

Comparative Study of Widal and Dot Elisa in the Diagnosis of Typhoid Fever



MEDICAL SCIENCE

KEYWORDS : Typhoid fever, Widal test, Dot ELISA, Serological diagnosis

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ABSTRACT

Typhoid fever is a life threatening systemic illness affecting mainly the developing countries of the world. Correct diagnosis must be made at the crucial time for early treatment and control of transmission. Diagnosis of Typhoid fever by the following tests: Widal test, Dot ELISA and to compare the effectiveness of Widal test and Dot ELISA in the diagnosis of typhoid fever. Patients attending the Tirunelveli Medical College Hospital with acute febrile illness and clinically suspecting Typhoid fever formed the subject of the study. From the blood samples collected both Widal and Dot ELISA tests were performed. A total of 86 Samples were collected and tested. Out of 86 cases, 9 were Widal Positive whereas 23 were Dot ELISA Positive. Dot ELISA is a practical alternative to Widal test in the diagnosis of Typhoid fever.

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. There are at least 16 million new cases of typhoid globally. The annual incidence of typhoid fever has been reported as more than 13 million cases in Asia and causing more than six lakhs deaths worldwide annually¹. 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.

Widal test has been used in the diagnosis of typhoid illness for long time in this country but it remains a serological test with a moderate sensitivity and specificity. Therefore, a fast, reliable, and easy to perform serodiagnostic 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. Typhidot is a rapid serological test for the diagnosis of typhoid fever. However, its usefulness in terms of Specificity and sensitivity as compared to Widal test has not been studied so far in our region.

Few studies conducted in south India and other parts of Asia have reported encouraging in terms of diagnostic yields as compared to Widal test and to ascertain any difference of its specificity and sensitivity due to different strains of *Salmonella typhi* in this region.

Materials and Methods

Study population

This prospective study was conducted in Tirunelveli Medical College for a period of six months (April to September 2013). Patients attending the Tirunelveli Medical College hospital with complaints of acute febrile illness and clinically suspecting typhoid fever formed the subject of the study. Eighty six clinically suspected cases of typhoid of all ages and sexes were randomly chosen and formed the subjects of the study.

Before the collection of the specimen the patients were informed about the study and the details of the clinical symptoms and signs were recorded in the Proforma and examination of the patient was done to look for positive signs and symptoms. Informed consent was obtained from all patients.

Typhipoint Test

Typhipoint is a Dot Enzyme Immuno Assay test kit for the detection of specific IgM Antibodies against extracted protein of

S.typhi in human serum or plasma sample. A 50 KD *S.typhi* antigen from outer membrane protein is fixed on a nitrocellulose Membrane. Patient's serum is added. After incubation for 15 minutes it is washed. Anti Human IgM is added and incubated for 30 minutes. After washing add colour development solution. Incubate for 10 minutes. Stop the reaction with deionised water. Results are read visually. It does not need ELISA washer or reader. It can be done at field conditions.

Widal test

When a patient's serum containing antibodies to *S. typhi* and *S.paratyphi* is mixed with the respective antigen and incubated, agglutination takes place which indicates the presence of antibodies to a particular antigen (O, H, AH, BH). Serum samples from the blood that were kept for serum separation were used. The serum sample was stored at 2-8°C if not immediately used. The specimen (if refrigerated) and the kit were allowed to room temperature before testing.

A sets of 8 clean dry tubes (10 x 75 mm) were used for O, H, AH and BH antibody. The tubes are mixed well and then incubated at 37°C for 16-20 hours and observed for agglutination. 'O' Ag showed granular agglutination and 'H' Ag showed floccular or fluffy appearance. The highest dilution titre showing clearly visible agglutination with naked eye was noted. Saline control remained unchanged. The positive and saline controls were used along with the serum sample

Results

86 Samples were collected and tested. 23 Patients who had a positive result in either Widal or Dot ELISA or both were considered positive. 22 were positive by Dot ELISA, 9 were positive by Widal and 8 were positive by both tests. (Table-1) Males (15.1%) showed higher incidence of typhoid than females (12.8 %). The age group of 21 to 30 year old subjects showed the highest incidence (14 %) of typhoid. Children less than ten years of age had an incidence of 3.5%. Age wise distribution of Typhoid fever by Widal and Dot ELISA tests was depicted in Figure-1.

Discussion

Typhoid fever is widely recognized as a major public health problem in developing countries in Asia due to its huge population and suboptimal basic needs accounting for more than 13 million cases annually.¹ Even though isolation of *S.typhi* from Blood Culture / Bone marrow culture is the gold Standard for diagnosis of typhoid fever, it has its limitations. It needs proper sample collection, a full fledged microbiological laboratory, and technical expertise.

Besides when antibiotics are administered early, culture will

be negative. The purpose of the study is to analyse whether dot ELISA can be used at field conditions as a rapid diagnostic test to diagnose Typhoid fever suboptimal specificity and sensitivity are the limitations of Widal test. There is a need to determine the baseline titre in endemic areas. Decreased sensitivity is due to the long latent period of average 7days after which the test may become positive. Decreased specificity is due to prior infection, vaccination with TAB vaccine, cross reaction with other gram negative infections. Various studies have shown Dot ELISA to be more sensitive and specific for diagnosis of typhoid fever. Patients positive by either Widal or dot ELISA are considered to be positive.

In this study the highest incidence of typhoid is seen in the 21 -30 age group (14 %) Children positive for typhoid were 2.3 % in 0 to 10 years age group and 7 % in 11 to 20 Years. This is in contradiction to the WHO bulletin, which states that typhoid in India is common in children ¹.Walia et al too found, that most typhoid cases occurred in children of 5-12 years²In our study males were predominantly affected (15.1%). This correlates with an Iraqi study by Mohammed Abdul where 61% of typhoid patients were male³.This may be due to increased outdoor activity of Indian males exposing them to contaminated food and water.

In the present study out of 86 patients dot ELISA was positive in 23 cases (26.7%) and Widal was positive in 9 cases (10.5 %). In a study by Narayanappa, Rachana et al, out of 105 cases widal was positive in 48 cases (45.7%) and Typhoid M was positive in 78 cases (74.3 %)⁴.

In the current study dot ELISA detected 14 cases (63.6%) of less than seven days duration, widal detected 4 cases (44.4%). In a study by Narayanappa, Rachana et al, out of 33 children having fever of less than 1 week and blood culture positive for *S. typhi*, Typhidot was positive in 32cases (96.9 %) compared to 8% cases positive by Widal test (24.2 %)⁴. Retnosari S, Tumbelaka AR, Akib AP et al conducted a study on 36 paediatric patients with duration of fever less than 8 days with blood culture positivity in 24 % patients and widal positivity in 11% patients. 72% positive for typhidot. This shows that Dot ELISA can detect typhoid earlier (fever of less than 1 week duration) than Widal.^{5,6}

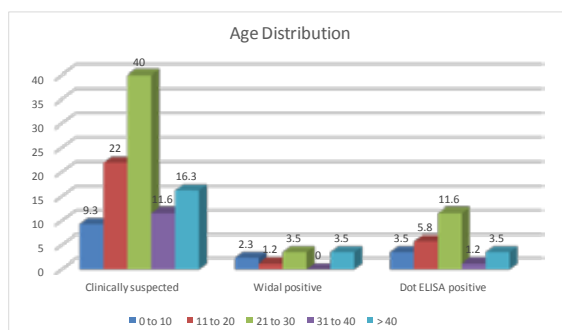
Conclusion

Prompt diagnosis is essential for the patient and the community management of typhoid fever. Blood culture and Widal test are used as conventional methods in the diagnosis of typhoid fever. The new rapid serological test Dot ELISA has been introduced commercially for the diagnosis of typhoid fever. Limitations of dot ELISA are inability to detect *S.paratyphi* cases, negative in fevers of less than three days duration and non availability of a bacterial isolate for drug susceptibility testing. In the present study we conclude that Dot Elisa is a practical alternative to Widal test in the diagnosis of Typhoid fever on account of its increased sensitivity, early detection of cases, ease of procedure with minimal infrastructure and availability of results on the same day.

Table 1: Results of Widal and Dot ELISA tests

WIDAL	DOT ELISA	No.	PERCENTAGE
+	+	8	9.3 %
+	-	1	1.2 %
-	+	15	17.4 %
-	-	62	72 %

Fig 1: Age wise distribution of Typhoid fever by Widal and Dot ELISA tests



REFERENCE

1. WHO .int/bulletin volume 86/4/06 | 2. Walia.M, Gaiind R et.al; Age related clinical and microbiological characteristics of enteric | fever in India.Trans R soc of tropical medicine .2006.oct 100(10); 942-8. | 3. Ismail Kader South East Asian J trop Med Pub Health 1991 22:563 – 566 | 4. Narayanappa D, Rachana et al. Comparitive study of Dot Enzyme Immuno Assay and | Widal test in the diagnosis of Typhoid fever. Indian Pediatrics 2010; 47; 331-333 | | 5. Retnosari S, Tumbelaka AR, Akib AP, Hadinegoro SRS. Clinical and laboratory | features of typhoid fever in childhood, focus on relationship between prior antibiotic | administration with blood culture, Widal and typhidot results. Paediatr Indones | 2001; 41:149-54 | 6. Choo KE, Oppenheimer SJ, Ismail AB.Rapid Serodiagnosis of Typhoid fever. | 1994; 9: 172 – 176 |