Research Paper

Volume : 3 | Issue : 7 | July 2014 • ISSN No 2277 - 8179

Diagnostic Utility of Typhoid Elisa Test In Comparison to Other Conventional Methods for Diagnosis of Typhoid Fever

Medical Science

KEYWORDS : Widal test, Typhoid IgM ELISA

Dr. Neha Makwana	MD Microbiology, Department of Microbiology, PDU Medical College, Rajkot.
Dr. Pratik R. Shah	Assistant Professor, Department of Surgery, PDU Medical College, Rajkot.
Dr. Madhulika Mistry	Assistant Professor, Department of Microbiology, PDU Medical College, Rajkot.
Dr. Yogesh Goswami	Professor & Head, Department of Microbiology, PDU Medical College, Rajkot

ABSTRACT

Typhoid fever is endemic in many parts of the world and continues to be one of the leading causes of morbidity and mortality in our country. We have studied typhoid IgM ELISA test, a rapid serological test for diagnosis of typhoid fever and its usefulness for an early diagnosis, its sensitivity and specificity as compared to widal test. Out of 100 samples 22 (22%) cases were positive by blood culture, 30 (30%) were positive with Widal test and 36 (36%) were positive with typhoid ELISA test. The sensitivity, specificity, positive and negative predictive value of Widal test was 63.6%, 79.4%, 46.6% and 88.5% respectively. The sensitivity, specificity, positive and negative predictive value of typhoid IgM ELISA test was 95.4%, 80.7%, 58.3% and 98.4% respectively proving the superiority of typhoid IgM ELISA test.

Introduction:

Typhoid fever is a life threatening systemic infection occurring in lesser-developed areas of the world and continues to be a major public health problem[1]. Recent surveillance studies have indicated that infection by S. Typhi causes 21 million illnesses and 200,000 deaths annually[1]. There are at least 17 million new cases of typhoid globally. India is the second most populous country of the world with majority inhabiting the rural areas with little access to modern diagnostic tools. Blood culture and widal test are routinely employed investigations for diagnosis of typhoid fever in all clinical settings[2]. Widal test has been used in the diagnosis of typhoid illness for long time but it remains a serological test with a moderate sensitivity and specificity[3]. Therefore, a fast, reliable, and easy to perform test with a higher sensitivity and specificity than Widal test is required, for rapid diagnosis and management of typhoid cases, thereby enabling clinicians to initiate an early therapy, reducing morbidity and its complications. Typhoid IgM ELISA is a rapid serological test for the diagnosis of typhoid fever[4]. In view of this, we observed that typhoid ELISA is a superior diagnostic tool assuming blood culture as a gold standard for enteric fever, as compare to conventional Widal test which has low sensitivity and specificity[5].

Material and methods:

The study was conducted over a period of 2 year (Sep 2011 to Aug 2013) in the Department of Microbiology, Govt. Medical College, Rajkot. Serum samples from typhoid patients were collected before treatment and processed within a few hours of collection. Typhoid ELISA is an enzyme immune assay, which detects IgM antibodies to a specific 50 kD outer membrane protein (OMP) antigen of Salmonella typhi.[6] Typhoid ELISA becomes positive as early as in the first week of fever. The results can be visually interpreted and is available within hour.[7] Bring all specimens and kit reagents to room temperature (18-26°C) and gently mix. Place the desired number of coated strips into the holder. Negative control, positive control, and calibrator are ready to use. Prepare 1:101 dilution of the test sample by adding 5µl of the sample to 0.5 ml of sample dilution. Mix well. Dispense 100µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense100µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature. Remove liquid from all wells. Wash well three times with 300µl of 1X wash buffer. Blot on absorbance paper. Dispense 100µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature. Remove enzyme conjugate from all wells. Wash well three times with 300µl of 1X wash buffer. Blot on absorbance paper. Dispense 100µl of TMB substrate and incubate for 10 minutes at room temperature. Add 100µl of stop solution. Read O.D. at 450 nm using ELISA reader within 15 minutes.

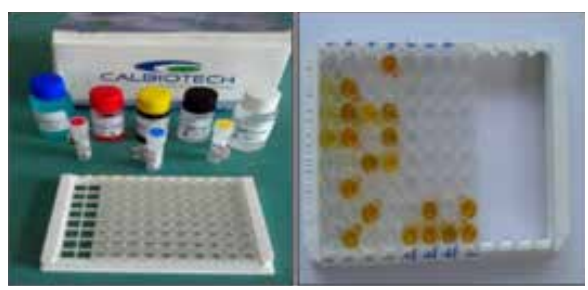

Figure 1: Kit and test results of Typhoid ELISA

Observation and Results:

Out of 100 isolates 61 were from male while 39 from female. The youngest patient was 6 years old and oldest was 61 years. The mean age was 23 years [table 1]. In this study 75% of the patients were from lower socio-economic group followed by middle socio-economic class (25%). Out of 100 samples 22 (22%) cases were positive by blood culture, 30 (30%) were positive with Widal test and 36 (36%) were positive with typhoid ELISA test (some subjects were represented in more than one test) [table 2]. 75 cases were during the month of July to October which coincides with the rainy season [table 3]. The mean duration of fever was 14.24 days [table 4]. All three tests were positive in 14 (14%) cases. Blood culture and S. typhi IgM ELISA positive but Widal test negative was in 7 (7%) cases. Only Blood culture positive, Widal and S. typhi IgM ELISA test negative was in 1 (1%) case. Blood culture negative, Widal and S. typhi IgM ELISA positive were in 13 (13%) cases. Blood culture and S. typhi IgM ELISA were negative while Widal positive was in 3 (3%) patients. Blood culture and Widal negative while S. typhi IgM ELISA was positive in 2 (2%) cases. All three tests were negative in 60 (60%) cases.

Age group (years)	Number of patients	Percentage (%)
0 to 10	14	14
11 to 20	33	33
21 to 30	25	25
31 to 40	15	15
> 40	13	13
Total	100	100

Table 1: Age distribution of selected cases

Results	Blood Culture	Widal	S. typhi IgM ELISA
Positive	22	30	36
Negative	78	70	64
Total	100	100	100

Table 2: Results of blood culture, Widal and S. typhi IgM ELISA of patients

Month	Number of suspected cases
Sept/Oct-11	18
Nov/Dec-11	05
Jan/Feb-12	02
Mar/Apr-12	01
May/June-12	03
July/Aug-12	17
Sept/Oct-12	19
Nov/Dec-12	04
Jan/Feb-13	02
Mar/Apr-13	02
May/June-13	06
July/Aug-13	21
Total	100

Table 3: Month-wise distribution

Fever (days)	No. of patients	Blood Culture	Widal	S. typhi IgM ELISA
0-5	5	1	0	0
6-10	30	18	13	15
11-15	28	2	12	13
16-20	10	1	5	8
21-25	17	0	0	0
>25	10	0	0	0
Total	100	22	30	36

Table 4: Test result pattern in different fever duration

Positive		Blood Culture		Total
		Negative		
Widal Test	Positive	14	16	30
	Negative	08	62	70
Total		22	78	100

Table 5: Comparison of Widal test with blood culture

Positive		Blood Culture		Total
		Negative		
S. typhi IgM ELISA	Positive	21	15	36
	Negative	01	63	64
Total		22	78	100

Table 6: Comparison of S. typhi IgM ELISA with Blood Culture

Discussion

Early diagnosis of typhoid fever is essential for the patient as well as community. The present study was undertaken to evaluate various laboratory diagnostic tool for diagnosis of typhoid fever. In this study, we found that males were more prone to typhoid than females with male: female ratio of 1.56:1. Maximum numbers of the patients were of 11-20 (33%) years group. Majority of the patients belonged to lower socio-economic class. Month wise distribution of cases showed an increase incidence in the month of July to October (rainy season). Mean duration of fever was 14.24 days with more number of clinically suspected cases (58%) in 6-15 days.

Parameter	Widal	S. typhi IgM ELISA
Sensitivity	63.6	95.4
Specificity	79.4	80.7
Predictive value of a positive test	46.6	58.3
Predictive value of a negative test	88.5	98.4
Likelihood ratio for positive test	3.10	4.96
Likelihood ratio for negative test	0.46	0.06
Accuracy	0.76	0.84

Table 7: Comparison between Widal and Typhoid ELISA

The sensitivity, specificity, positive and negative predictive value of Widal test was 63.6%, 79.4%, 46.6% and 88.5% respectively [table 5]. The sensitivity, specificity, positive and negative predictive value of typhoid IgM ELISA test was 95.4%, 80.7%, 58.3% and 98.4% respectively [table 6].Blood culture and Widal test are used as conventional methods in the diagnosis of typhoid fever. The new serological S. typhi IgM ELISA test has been proved more valuable for the diagnosis of typhoid fever. Blood culture is the gold standard for diagnosis of typhoid fever with high specificity and sensitivity which is higher in first week of illness, decline with the increase in the duration of illness. The disadvantages are it is time consuming and affected by prior antibiotic treatment. Widal test is inexpensive, simple, readily available and can be done on OPD basis. It detects antibodies against somatic and flagellar antigens of *S. typhi* has disadvantages like sharing of antigenic determinants with other salmonellae, effect of prior antibiotic treatment, previous immunization with TAB vaccine which affects the interpretation of results and it is unreliable with the single titres as a diagnostic tool. Majority of cases are positive in 2nd week of the disease. S.typhi IgM ELISA test which has been designed to detect the IgM antibodies against the outer membrane protein of *S. typhi* offers the advantage of speed, simplicity, and early diagnosis but only limitation is that no isolates are available for antimicrobial susceptibility testing.

In the present study, we have compared the sensitivity and specificity of Widal test and S. typhi IgM ELISA test in the diagnosis of Typhoid fever. Widal test showed the sensitivity and specificity of 63.6 % and 79.4%, respectively, whereas S. typhi IgM ELISA test shows the sensitivity and specificity of 95.4% and 80.4%, respectively. Hence, the sensitivity of S. typhi IgM ELISA test is more compared to Widal test and specificity of both the tests remains same.

Conclusion:

We conclude that the S. typhi IgM ELISA test appears to be a practical alternative to Widal test in the diagnosis of typhoid fever even in the resource poor laboratories as it neither requires much laboratory equipments nor laboratory expertise to conduct the test, but whenever feasible confirmation with blood culture is strongly encouraged especially with the appearance of drug resistance strains in the community.

REFERENCE

1. Park K, Park's textbook of preventive and social medicine, 22th ed. Jabalpur: M/S Banarsidas Bhanot 2013; 213-16. | 2. Koneman EW, Alens D: Color atlas and textbook of diagnostic microbiology; 6th ed. Philadelphia: Lippincott; 2006; 251-5. | 3. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's Diagnostic microbiology, 12thed. Mosby: Elsevier; 2007. | 4. Topley and Wilson's Microbiology and microbial infections; 9th ed. Systematic Bacteriology. Vol. 2, London: Arnold; 969-95. | 5. Mackie and McCartney – Practical Medical Microbiology; 14th ed. Edinburgh: Churchill Livingstone; 2011; 385-404 | 6. Brooks GF, Carroll KC, Butel JS, Morse SA. Medical microbiology; 24th ed. McGraw-Hill Lange; 2007 | 7. Jesudason MV, Sivakumar S. Prospective evaluation of a rapid diagnostic test Typhidot® for typhoid fever. Ind J Med Res 2006; 123:513 | 8. Kawano RL, Leano SA, Agdamag DMA. Comparison of serological test kits for diagnosis of typhoid fever in the Philippines. J Clin Microbiol 2007; 45:246-7. | 9. Pastoor R, Hatta M, Abdoel TH, Smits HL. Simple, rapid and affordable point- of-care test for the serodiagnosis of typhoid fever. Diagn Microbiol Infect Dis 2008; 61:129-34. | 10. Atif Sitwat Hayat, Naila Shaikh et al.A study of evaluation of typhidot (IgM) in early and rapid diagnosis of typhoid fever; Professional Med J Apr-Jun 2011;18(2): 259-264 | 11. Moustafa A. Fadeel, Brent L. House et al. A study of evaluation of a newly developed ELISA against Widal, TUBEX-TF and Typhidot for typhoid fever surveillance; J Infect Dev Ctries 2011; 5(3): 169-175. | 12. Balakrishna T. P. Et al. a comparative study of Typhidot and Widal test in the diagnosis of typhoid fever; RGUHS: 2009; 92-103. |