

A Comparison of Typhidot test with Widal test and Blood culture for the Diagnosis of Enteric Fever (Typhoid fever) in a Tertiary Care Hospital

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ABSTRACT

Aim: To Comparison of Typhidot test with Widal test and Blood culture for the Diagnosis of Enteric Fever

Period: March to September 2017

Settings: Microbiology lab. Skeikh Kahlifa Bin Zayed Al-Nahyan Hospital Rawalakot AJ&K.

Methods: A total of 150 samples were included in this study. Blood samples were obtained for culture, Widal test and Typhidot test.

Results: Out of these 150 specimen 31.33% were positive by culturing the blood, Widal test showed 40.76% positive results and 51.34% when Typhidot test was performed.

Conclusion: Typhidot-M is good alternative to Widal test if it performed within a week of infection.

Keywords: Typhidot test, widal test, enteric fever

INTRODUCTION

Enteric fever or typhoid fever is mainly caused by *Salmonella enterica* serotype typhi. It is one of the leading cause of morbidity and sufferings worldwide. It is estimated that it is the sole cause of huge number of infections and deaths in the world. It is responsible for 16.6 million infections and about 1.6 million deaths in the world each year¹.

Enteric fever is endemic in South Asia, Pakistan, India and Bangladesh. It is also endemic in Africa and other Asian countries. It reported that more than 13 million cases are reported in Asia each year¹. According to one of the statistics mortality rate in 9.3 per 100000 patients in Pakistan due to typhoid fever². According to WHO 90% of all morbidity and deaths are reported in Asia³. The most important factors that lead to the diseased condition includes poor socioeconomic conditions and sanitation on the top among others in our country⁴. The economic impact of typhoid fever is vast as well as social because its prolonged course of the disease⁵. Enteric fever affects all ages in both gender but it is more severe in children younger than 5 years of age with grave complications and longer hospital stay⁶.

The clinical manifestations are wide range and vague. Diagnosis is based on isolating the organism from blood, urine, stool or bone marrow culture. Blood culture is positive earlier than stool culture that's why blood culture is routinely performed for the isolation of the organism⁷.

Blood culture is gold standard for the diagnosis of enteric fever. Only 30 to 40% of blood samples are positive for blood culture because most of the patients start antibiotics prior to obtaining blood sample for culture⁸. The facility for blood culture is limited to tertiary care centres and it takes time of 2 to 3 days before declaring culture as positive or negative, therefore clinician rely routinely rely on rapid antigen based methods like Widal and Typhidot test for typhoid fever. These tests are not gold standard and therefore, these are not encouraged internationally^{9,10}. However in Pakistan Widal and Typhidot tests are routinely done because these are cheap, readily available and give rapid results for the diagnosis.

Widal test is done on paired serum samples with 4 fold rise in antibody titres. A single test is not as much as compared to two samples which are taken 7 to 14 days apart and show a rise in antibody titre. Therefore a single acute phase sample may be misleading in diagnosis and you must know the titre level of population. This raises questions on the reliability this test^{11,12}.

Typhidot test is Enzyme Linked immunosorbent assay (ELISA) that detects IgM for recent infection and IgG for late phase of infection. This test is considered to be superior to Widal test. However,

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modified Typhidot –M test may be false positive in second week of infection¹³.

This study was designed and conducted for the comparison of Typhidot and Widal tests with blood culture as well as their sensitivity and specificity for the diagnosis of enteric fever.

MATERIALS AND METHODS

This study was conducted in the Microbiology laboratory, Sheik Khalifa bin Zayed Al-Nahyan Hospital Rawlakot AJ&K, which is a three hundred bedded hospital that is attached to Poonch Medical College as teaching hospital. This study was conducted from March to September 2017. A total of 150 samples were included in this study irrespective of gender, age or any other underlying diseased condition. These blood samples were sent to the lab for the diagnosis of typhoid fever. Blood culture was performed in blood culture bottles and their sub cultures were performed on blood agar. If growth was obtained then the organism was confirmed by API20E and stabbing and incubating the organism in triple sugar iron medium.

Typhidot-M test (Typhidot-M kit), Widal tube test method and for the isolation of the organism blood culture was done. A titre of 1 in 160 or greater than that for O antigen and for H antigen a titre more than 1 in 320 was considered as positive that test is positive.¹⁴ Typhidot test was performed for the detection of IgM antibody. In this test, IgG is inactivated before carrying out the assay as for the Typhidot. The test uses a nitrocellulose membrane strip dotted with the 50 KDa specific proteins and a control antigen. 2.5 µL of patient serum and controls are pre-absorbed for at least 1 min with 90 µL of IgG inactivation reagent. 250 µL of sample diluent is then added into the reaction wells and the mixture incubated at room temperature on a rocker platform for 20 min. The strips are washed thrice for a total of 5 min, and 250 µL of antihuman IgM conjugate is added and incubated for 15 min. The strips are washed as before, and 250 µL of color development solution is added and incubated for 15 min. The reaction is stopped by washing the strips in distilled water, and the results are read. When both the dots on the test strip are as dark as or darker than their corresponding dots on the positive control strip, they are reported as positive.

Statistical analysis was performed by using Microsoft excel and the results were presented as tables and graphs. All blood samples that received for the diagnosis of typhoid fever from patients that were clinically suspected as suffering from typhoid fever, were included in this study irrespective of any underlying diseased condition. Those individuals who

have already started antibiotics were excluded from this study.

Sampling and examination of sample: All samples were fresh blood samples that were collected from patients that were clinically suspected that they are suffering from enteric fever. All samples were tested within 2 hours of receiving for Widal and Typhidot test. Blood culture was done by using blood culture bottles and 3 sub cultures were also performed before labelling the culture as no growth of salmonella.

RESULTS

All 150 samples were subjected to blood culture, Widal test and Typhidot test. Blood culture being the gold standard for the diagnosis of enteric fever was considered as ultimate diagnostic test in this study and the samples that showed positive growth for salmonella were considered as true positive. Out of these 150 samples 47 were positive for growth of organism and remaining were negative for bacterial growth, even their sub cultures for consecutive three days didn't show growth of salmonella. This is shown in table 1 below.

Table 1: No and %age of positive and -ve blood culture.

Total samples	Positive for blood culture	Negative for blood culture
150	47(31.33%)	103(68.67%)

Widal test was performed using serum of all blood samples in different concentrations and results were marked as positive or negative according to the set criteria of titres. The results of Widal test are shown in table 2 below.

Table 2: No & %age of positive and negative for Widal test

Total Samples	Positive for Widal test	Negative for Widal test
150	61(40.67%)	89(59.33%)

Typhidot test was performed for the IgM antibody on patients' serum samples and results are shown in table 3 below.

Table 3: No & %age of positive and -ve for Typhidot test

Total samples	Positive for Typhidot test	Negative Typhidot test
150	77(51.34%)	73(48.66%)

DISCUSSION

Enteric fever or typhoid fever is an infectious disease which prevails more frequently in developing and developed countries. Poor socioeconomic conditions and lack of proper medical care and facilities in the underdeveloped and developing countries also play

an important role in the spread of disease¹⁵. With the increase of irrational use of antibiotics drug resistant strains of the organism have emerged. Therefore proper and prompt diagnosis and treatment are necessary¹⁶. Blood culture is considered to be gold standard for the diagnosis of typhoid fever but it takes time.

In this study blood culture of clinically suspected cases was positive in 31.33% of cases. This is comparable to some other studies conducted. In a study conducted it came out to be 30% which is close to our study¹⁷. Blood culture positivity is different in different studies. Similarly, in our study Widal test was positive for 40.67% of samples and Typhidot in 51.34% samples. These results can be compared to the studies conducted¹⁷. Thus blood culture is the gold standard for diagnosis while second reliable are Widal and Typhidot tests in our settings. Widal is less reliable because the antigens are shared by members of Enterobacteriaceae.

CONCLUSION

Gold standard for the diagnosis of typhoid fever is blood culture. Results of Widal and Typhidot should be confirmed by blood culture and sensitivity should be tested especially in those settings where the organism has become resistant to antibiotics. However, Typhidot-M is good alternative to Widal test if it performed within a week of infection. Clinician should properly diagnose and treat enteric fever to mimic its resistance to antibiotics.

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