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Comparative study between WIDAL and DOT ELISA in the diagnosis of Typhoid fever

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ABSTRACT

Background: Typhoid fever, also known as enteric fever, is a communicable disease, found only in man and occurs due to systemic infection mainly by *Salmonella typhi* organisms. Blood culture is regarded as the gold standard for diagnosis and carry 70-75% diagnostic yield in the first week of illness. **Aims and Objective:** To compare the sensitivity and specificity of Widal test and dot ELISA with blood culture in the early diagnosis of Typhoid fever. **Materials and Methods:** A Cross-Sectional study was carried out in the Department of Microbiology, Era's Lucknow Medical College and Hospital for a period of 18 months. Patients presenting with acute febrile illness suspicious of typhoid fever accompanied by clinical signs and symptoms of typhoid fever in the absence of any other known febrile illnesses, were included in the study. Widal and Dot ELISA was performed using serum samples and for blood culture aseptically collected blood was used. **Results:** Sensitivity, Specificity, PPV, NPV of DOT ELISA as compared to Blood culture for typhoid positivity was found to be 92.6%, 83.7%, 55.6% and 98.1% respectively. Diagnostic accuracy of DOT ELISA as compared to Blood culture was found to be 85.3%. **Conclusion:** For both early and late diagnosis of typhoid fever with high sensitivity as well as accuracy for identification of typhoid fever, the rapid diagnostic test (Dot Elisa) is better than the Widal test. However, it may be an increased burden to healthcare owing to a low positive predictive value in a low prevalence scenario.

Key words: DOT ELISA; *Salmonella typhi*; Widal test; Blood culture

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INTRODUCTION

Typhoid fever, also known as enteric fever, is a communicable disease, found only in man and occurs due to systemic infection mainly by *Salmonella typhi* organisms. It is an acute generalized infection of the reticuloendothelial system, intestinal lymphoid tissue, and the gall bladder. It is a potentially fatal multisystemic illness that causes nearly 220,000 deaths annually and 22 million illnesses per year. It predominantly affects the children of school-age or younger.[1-2] In order to distinguish typhoid fever from other diseases, correct diagnosis is always difficult, both in the clinic and in the laboratory, but successful treatment selection is crucial. Doctors frequently initiate typhoid treatment empirically, including in heavily resourced western countries, while waiting for validation of the diagnosis.[3] Isolation of Salmonella from blood, urine or stool is the most reliable means of confirming an infection. Blood culture is regarded as the gold standard for diagnosis and carry 70-75% diagnostic yield in the first week of illness.[4] Although in the developed world, molecular diagnostic tests such as polymerase chain reaction are used to confirm the diagnosis.[5-8] However, these techniques are not suitable for the developing and resource-starved environments like India where even basic laboratory facilities are not available in primary care settings. A serological examination, Widal, is readily available, affordable and has been in use for several years in all clinical environments. However, questions about its validity have been raised as the diagnostic importance titers vary in various geographical regions, in different populations and in the presence of other febrile illnesses. DOT ELISA, commercially available for the diagnosis

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of typhoid fever, is stated to be a serodiagnostic test that is fast , accurate and simple to administer with greater sensitivity and specificity than the Widal test.[9] Studies from other Countries of Asia and India have found it to be of practical alternative to Widal test in the diagnosis of typhoid fever.[4,10-12] However, its sensitivity and specificity varies in different studies. Uttar Pradesh, which is one of the most populous state in India and relatively has poor sanitary condition has conducive environment for typhoid fever but there are only limited studies comparing the conventionally used Widal test against dot ELISA for diagnosis of typhoid fever. Hence the present study was undertaken to systematically evaluate the utility of dot ELISA and Widal test in diagnosis of typhoid fever in terms of sensitivity and specificity.

Aims and Objectives

To compare the sensitivity and specificity of Widal test and dot ELISA with blood culture in the early diagnosis of Typhoid fever and to determine the sensitivity and specificity of Widal test and dot Elisa in patients of typhoid fever.

MATERIALS AND METHODS

A Cross-Sectional study was carried out in the Department of Microbiology, Era's Lucknow Medical College and Hospital from the month of June 2016 to November 2017. Serum samples of 150 patients with febrile illness (children and adults) having Clinical suspicion of typhoid fever were included. All the patients with febrile illness (children and adults) having Clinical suspicion of typhoid fever, in the absence of any other known febrile illnesses, were included in the study.

Serology and Microscopy

For widal Qualitative slide agglutination and semi quantitative tube agglutination (titration) was performed using febrile antigen kits of Salmonella typhi134 (ARKRAY Healthcare Pvt. Ltd., Surat, India).

For Dot ELISA assessment, Genomix Typhoid Rapid Diagnostic Casette (Serum/Plasma) was used. Blood culture test was performed using BD BACTEC™ Plus Aerobic/F Culture vials.

RESULTS

Table 1 shows the distribution of patients according to age. Age of patients enrolled in the study ranged between 2 years & 65 years, median age of patients was 26 years while mean age was 29.09±13.19 years. Majority of the patients were aged 11-30 years (54.00%). Most common age group was 21-30 years (30.00%) followed by 11-20 years (24.00%) and 31-40 years (18.00%) while least common age group was >60 years (2.00%) followed by ≤10 years & 51-60 years (6.00% each) and 41-50 years (14.00%). Min-Max (Median): 2-65 (26.00); Mean±SD: 29.09±13.19.

In Table 2, depicts gender wise distribution of study population. Out of 150 patients enrolled in the study, majority (n=93; 62.0%) were males and rest 57 (38.0%) were females. Male: Female ratio was 1:0.61.

In Table 3, it was observed that approximately one-third of the patients had fever below 100°F i.e. low grade of fever (n=54; 36.0%) and rest 96 (64.0%) had fever >100°F.

Table 4 depicts duration of fever at the time of hospital visit among study population that ranged between 5 and 9 days, median duration of fever was 6 days and mean duration was 6.36±1.22 days. Majority of the patients had duration of fever <7 days (78.7%), fever >7 days was found among 32 (21.3%) patients only.

Table 5 shows the agreement of DOT ELISA and Blood culture was found for 128/150 cases i.e. 85.33% agreement.

Table 1: Age wise Distribution of Study Population

S. No	Age Group (years)	No. of patients	Percentage
1.	≤10 years	9	6.0
2.	11-20 years	36	24.0
3.	21-30 years	45	30.0
4.	31-40 years	27	18.0
5.	41-50 years	21	14.0
6.	51-60 years	9	6.0
7.	>60 years	3	2.0

Table 2: Gender wise distribution of study population

S. No	Gender	No. of patients	Percentage
1.	Female	57	38.0
2.	Male	93	62.0

Table 3: Grade of Fever among Study Population

S. No	Grade of Fever	No. of patients	Percentage
1.	Low (<100°F)	54	36.0
2.	High (>100°F)	96	64.0

Table 4: Duration of Fever (days) among Study Population

S. No	Duration of Fever (days)	No. of patients	Percentage
1.	5 days	45	30.0
2.	6-7 days	73	48.7
3.	>7 days	32	21.3

Level of agreement was found to be substantial (κ=0.606) and statistically significant.

Sensitivity	Specificity	PPV (Positive predictive value)	NPV (Negative Predictive Value)	Diagnostic accuracy
92.6	83.7	55.6	98.1	85.3

Sensitivity, Specificity, PPV, NPV of DOT ELISA as compared to Blood culture for typhoid positivity was found to be 92.6%, 83.7%, 55.6% and 98.1% respectively. Diagnostic accuracy of DOT ELISA as compared to Blood culture was found to be 85.3%.

Table 6 shows the agreement of Widal and Blood culture was found for 119/150 cases i.e. 79.33% agreement. Level of agreement was found to be Moderate (κ=0.411) and statistically significant.

Sensitivity, Specificity, PPV, NPV of Widal as compared to Blood culture for typhoid positivity was found to be 66.7%, 82.1%, 45.0% and 91.8% respectively. Diagnostic accuracy of DOT ELISA as compared to Blood culture was found to be 79.3%.

Table 7 depicts Comparison of Widal/Dot ELISA positivity with duration of fever. For both <7 days and >7 days duration of fever, the positivity rate was lower for Widal as compared to that for Dot ELISA.

DISCUSSION

In the recent years, a number of rapid diagnostic tests for the diagnosis of typhoid have come up with a reasonable sensitivity and specificity and are gaining popularity owing to ease of testing and rapidity of results.

Table 5: Level of Agreement among Blood Culture and DOT ELISA

DOT ELISA	Blood culture		TOTAL
	Positive	Negative	
Positive	25	20	45
Negative	2	103	105
	27	123	150

κ=0.606; p<0.001

Table 6: Level of Agreement among Blood Culture and Widal

Widal	Blood culture		TOTAL
	Positive	Negative	
Positive	18	22	40
Negative	9	101	110
	27	123	

κ=0.411; p<0.001

For this purpose, a total of 150 suspected cases were enrolled in the study. The age of patients ranged from 2 to 65 years. Mean age was 29.09±13.19 years. A total of 90 (60%) patients were within 30 years of age. Epidemiological studies in general report younger population, especially school-age children, particularly those in age group 5-15 years to be most affected by typhoid fever [2,13]. In present study too, a substantial number of patients were aged <20 years (n=45; 30.0%), however, the study did not show a predominance of children among suspects. There could be primarily three reasons for this – first, our facility was a tertiary care referral facility; secondly, the source of data was from a facility which had a substantially larger capacity for Medicine patients as compared to Pediatric patients and thirdly but most importantly the sampling frame ruled out inclusion of all those patients who had antibiotic treatment within two weeks before coming to the hospital. As a matter of fact, most of the children being referred to our facility were referred from primary or secondary care facilities that already had initiated antibiotic treatment before the admission of child patients in the facility.

In present study, majority of patients were males (62%). Male to female ratio was 1.63. Although epidemiological studies place females at a higher risk[14], however, most of the hospital-based studies among suspected cases show a dominance of males. In a recent study among cases of acute febrile illness, Salagre et al[15] reported the proportion of males to be 67.8%. Choudhary et al[16] reported a much higher gender ratio (4:1) with 80% patients as males. Studies from states like Kerala, where population gender ratio is not as skewed as in Northern India too, cases of acute febrile illness generally show a dominance of males (88.54% vs 11.46%). Thus, despite a relatively similar risk the higher proportion of males in hospital-based studies indicates a gender-related bias in health services utilization pattern in our society.

In present study as many as 96 (64%) of patients had high grade fever. This is contrary to the described clinical profile of patients who are marked with prolonged low-grade fever. However, with the second week the fever becomes high-grade following a persistent rise[17]. On evaluating further, we found that only 30% patients had fever for up

Table 7: Comparison of Widal/Dot ELISA positivity with duration of fever

Duration of fever	No.	Dot ELISA positive		Widal positive	
		No.	%	No.	%
≤7 days	118	29	24.58	25	21.19
>7 days	32	16	50.00	15	46.88

to 5 days while 73 (48.7%) had fever for 6-7 days (i.e. at the verge of start of second week) and 32 (21.3%) were already in second week of fever and hence the dominance of high grade fever patients in present study could be well justified.

In present study, when compared to blood culture the Sensitivity, Specificity, PPV, NPV of DOT ELISA was 92.6%, 83.7%, 55.6% and 98.1% respectively whereas for Widal test it was 66.7%, 82.1%, 45.0% and 91.8% respectively. Thus, Dot Elisa was found to be relatively more sensitive and almost similarly specific as compared to Widal.

Similar to our study, a number of other studies have found the sensitivity and specificity of Widal test to be lower than Dot Elisa in their study.[18-22] However, similar to our study, these studies except the one by Beig et al[18] observed the difference in specificity of two methods to be nominal. However, there is only one study that finds both sensitivity and specificity of Widal to be higher than Typhido.[11]

In present study, we also made an attempt to compare the positivity rate of Dot Elisa and Widal tests for cases presenting before 7 days and after 7 days of symptoms and found that for both the time intervals, the detection rate was higher for Dot Elisa. It was found that for <7 days Dot Elisa detected a total of 4 more cases as compared to Widal whereas for those presenting at >7 days only 1 more case as compared to Widal was detected, thus giving an inference that of two modalities, Dot Elisa can provide better results even in early diagnosis and hence should be recommended as an early diagnostic modality.

The findings in present study have direct clinical implication. With a high sensitivity as well as a reasonable specificity, Dot Elisa could be recommended as a preferred diagnostic modality for diagnosis of Typhoid fever, however, it must be kept in mind that owing to a high burden of false positivity, the burden of healthcare increases twice while using this rapid diagnostic test. Thus, the findings of present study corroborate the contemporary evidence that supports the use of Dot Elisa as a preliminary detection modality for detection of typhoid fever.

CONCLUSION

The results of the present study thus assess the efficacy of the rapid diagnostic test (Dot Elisa) over the Widal test for both early and late diagnosis of typhoid fever, which has a high sensitivity as well as specificity for identification of typhoid fever. However, it may be an increased burden to healthcare owing to a low positive predictive value in a low prevalence scenario. This is an issue of concern that needs to be addressed further.

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Author's Contribution:
SH, MS, VK-Concept and design of the study; prepared first draft of manuscript; **SH-** Interpreted the results; reviewed the literature and manuscript preparation; **VK, SH, SY-** Concept, coordination, review of literature and manuscript preparation; **ZS-** Coordination for sample collection and over all coordination; **SAA-** Statistically analyzed and interpreted preparation of manuscript and revision of the manuscript

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