health problem, both in developing as well as developed economies.^[1] About 21 million cases and 128 000 to 161 000 typhoid-related deaths occur annually by *Salmonella typhi*.^[2] An estimate of annual typhoid incidence rate of 493.5 cases per 100, 000 person-years has been reported from India.^[3,4]

Overcrowded populations and poor hygienic conditions predispose developing countries to being endemic for typhoid fever, with the highest incidence found in the Indian subcontinent and Southeast Asia.^[5]

The major cause of enteric fever are *Salmonella enterica* serovar *Typhi* and also, to a lesser extent, strains of *S.enterica* belonging to serovars Paratyphi A, B, C. Enteric fever is mostly seen in low or middle income countries with inadequate sanitation and hygiene, particularly regarding food, water and disposal of human excreta. In such places there are also other causes of febrile illnesses, such as vector borne malaria, dengue fever, and rickettsiosis as well as environmentally transmitted leptospirosis and melioidosis.^[6-10]

Enteric fever is diagnosed clinically and treated presumptively which leads to delay in diagnosis and emergence of drug resistance.^[11] Therefore, reliable laboratory tests are essential to establish etiologic diagnosis so that appropriate treatment can be given.^[12]

Blood culture remains the gold standard test for diagnosis of enteric fever till today, but has poor sensitivity 40-60%,

requires time, trained labour, infrastructure, equipment and indiscriminate use of antibiotics leads to poor results.^[13]

The sensitivity of stool and urine cultures is low and become positive only after first week of infection. Bone marrow cultures are more sensitive but its utility for diagnosis is limited in public health settings.

Widal test is most widely used serological test as it is readily available, inexpensive and has been used in clinical settings for years. But lacks sensitivity and specificity and reliance on it alone in areas where enteric fever is endemic, in different populations and in the presence of other febrile illnesses leads to errors in diagnosis.^[14-16]

Hence we need a test which is fast, reliable and easy to perform serodiagnostic test with higher specificity, sensitivity than Widal test. Rapid diagnosis of Enteric fever is required to reduce morbidity and its complications.

The early rising antibodies to the lipopolysaccharide (LPS) O are predominantly IgM in nature. The *S. typhi* specific IgM antibodies can be used as an early marker to detect a recent infection.^[17]

Currently, another serological test that detects IgM antibody (Enzyme linked immunosorbent assay) is commercially available for the diagnosis of typhoid fever. This has been reported as a fast, reliable, and easy to perform sero diagnostic test with higher sensitivity and specificity than Widal.

Few studies conducted in South India and other parts of Asia have shown encouraging results in terms of

diagnostic tool and have found it to be of practical alternative to Widal test in the diagnosis of typhoid fever [18-22]

The present study was undertaken to compare results of Tube Widal test and IgM ELISA along with blood culture and to evaluate efficacy of IgM ELISA in diagnosis of Enteric fever.

2. Material and Methods

The present study was carried in the department of Microbiology, at a tertiary care teaching hospital BJGMC & SGH Pune over a period of 24 months. Study included patients with complain of continuous fever for 3 days or more of duration. Total 100 blood samples were collected from clinically suspected Enteric fever patients attending Medicine and paediatric opd at our hospital. Blood culture, Tube Widal test and IgM ELISA was performed.

Blood culture was done by conventional method using trypticase soy broth and identification of organisms was done by gram staining, standard biochemical tests and confirmed by agglutination with the Salmonella polyvalent 'O', 09 and the H:d antisera, antimicrobial susceptibility tests were also performed according to CLSI (M100S-26) guidelines.

The Tube Widal test was done as per manufacturer kit insert (STAR Diagnostics) and agglutination was considered positive if with titre equal or more than S.typhi O antigen 1: 80, S.typhi H antigen 1: 80, was observed.

IgM ELISA test (AB Diagnostics Ltd) was performed according to manufacturer kit insert.

Procedure for IgM ELISA:

- Treat the patient serum with RF absorbent with mixture of 2 µl patient serum+ 21µl of RF absorbent in small tube.
- Add 177µl of sample diluent to each serum mixture, dilution is 1:100 for positive and negative control and Incubate for 15 minutes.
- Remove all liquids from well, wash 3 times with 300µl of 1X washing buffer. Blot on absorbed paper.

- Dispense 100µl of enzyme conjugate of anti-human IgM hrp conjugated to each well and incubate for 30 minutes.
- Remove conjugate by washing 3 times with 300µl of 1X wash buffer. Blot on absorbent paper.
- Dispense 100µl of TMB substrate and incubate for 10 minutes and add 100µl stop solution
- Results is read by OD at 450 nm using ELISA reader within 10 minutes

Statistical analysis: To compare diagnostic efficacy of IgM ELISA and Widal test, various parameters like Sensitivity, Specificity, Positive predictive value (PPV), Negative predictive value (NPV) were calculated. Chi Square test was used to assess the difference between tests. For all statistical tests, a *P* value less than 0.05 was taken to indicate a significant difference.

Ethics clearance: Ethical clearance was obtained from institution ethical committee ref no D-1214135-135 dated 16 December 2014. Awritten consent was obtained from all patients prior to sample collection.

3. Results

A total of 100 clinically suspected enteric fever cases, maximum were in age group of 11-20 (34%), followed by followed by 21-30 years (20%). Male to female ratio is 51:49 (table1).

Total 100 clinically suspected enteric fever cases; alternative diagnosis was made after 48-72 hours in 33 cases were febrile illness due to other causes that constituted the control group (table2). Among 67 clinically diagnosed enteric fever patients, only 8.9% were blood culture positive for *Salmonella species.*, 58.21% were tube widal positive and IgM ELISA was positive in 26.87% patients (table3, 4). Positivity percentage of IgM ELISA was 47% in first week of illness, 18% in second, it was significantly decreased ($p < 0.05$) to 9% in the third week whereas positivity percentage of Tube Widal test was (39.13%) in first week which was increased to 78% in second week & was 36.36% in third week (table 5). Over all sensitivity specificity, positive predictive value, negative predictive value of IgM ELISA was observed 83.33%, 78.69%, 90.00%, 38.75% as compared to Tube Widal test 50.00%, 40.98%, 7.69%, 89.29% respectively considering blood culture as gold standard. (table 6).

Table 1: Age and sex wise distribution of clinically suspected enteric fever cases (n=100)

Age group	Males (Number)	Females (Number)	Total (Number, %)
1-10	4	3	7 (7%)
11-20	17	17	34 (34%)
21-30	9	11	20 (20%)
31-40	6	7	13 (13%)
41-50	11	5	16 (16%)
51-60	2	1	3 (3%)
61 and above	2	5	7 (7%)
Total	51	49	100

Table 2: Distribution of causative agents of febrile illness due to other causes or control group n=33

Alternate Diagnosis	Number of cases
Primary septicemia	9
Malaria	6
UTI	6
Dengue	5
URTI	3
Meningitis	2
Rickettsial Infections	2
Total	33

Table 3: Comparison of Tube Widal test with blood culture in clinically diagnosed enteric fever cases (n=67)

	Blood culture positive for <i>salmonella</i> species (n=6)	Blood culture negative for <i>salmonella</i> species (n=61)
Tube Widal positive (n=39)	3	36
Tube Widal negative (n=28)	3	25

Table 4: Comparison of IgM ELISA with blood culture (n=67)

	Blood culture positive for salmonella species (n=6)	Blood culture negative for salmonella species (n=61)
IgM positive	5	13
IgM negative	1	48

Table 5: Comparative of blood culture, Tube Widal and IgM ELISA according to duration of illness in clinically diagnosed enteric fever cases (n=67)

Duration of illness	Total No. of samples	Blood culture Positive for <i>salmonella</i> species (n=6)	Tube Widal positive (n= 39)	IgM ELISA positive (n= 18)
1 st week	23	3 (13.04%)	9 (39.07%)	11 (47%)
2 nd week	33	3 (9.09%)	26 (78.78%)	6 (18%)
3 rd week	11	0	4 (36.36%)	1 (9%)
Total	67	6	39	18

Table 6: Comparative evaluation of Tube Widal and IgM ELISA tests in clinically diagnosed enteric fever cases (n=67) [Blood culture as gold standard]

Tests	Sensitivity	Specificity	Positive Predictive Value (PPV)	Negative predictive value (NPV)
Tube Widal test	50.00%	40.98%	7.69%	89.29%
IgM ELISA	83.33%	78.69%	90.00%	38.75%

4. Discussion

Early and rapid detection of enteric fever is essential to reduce morbidity and mortality caused due to enteric fever.

The present study was carried out to compare Tube Widal test and IgM ELISA test and to evaluate the efficacy of IgM ELISA test as rapid diagnostic tool. A total of 100 clinically suspected enteric fever patients were included in the study.

Out of these 100 clinically suspected enteric fever patients, predominant age group was 11-20 (34%) followed by 21-30 (20%) similar to N. Makwana et al^[23] (2014) observed numbers of the patients were of 11-20 (34%) years group. Out of 100 clinically suspected patients, 51% were males and 49% were females with male to female ratio of 51:49 consistent with A. Akhtar et al^[24] (2015) reported (52.9%) of males and 49 (47.1%) were females in their study. Out of these 100 patients, in 33 (33%) patients alternative diagnosis was made after 48-72 hours. These patients were considered as febrile cases due to other causes and considered as control group. These consisted of 9 cases of primary septicaemia, 6 cases with malaria and 6 with

urinary tract infections, 5 cases with dengue, 3 with upper respiratory tract infections, 2 with meningitis and 2 with rickettsia infections Jindal N et al (2014)^[20] made similar observations a total of 100 cases presented to hospital with suspected typhoid fever but in 38 cases (38%), alternative diagnosis was made.

In our study we have also compared Tube Widal test with blood culture in clinically diagnosed enteric fever cases. Isolation rate for *Salmonella* species out of total samples (n=67) was 8.9%. A. Adhikari et al^[13] reported isolation rate for *salmonella species* 8.9%, Chawla V et al^[25] (1970) reported that isolation rate of *Salmonellae* varies from 8.6%-14% in different parts of India.

We observed tube widal positivity in relation with blood culture, Out of 6 blood culture positive for *Salmonella* species, 3 were widal positive and 3 were widal negative. The 3 blood culture positive which came widal negative were the patients who presented with symptoms in their first week of illness as compared to the 3 which were blood culture and widal positive were from second week of illness. In blood culture negative (n=61), 36 were widal positive this could be due to late presentation of patients in our tertiary care centre. Sensitivity, specificity of tube

Widal test in comparison with blood culture is 50.00%, 40.98%.

In our study among 6 blood culture positive cases for *Salmonella* species, 5 were IgM ELISA was positive and 1 was IgM ELISA negative which was *S. paratyphi A*. Sensitivity, specificity, Positive predictive value, and Negative predictive value for IgM ELISA was 83.33%, 78.69%, 27.78%, 97.76% with blood culture as gold standard. In our study *S. paratyphi A* was IgM ELISA negative false negative results may be failure of the test to detect antibodies or antibodies did not reach the detectable level. Begum.Z et al.^[26] (2009) reported sensitivity, specificity, positive predictive value, negative predictive value as follows 92.85%, 90.00%, 76.47%, 97.29%. Similar findings were also reported in study from N.Makwana et al.^[23] (2014) 95.4%, 80.7%, 58.3% and 98.4%. N.Jindal et al.^[20] (2014) study from Punjab reported sensitivity and specificity of IgM ELISA sensitivity 83.8%, 92.11%.

In this study, we compared blood culture, Tube widal and IgM ELISA results in respect with duration of illness of enteric fever, we observed. Out of the total number of patients who presented with first week of illness (n=23) IgM ELISA have shown maximum positivity percentage 47%, followed by tube widal test 39 % and blood culture was positive in 13.04% patients. Total number of patients who presented with second week of illness (n=33), in these patients tube widal test have shown maximum positivity percentage 78.78%, followed by IgM ELISA with positivity percentage of 18% and blood culture positivity percentage was 9.09% respectively. Out of total number of patients who presented in third week of illness, Tube widal test positivity percentage was 36.36%, IgM ELISA positivity percentage 9%, while none of blood cultures were positive in patients who presented with third week of illness. IgM ELISA have shown maximum positivity percentage in first week of illness In our evaluation, the positivity percentage of IgM ELISA was high in the first week of illness onset, this is because the early rising antibodies to the lipopolysaccharide (LPS) O are predominantly IgM in nature.^[17] The *S. typhi* specific IgM antibodies occur earlier in the course of the illness. Tube widal test was sensitive in second and third week patients are presented late in the course of their illness and fever, so they have sustained peak antibody titre at the time of admission this could be explanation for maximum widal positivity in second and third week of illness similar findings were reported by Ali Musa et al.^[27] (2011) maximum widal positivity in second and third week of illness.

In our study we compared Tube Widal test and IgM ELISA with blood culture as gold standard we observed IgM ELISA has greater sensitivity 83.33%, and 78.69% specificity, positive predictive value (PPV) 90.00% and negative predictive value (NPV) 89.29%. In comparison with tube widal test sensitivity 50.00%, specificity 40.98%, PPV 7.69%, NPV 89.29%.

In our study we observed IgM ELISA was more sensitive and specific than Tube Widal. This is consistent with other

studies such as Fadeel et al.^[28] (2011) Tube widal sensitivity specificity 78%, 75% and IgM ELISA sensitivity, specificity, 79%, 78%, Sanjeev^[29] et al (2013), 78.78%, 58.82%, 100%, 76%.

5. Conclusion

Current guidelines of Integrated disease surveillance programme (IDSP) recommends to use blood culture for diagnosing and reporting enteric fever, however our study suggest that blood culture should be supplemented with IgM ELISA in first week of illness, As in our current study results indicate that additional 13 patients of enteric fever cases were confirmed by IgM ELISA which were not picked up by blood culture.

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