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RESEARCH ARTICLE

A COMPARATIVE STUDY OF WIDAL, DOT-EIA WITH BLOOD CULTURE IN DIAGNOSIS OF ENTERIC FEVER AT PEDIATRIC AGE GROUP

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ABSTRACT

Aim To compare the Widal and dot-EIA with the blood culture in the diagnosis of Typhoid Fever. **Methodology:** Clinically suspected typhoid fever of aged 1 to 12 year are included. Case details & appropriate investigations are done & results are analysed. **Results** total of 140 children were screened for enteric fever, out of which 30 cases positive for Widal, 39 Dot-EIA & 20 for blood cultures. **Conclusion** dot-EIA have a higher sensitivity compared to Widal test. Dot-EIA could be a practical alternative to Widal test even in the resource constrained laboratories.

INTRODUCTION

Enteric fever (more commonly termed typhoid fever) is a global health problem. Its real impact is difficult to estimate because the clinical picture is confused with those of many other febrile infections. Additionally, the disease is underestimated because there are no bacteriology laboratories in most areas of developing countries[1]. Typhoid fever is caused by S. enterica serovar Typhi (S.Typhi), a Gram negative bacterium. A very similar but often less-severe disease is caused by Salmonella Paratyphi A and rarely by S. Paratyphi B (Schotmulleri) and S. Paratyphi C (Hirschfeldii) [3]. A case or carrier is infectious as long as bacilli appears in stool or urine.

After ingestion of contaminated food or water, typhoid organisms pass through the pylorus and reach the small intestine. They rapidly penetrate the mucosal epithelium via either microfold cells or enterocytes and arrive in the lamina propria, where they rapidly elicit an influx of macrophages that ingest the bacilli but do not generally kill them. Some bacilli remain within macrophages of the small intestinal lymphoid tissue. Other typhoid bacilli are drained into mesenteric lymph nodes where there is further multiplication and ingestion by macrophages. It is believed that typhoid bacilli reach the bloodstream principally by lymph drainage from mesenteric nodes, after which they enter the thoracic duct and then the general circulation. As a result of this silent primary bacteraemia the pathogen reaches an intracellular haven within 24 hours after ingestion throughout the organs of the reticuloendothelial system (spleen, liver, bone marrow, etc.), where it resides during the incubation period, usually of 8 to 14 days. The incubation period in a particular individual depends on the quantity of inoculum. Incubation periods ranging from 3 days to more than 60 days have been reported[1].

Blood cultures

The gold standard diagnostic method for diagnosis of enteric fever. The sensitivity of blood culture is highest in the first week of the illness and reduces with advancing illness. Failure to isolate the organism may be caused by several factors which includes inadequate laboratory media, the volume of blood taken for culture, the presence of antibiotics and the time of collection. Sufficient amount of blood should be collected for culture as the median bacterial count in the peripheral blood is only 0.3 CFU/mL[4]. At least 5ml of blood in children should be collected

Blood culture is not always available and, when it is, it is time consuming. As a result, diagnosis may be delayed or overlooked and patients without typhoid fever may receive

unnecessary and inappropriate antimicrobial treatment. For this reason, in developing countries typhoid rapid antibody tests can facilitate diagnosis and disease management[7].

Widal test

Widal measures agglutinating antibodies against lipopolysaccharide (LPS; O) and flagella (H) antigens of the S. Typhi in the serum of individuals with suspected enteric fever. The Widal test ideally requires both an acute and a convalescent-phase serum sample taken approximately 10 days apart, and a positive result is determined by a fourfold increase in antibody titer. For practical purpose and for optimal result this test should be done after 5 to 7 days of fever by tube method and level of both H an O antibodies of 1 in 160 dilution (fourfold rise) should be taken as cut-off value for diagnosis[4]. Disadvantage: A single sample test may plagued by false-negative and false-positive results. A large number of cross-reacting antigenic determinants of typhoidal and nontyphoidal Salmonella organisms and other Entero bacteriacae are now recognized, as are several other diseases caused by non- Salmonella organisms such as malaria, dengue and TB[2]. Poor specificity, is a consequence of pre-existing base line antibodies in endemic areas, cross reactivity with other Gram negative bacteria and non typhoidal salmonella, anamnestic reactions in unrelated infections and prior TAB or oral typhoid vaccination[6].

Enzyme Immunoassay Test or Dot-EIA Test

A dot enzyme immunoassay (EIA) that detects IgG and IgM antibodies against a 50 KD outer membrane protein distinct from the somatic (O), flagellar (H) or capsular (Vi) antigen of S. typhi is commercially available as Typhipoint. This dot EIA test offers simplicity, speed, early diagnosis and high negative and PPVs. The detection of IgM reveals acute typhoid in the early phase of infection, while the detection of both IgG and IgM suggests acute typhoid in the middle phase of infection. In cases of reinfection there is a secondary immune response with a significant boosting of IgG over IgM, such that the later cannot be detected and its effect masked. A possible strategy for solving this problem is to enable the detection of IgM by ensuring that it is unmasked[4]. Since IgG antibodies can persist for more than 2 years after typhoid infection the detection of specific IgG antibodies cannot differentiate between acute and convalescent cases. Disadvantage: Dot-EIA test might find masking effect in which IgM was masked by high IgG levels where IgG was likely to come from past infection.

MATERIALS AND METHODS

A comparative study of Dot-EIA & Widal test in the diagnosis of typhoid fever was conducted from November 2014 to August 2016. Inclusion criteria: 140 clinically suspected cases of typhoid fever, of 1 to 12 years age groups and both sexes attending Rajah Muthiah Medical College, Chidambaram using simple random method, constituted the study group. Exclusion criteria: Fever patient with alternative diagnosis, paratyphoid fever (culture confirmed), Child who are all vaccinated for typhoid fever were excluded from the study.

Collection of specimen: Blood samples were collected, 7ml from children. Around 5ml was inoculated into the Brain Heart Infusion biphasic media and transported to the laboratory. Remaining 2 ml used for serology test.

Widal tube test: Widal testing was performed on serum sample using Widal tube-test kits. Interpretation of results; A titre of $\geq$ 160 for "O" agglutinins and a titre of $\geq$160 for "H" agglutinins was considered consistent with typhoid fever.

Dot-EIA test: Dot-EIA test was done on serum by using the Dot-EIA kit. It is a qualitative antibody detection test with total assay time of 1 hour. Dot-EIA contain reagents and antigen dotted strips for detection of specific IgM and IgG antibodies to *Salmonella. Typhi* inclusive of controls.

RESULTS

A total of 140 cases clinically diagnosed as typhoid fever were studied. Out of them, 20 cases (14.29%) were positive by blood culture, 30(21.43%) by Widal test and 39 cases (27.86%) by dot-EIA test [Table: 1]. In the study group, 77 were boys and 63 were girls. Out of 20 blood culture positive cases (taken as gold standard) only11 were positive by Widal test & 9 were negative. Out of 30 positive by Widal test only 11 were positive and 19 negative by blood culture. Widal test has a sensitivity of 55%, specificity of 84.17%, positive predictive value of 36.67%, negative predictive value of 91.82% in comparison with blood culture results [Table:2]. Out of 140 cases 39 were positive by dot-EIA test. Out of 39 dot-EIA positives, 18 were positive and 21 negative by blood culture. Dot-EIA test has a sensitivity of 90%, specificity of 82.50%, positive predictive value of 46.15%, and negative predictive value of 98.02% in comparison with blood culture results. Out of 20 blood culture positive cases 18 were positive and 2 were negative by Dot-EIA test.

Table 1 Results of Blood Culture, Widal And Dot-Eia Among Selected Cases

Results	Blood culture		WIDAL		Dot-EIA	
	N	%	N	%	N	%
Positive	20	14.3	30	21.4	39	27.9
Negative	120	85.7	110	78.6	101	72.1
Total	140	100	140	100	140	100

Table 2 Comparison of Present Study Results With other Studies

Study	BC	WIDAL				Dot-EIA			
	+Ve %	Sen	Spe	PPV	NPV	Sen	Spe	PPV	NPV
Present Study	14.3	55	84.17	36.67	91.82	90	82.50	46.15	98.02
Santhoshkumar K et al	14.2	85.71	43.79	20.17	94.87	96.43	54.44	25.96	98.92
Balakrishna T P et al	14	53	83	34.88	91.72	92	83	47	98
Kiran Yadav et al	20	45	86	86	94	90	100	100	93
Jindal N et al	11.29	72.58	34.22	-	-	83.80	92.10	-	-
Appalaraju B et al		82	65	-	-	100	63	-	-
Sanjeev H et al	66	78.78	58.82	78.79	58.82	100	76.5	89.18	100

BC blood culture. -+^{ve} % - positive percentage. Sen- Sensitivity. Spe- specificity. PPV- positive predictive value. NNV- negative predictive value.

DISCUSSION

Blood culture: The considerable advantage of routine blood cultures in investigation of suspected typhoid fever is, not only that, they are 100% specific, but they also provide, isolate for antimicrobial sensitivity testing which is vital in today's scenario of multidrug resistance. In this study 14.3% of suspected enteric fever cases are positive for blood culture for S.Typhi. Santhosh kumar K *et al*[9] have similar blood culture positivity. Jinadal N[11] *et al* have lower blood culture positivity and Kiran Yadav *et al* have higher blood culture positivity. The lower percentage of culture positivity may prior start of antibiotics and late presentation to our center.

Widal test: In this study Widal test has a sensitivity of 55%, specificity of 84.17%, positive predictive value of 36.67%,

negative predictive value of 91.82% in comparison with blood culture results. Sensitivity and specificity of our study is in consistent with Balakrishna T P *et al*[6] and Kiran Yadav *et al*[10]. Higher sensitivity and lower specificity than our study seen in Jindal N *et al*[11].

Dot-EIA test

In this study Dot-EIA test has a sensitivity of 90%, specificity of 82.50%, positive predictive value of 46.15%, and negative predictive value of 98.02% in comparison with blood culture results. Sensitivity of this study is comparable with Balakrishna *et al*[6] and Kiran Yadav *et al*[10]. The specificity of this study is comparable with Balakrishna *et al*. few studies like Appalaraju B *et al*[8] and Sanjeev H *et al*[13] have 100% sensitivity. Kiran Yadav *et al* have 100% specificity. The positive predictive value and negative predictive value of our study is in consistent with Balakrishna T P *et al*[6]. Kiran Yadav *et al*[10] have higher positive predictive value.

CONCLUSION

Widal test showed the sensitivity and specificity of 53% & 83% respectively. Dot-EIA test showed sensitivity of 92% and specificity of 83% in culture proven cases. Hence the sensitivity of Dot-EIA test is more as compared to Widal test and specificity of both tests remains same. So we conclude that the Dot-EIA appears to be a practical alternative to Widal test in the diagnosis of typhoid fever, even in the resource constrained setup, as it neither requires much lab equipment, nor expertise to conduct the test, but whenever feasible confirmation with blood culture is strongly encouraged with the appearance of drug resistant strains.

Bibiliography

1. Background document: The diagnosis, treatment and prevention of typhoid fever. world health organization.2003; WHO/V&B/03.07: 1-38
2. Christopher M Parry, Lalith Wijedoru, Amit Arjyal & Stephen Baker. The utility of diagnostic tests for enteric fever in endemic locations. Expert Review of Anti-infective Therapy. 2011; 9(6): 711-725.
3. Zulfiqar Ahmed Bhutta. Nelson textbook of pediatrics. 20th ed. Philadelphia: Elsevier 2016; 1388-1392
4. Parthasarathy A, Ritabrata Kundu, Rohit Agarwal. Textbook of pediatric infectious diseases. Indian academy of pediatrics infectious disease chapter. New delhi. Jaypee brothers medical publishers. 2013; 418-23
5. Chandrashekar, Anil Kumar YC, Kirandeep Sodhi and Dalal S.S. A Study of clinical and laboratory profile of enteric fever in pediatric age group. *International Journal of Basic and Applied Medical Sciences* 2013; 3(3): 16-23
6. Balakrishna T.P, S. Sumathi, Anuradha. K3, D. Venkatesh, S. Krishna. A comparative study of dot-EIA and Widal test in the Diagnosis of typhoid fever. *Journal of Evolution of Medical and Dental Sciences*. 2013; 2(21):3720-25.
7. Karen H Keddy, Arvinda Sooka, Maupi E Letsoalo, Greta Hoyland, Claire Lise Chaignat, Anne B Morrissey & John A Crump. Sensitivity and specificity of typhoid fever rapid antibody tests for laboratory diagnosis at two sub Saharan African sites. Bulletin of the World Health Organization.2011:640-47
8. Appalaraju B, Anila A.Mathews & Priya P. The clinical utility of Dot-EIA in the diagnosis of typhoid fever. *Journal of pharmaceutical and biomedical sciences* (J Pharm Biomed Sci.) 2013, 29(29): 831-835.
9. Santosh Kumar K, M Suganya, B Sathyamurthi, Heber Anandan Reliability of Dot-EIA Rapid Immunoglobulin M and Immunoglobulin G in the Diagnosis of Typhoid Fever. *International Journal of Scientific Study*.2016; 4(2): 256-59.
10. Kiran Yadav, Suresh Kumar Yadav and Geeta Parihar. A Comparative Study of dot-EIA and Widal test for Rapid Diagnosis of Typhoid Fever. *International Journal of Current Microbiology and Applied Sciences*. 2015; 4(5): 34-38
11. Jindal N, Bansal R, Grover P, Malhotra R and Singh S. Rapid diagnosis of typhoid fever-a comparative study of dot-EIA and widal test. *International Journal of Bioassays*. 2014; 3(11): 3438-40.
12. Hajir Abd-Alhafeez Abd-Alrahman and Mohammed Nafi. Comparison of dot-EIA-eia and widal test in respect to polymerase chain reaction as diagnostic procedures for early diagnosis of typhoid fever. *Journal of Biomedical and Pharmaceutical Research*. 2014; 3(5): 18-20
13. Sanjeev H, Sweetha Nayak, Pai Asha K.B., Rai Rekha, Vimal Karnaker & Ganesh H.R. A systematic evaluation of rapid dot-eia, blood culture and widal test in the diagnosis of typhoid fever. Nitte University *Journal of Health Science*. 2013; 3(1): 21-24.