

Role of Widal Test in the Diagnosis of Typhoid Fever in Context to Other Test

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Abstract Typhoid is one of the infectious diseases of human. Outbreaks of typhoid fever caused by *S. typhi* and *S. paratyphi A* are still a serious health problem worldwide. There are number of test available now days from molecular to immunological and biochemical to microbiological. In our study we have tried to establish the relevance of widal test in the diagnosis of typhoid fever. Both the tube and slide agglutination widal test were used to investigate the incidence of typhoid fever in 80 test individuals comprising 60 typhoid fever patients and 20 apparently healthy individuals comprised of adult and children. The test results were compared with those of standard blood culture tests. We tested different individuals and were categorized according to their age above 10 years (20), and between 5- 10 years (20), and children under 5 years (20). Results showed that the accuracy of the widal tube and slide agglutination tests as used in the study in terms of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 71%, 62%, 91% and 31%, respectively. It is concluded that widal test will remains relevant as a diagnosis tool for typhoid fever, which is more convenient, cheaper and faster than the other molecular and serological test.

Keywords Typhoid, Widal Test

1. Introduction

About 16 million new cases of typhoid fever with 600000 deaths caused by *Salmonella enterica* serotype Typhi (*S. Typhi*) occur globally each year[1] with the highest incidence (1000 cases per 100000 people per year) in Southeast Asia[2]. The incident rate of typhoid in Asia-pacific region is estimated at more than 100 cases/100,000 population per year. The highest burden of disease has been observed in children[3]. Widal a serological diagnosis test for enteric fever was founded in 1896 by Georges Fernand Isidore Widal[4]. It is an agglutination reaction demonstrating the presence of lipopolysaccharide (LPS) somatic (O) and flagella (H) agglutinins to *Salmonella typhi* in the serum of a patient using suspensions of O and H anti gens[5]. Commercial kits are available for antigens of *Salmonella paratyphi A*, B and C. The recommended method of performing the widal test is by the tube agglutination technique were serial two-fold dilutions of the subject's serum from 1:20 to 1:1280 are tested[6]. Now a days a rapid slide test is most commonly used technique in local laboratories and hospitals because of its convenience. The widal test has been used extensively in the serodiagnosis of typhoid fever and so remains the only practical test available in most developing

countries[7]. Isolation of *Salmonella typhi* from the blood, faeces, urine, bone marrow, or other body fluids are an important diagnostic tool. In addition, unavailability of microbiological facilities and the long waiting time for culture results have been identified as reasons for the preference for the widal test[8]. One of the major drawback of widal test is cross-reactivity due to which some other bacteria of same genus often produces false positive results, so the positive results must correlate clinically before prescribing medicine. Typhidot is another rapid test used to ascertain the diagnosis of typhoid fever, but not cost effective as widal. So widal test is the choice for typhoid fever especially in rural area.

2. Materials and Methods

Blood samples were collected from apparently healthy individual and out-patients visiting the laboratory sections of the Rajendra Institute of Medical Sciences (RIMS) Ranchi and Indira Gandhi Institute of Medical Sciences (IGIMS) Patna, India, The samples were taken by venipuncture from patients with confirmed typhoid who were yet to undergo treatment. The fresh bloods were dispensed into ethylene diamine tetra acetic acid (EDTA) vial. The patients were also given sterile, dry, wide-necked, leak-proofed universal laboratory bottles for the collection of mid-stream urine for culture. Stools for culture were collected in clean wide-mouth containers and the patients were instructed to avoid contaminating the feces with urine. Each sample container was labeled with the name of the patient, date and time of

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Published online at <http://journal.sapub.org/ajb>

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collection.

2.1. Screening Analysis

The Sera for widal test were collected from fresh blood samples by centrifugation. Using a Pasteur's pipette, six to eight drops of each serum were transferred onto eight rings on a white tile. The Salmonella antigen reagent was also dropped into the rings. Both were thoroughly mixed using an applicator stick and the tile gently swirled for one minute for observable agglutination. The reacting antigens were recorded positive (+) while nonreactive antigens were classified as negative (-). Reactive titers of 1:80 and above were regarded as positive (+) while titers less than 1:80 were negative (-). All Negative slide tests were confirmed by the tube test.

2.2. Analysis of Samples

The blood samples were examined using two methods, the widal test examination for the diagnosis of Typhoid fever, and blood culture for the isolation of salmonella typhi, the causative organism of Typhoid fever. The widal tube agglutination test was first done on each blood sample so as to determine the serum/antigen titre level before performing the widal slide agglutination test.

This would help in the proper interpretation of the results of the results of the slide agglutination test which is mostly used in our laboratories. Blood culture was then done on each sample for the definitive diagnosis of typhoid fever.

2.3. Widal Slide Agglutination Test

After the tube agglutination test gave the titer values, the slide agglutination test was done for each serum sample. Equal amount (0.5ml) of undiluted patient's serum and antigens O_A, O_B, O_C, and O were placed side by side on a plastic agglutination slide and rocked by hand for one minute. The same procedure was repeated for the H_A, H_B, H_C and H antigens O_A, O_B and O_C represent S. paratyphi O antigen

while H_A, H_B and the represent S. paratyphi H antigen. O and H represent S. typhi O and H antigens, respectively. Agglutinations were noted as either positive or negative, agglutinations greater than or equal to the titer shown in the tube agglutination test were regarded as significant and counted as positive.

2.4. Biochemical Tests

Biochemical tests were carried out on blood culture isolates of each sample to confirm the presence of Salmonella which are as follows Gram reaction, motility test, lactose fermentation, glucose fermentation test and citrate utilization test. The procedures for the test are according to monica chessbrugh 1998.

3. Results

The results of the widal tube agglutination test are presented in Table1. Titer values from 1:80 and above were regarded as significant and therefore positive for the Salmonella antigen. A total of 50 (62.5% approx) out of the 80 children had significant (O titre \geq 1:80, H titre \geq 1:80) slide agglutination titer values and therefore were regarded as positive.

Blood culture is widely accepted as the definitive tool for the diagnosis of typhoid fever. Isolation of the bacteria (Salmonella typhi) in the blood indicates bacteria. In this study, 44 (55%) of the 80 childrens positive for salmonella species infection as shown by growth on MacConkey agar (Table 3).

Biochemical tests carried out on the blood culture isolates of each sample were Gram staining, motility test, lactose fermentation test, glucose fermentation test and citrate utilization test, for the two zones. The results are presented in table 4.

Table 1. "O" Agglutination titer in children of different age groups

Groups	Number of Cases	1:80 (%)	1:160 (%)
Normal children	20	1(5)	0
Children between 5-10 years of age with typhoid fever	20	1(5)	4(20)
Children between 10-15 years of age with typhoid fever	20	0(0)	4(20)
Children under 5 years of age with typhoid fever	20	2(10)	5(20)

Table2. "H" Agglutination titer in children of different age groups

Groups	Number of Cases	1:80 (%)	1:160 (%)
Normal children	20	1(5)	0
Children between 5-10 years of age with typhoid fever	20	2(10)	10(50)
Children between 10-15 years of age with typhoid fever	20	1(5)	10(50)
Children under 5 years of age with typhoid fever	20	3(15)	9(45)

Table 3. Correlation of widal and blood culture

Groups	Positive widal cases	Positive Blood culture cases
Normal children	3	0
Children between 5-10 years of age with typhoid fever	17	16
Children between 10-15 years of age with typhoid fever	14	11
Children under 5 years of age with typhoid fever	19	17

Table 4. Results of Biochemical test

Groups	No. of Isolates Examined	Gram Staining	Motility	Lactose Ferm	Glucose Ferm	Citrate Utilization
Normal children	20	Very few –	Very few +	Very few –	Very few +	Very few –
Children between 5-10 years of age with typhoid fever	20	–	+	–	+	–
Children between 10-15 years of age with typhoid fever	20	–	+	–	+	–
Children under 5 years of age with typhoid fever	20	–	+	–	+	–

4. Discussion

Typhoid fever is a major public health problem associated with significant morbidity and mortality in many countries[9]. Blood culture has remained the gold standard test in diagnosis of typhoid fever, but its utility in early diagnosis is limited in early phase of illness thereby making the isolation of the organism difficult. Although the Widal test at this cut-off titer performed relatively well in terms of sensitivity, specificity and NPV, its PPV was low. It has been argued that PPV is the most important measure of a clinical diagnostic method since it represents the proportion of patients with positive test results that are correctly diagnosed[10]. The PPV is not intrinsic to the test; it is affected by prevalence of the disease.

There are several difficulties associated with evaluation of the Widal test. Firstly, levels of agglutinins detectable in the non-infected populations of different areas vary considerably by time and place depending on the endemicity of the disease, which affects test performance. For example, the sensitivity and specificity of a Widal test. Widal positivity is more of an epidemiological evaluation rather than clinical because a rising titer repeated after two weeks duration should be demonstrated before it is of clinical significance, although this has also been under serious criticism in recent years. Sharing of O and H antigens by other *Salmonella* serotypes and other members of *Enterobacteriaceae* makes the role of widal test even more controversial in diagnosing typhoid fever[11].

5. Conclusions

It is concluded, that even today, the Widal test remains one of the best, easily accessible, cheap and simple method in comparison to other molecular and biochemical test for the diagnosis of typhoid fever.

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