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**COMPARATIVE EVALUATION OF DOT ENZYME IMMUNOASSAY (TYPHIDOT)
AND WIDAL TEST WITH RESPECT TO BLOOD CULTURE IN THE
SERODIAGNOSIS OF TYPHOID FEVER**

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ABSTRACT

A total of 70 sera, 60 from suspected cases of typhoid fever and 10 from nontyphoid cases were tested by dot enzyme immunoassay (EIA) method (Typhidot) for IgM and IgG antibodies against *Salmonella enterica*, serotype Typhi. Widal test and blood culture were also done and compared with Typhidot test. On comparative evaluation with Widal test, Typhidot showed higher sensitivity (75%), specificity (90%), positive predictive value (PPV, 97.8%), negative predictive value (NPV, 37.5%). A high sensitivity of Typhidot (95.12%) was seen in culture proven typhoid cases. Typhidot test is a superior test than Widal test and should be preferred for the diagnosis of typhoid fever.

Keywords: Typhoid, Dot EIA, Widal, Comparative, Serological

INTRODUCTION

Typhoid fever caused by *Salmonella enterica*, serotype Typhi is still a continuing major health problem in most of the developing countries, especially in the Asia Pacific region, the Indian subcontinent, Central Asia, Africa and South America. The estimated incidence in Asia Pacific region is 540 cases per 100,000 populations [1]. However, WHO has estimated that there are 16.6 million cases of typhoid annually

with about 600,000 deaths [2]. The available conventional methods for isolation of serotype Typhi from blood, urine and stool is a good method of confirming the infection but have their own disadvantages. Due to these factors, in endemic areas, the diagnosis may be delayed or overlooked as cases of 'fever of unknown origin', thus leading to increased morbidity and mortality [3]. The serodiagnosis of typhoid fever by Widal test has been widely performed for

almost hundred of years in the laboratories of most of the developing countries but lacked an early diagnosis and had poor sensitivity and specificity due to associated fallacies. Hence, there has been always a need for standard rapid serodiagnostic tests for diagnosis of typhoid fever. One such test is the Typhidot test, a dot enzyme immunoassay for the rapid detection of specific IgM and IgG antibodies against a specific antigen on the outer membrane protein (OMP) to serotype Typhi, which has been claimed as an ideal test with rapidity, and good sensitivity and specificity [4].

In the present study, we evaluated the serodiagnostic sensitivity, specificity, positive and negative predictive values of typhidot test as compared to blood culture and Widal test for the diagnosis of typhoid fever.

MATERIALS AND METHODS

A total of 70 representative sera were collected from inpatients and outpatients department attending the Sir Sunderlal hospital, BHU, Varanasi after prior consent. Of these, 60 samples were from patients with clinical features suggesting typhoid fever and 10 samples from patients with nontyphoidal respiratory illness like respiratory infections and malaria. The study was performed in the serology section of the Microbiology department, Institute of Medical Sciences, BHU, Varanasi.

Blood culture, Widal test and Typhidot were performed for all the patients and compared for their sensitivity, specificity and predictive values. Typhidot kits (MBDR, Kuala Lumpur, Malaysia) were made available and reagents prepared according to manufacturers instructions. The assay was performed as per protocol. Briefly, the M and G strips were placed into the appropriate wells. Controls and test sera diluted 1:100 with the sample diluent were added and strips were allowed to thoroughly soak the sera and were incubated at room temperature on a rocker platform for 20 mins with constant shaking. The diluent was gently aspirated and the strips washed three times in the wash buffer. Prediluted antihuman IgG and IgM conjugated with HRP (horseradish peroxidase) was added to the wells separately and incubated for 15 mins at room temperature. The strips were similarly washed and then colour developing solution was added. The strips were incubated for 15 mins and finally washed with distilled water. Results were interpreted as positive when test sera showed dots with equal or higher intensity than the positive control. Negative sera showed no colour.

Widal test and blood culture was performed as per standard procedure [5].

RESULTS

Out of 60 sera tested from clinically suspected cases of typhoid fever, 41 were blood culture positive, 45 were typhidot positive and 35 were Widal positive. Their sensitivity, specificity, predictive values and diagnostic accuracy is shown in **Table 1**. Out of the 41 culture positive cases, 39 were

also positive for typhidot. The sensitivity of typhidot in culture proven cases was 95.12% as shown in **Table 2**.

From amongst the non typhoidal febrile illness control group, none were culture positive whereas only one case showed positive results with typhidot. In this group, Widal was positive in two cases.

Table 1: Comparative Evaluation of The Diagnostic Tests For Typhoid Fever

Test	%, (No. of samples/total no. of samples)			
	Sensitivity	Specificity	PPV	NPV
Blood culture	68.3, (41/60)	100, (10/10)	100, (41/41)	34.5, (10/29)
Widal	58.3, (35/60)	80, (8/10)	94.5, (35/37)	24.2, (8/35)
Typhidot	75, (45/60)	90, (9/10)	97.8, (45/46)	37.5, (9/24)

PPV= positive predictive value, NPV= negative predictive value

Table 2: Comparative Evaluation of Widal Test and Typhidot Test In Culture Proven Cases and Nontyphoidal Cases

Test	No.of cases positive in culture positive typhoid cases, n=41 (sensitivity %)	No.of cases positive in non typhoidal cases, n=10 (sensitivity %)
Widal	25 (60.97)	2 (20)
Typhidot	39 (95.12)	1 (10)

DISCUSSION

The present study showed the sensitivity of typhidot as 95.12%, which is in concordance with other comparable studies reporting sensitivity of 100% [6], 92.6% [7] and 92% [8]. These studies from India and others from abroad [9, 10] have clearly shown that typhidot is better than the widely performed Widal test for diagnosis of typhoid fever.

In this study, only two cases which were blood culture positive were not detected by typhidot, which could have been due to masking of the IgM antibodies by the IgG or due to decreased levels of IgM during later half of typhoid fever [7]. In addition, this study also showed that 6 cases of suspected typhoid fever with negative blood culture were detected positive by typhidot

test. This could be due to low sensitivity of culture especially in later weeks of fever, antibiotic treatment prior to or during collection of blood, laboratory errors and lack of automated systems [11].

Currently, blood culture and microbiological identification is considered the gold standard for diagnosis of typhoid fever. But this facility is rare in many developing countries as well as costly and requires expertise. Moreover, this test is useful only in the first week of typhoid fever. The other serological test that is widely used is the Widal test which lacks sensitivity and specificity and is not a good diagnostic test. As a result, most of the typhoid fever in developing countries is diagnosed clinically without any serological evidence and thus treated presumptively [12]. In this context, typhidot proves to be a reliable serological test in endemic areas where studies have shown acceptable sensitivity and specificity for this test. However, the cost effectiveness of typhidot and inability to detect *Salmonella Paratyphi A* and *B* infections as compared to Widal test cannot be overlooked.

CONCLUSION

Typhidot is a reliable serological test to diagnose typhoid fever than the widely used Widal test. Because of its high sensitivity, specificity and predictive values, this test

should be availed for prompt diagnosis of typhoid fever.

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