A COMPARATIVE STUDY OF WIDAL TEST WITH BLOOD CULTURE IN THE DIAGNOSIS OF TYPHOID FEVER IN FEBRILE PATIENTS ATTENDING GGH, ANANTHAPURAMU.

Microbiology

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ABSTRACT

Introduction Typhoid fever is a systemic prolonged febrile illness caused by certain Salmonella serotypes including Salmonella typhi, S. paratyphi A, S. paratyphi B and S. paratyphi C. 1 Human beings are the only reservoir host for typhoid fever. It is transmitted by feco-oral contamination of water and food in endemic areas especially by carriers handling food. The World Health Organization (WHO) estimates about 21 million cases of typhoid fever with >600,000 deaths annually. 2 The cases are more likely to be seen because of rapid population growth, increased urbanization, and limited safe water, limited infrastructure and health systems.

Aims & Objectives: The main aim of this study is to compare the result of Widal test and blood culture in the diagnosis of typhoid fever in febrile patients.

Material & Methods: This is a prospective study conducted in the GGH Ananthapuramu, for a period of 18 months i.e. from May 2018 to Oct 2019. Blood samples were collected from 542 febrile patients with symptoms clinically similar to typhoid fever. Blood culture was used to isolate S.typhi and S.paratyphi. Slide agglutination test and tube agglutination tests were used for the determination of antibody titer. An antibody titer of $\geq 1:80$ for anti TO and $\geq 1:160$ for anti TH were taken as a cut of value to indicate recent infection of typhoid fever.

Results: A total of the 542 patients were the study population, among which 384 (70.8%) were females, and 158 (29.1%) were males. Widal and blood culture was performed, 11 (2.02%) cases of S. typhi and 5 (0.9%) cases of S. paratyphi were identified using blood culture. The total prevalence of typhoid fever is 2.95%. The specificity, sensitivity, Positive Predictive Value, and Negative Predictive Value of the Widal test with O Ag is 70%, 81.25%, 10.93%, and 96%, respectively, and with H Ag is 40%, 96%, 18.1%, 98.8% respectively.

KEYWORDS

Widal test, Blood culture, Sensitivity, Specificity.

INTRODUCTION:

Typhoid fever is a systemic prolonged febrile illness caused by certain Salmonella serotypes including Salmonella typhi, S. paratyphi A, S. paratyphi B and S. paratyphi C. Human beings are the only reservoir host for typhoid fever. It is transmitted by feco-oral contamination of water and food in endemic areas especially by carriers handling food. The World Health Organization (WHO) estimates about 21 million cases of typhoid fever with >600,000 deaths annually.¹ The cases are more likely because of rapid population growth, increased urbanization, and limited safe water, limited infrastructure and health systems.^(2,3)

Accurate diagnosis of typhoid fever at an early stage is important not only for diagnosis of etiological agent, but also to identify individuals that may serve as a potential carrier, who may be responsible for acute typhoid fever outbreaks⁽⁴⁾. The Widal test has been used for more than 100 years as an important part of the diagnosis of typhoid fever^(5,6,7,8). It is a tube dilution test, which measures agglutinating antibodies against the lipopolysaccharide O and protein flagellar H antigens of S. typhi. The definitive diagnosis of typhoid fever is based on the isolation of S. typhi from blood, stool, urine or other body fluids^(9,10,11).

Aims & Objectives

The main aim of this study was to compare the result of Widal test and blood culture in the diagnosis of typhoid fever in febrile patients.

Methods:

This is a prospective study conducted in the GGH Ananthapuramu, for a period of 18 months i.e. from May 2018 to Oct 2019. Blood samples were collected from 542 febrile patients with symptoms clinically similar to typhoid fever. Blood culture was used to isolate S.typhi and S.paratyphi. Slide agglutination test and tube agglutination tests were used for the determination of antibody titer. An antibody titer of $\geq 1:80$ for anti TO and $\geq 1:160$ for anti TH were taken as a cut of value to indicate recent infection of typhoid fever.

Tube agglutination test: Patients serum sample was serially diluted by using fresh 0.95% saline preparation from 1:20 to 1:640 for anti TO

and anti TH separately in 12 test tubes. Then a drop of O antigens and H antigens are added in the test tubes, equal amount in all. Based on the manufacturer manual, an antibody titer of 1:80 and higher for anti TO and 1:160 and higher for anti TH antibodies were taken as a cut of value to indicate recent infection of typhoid fever.

Blood culture: Using a sterile syringe and needle, about 8–10 ml of blood from each adult study subject and about 3–5 ml of blood from each young child was collected. Then 5–7 ml from adults and 2-3 ml of blood from children was dispensed into the culture medium bottle containing 45 ml of BHI broth and then incubated at 37°C.

Sub Culturing And Biochemical Identification: After 24 hours of incubation sub-culturing was performed on XLD agar. After overnight incubation positive cultures were proceeded for further identification using biochemical tests, Triple Sugar Iron agar (TSI) citrate utilization test motility, urease test and lysine decarboxylation (LDC) test. Reagents were obtained from Himedia Mumbai. While Negative broth cultures were incubated for seven days and sub cultured before reported negative.

RESULTS:

A total of 542 patients attending GGH Ananthapuramu were analysed among which 384 (70.8%) were females, and 158 (29.1%) were males.

Qualitative Slide Agglutination Widal Test:

Qualitative slide agglutination test was performed as a primary screening test, it will detect the presence or absence of O antigen And H antigen of Salmonella typhi.

Among 542 cases 361(66.6%) cases show reactive agglutination for O antigen and 314(57.9%) cases show reactive agglutination for H antigen and 181(33.4%) patients have Non Reactive reaction result for H antigen and 228(42.1%) patients for O antigen.

Table 1: Qualitative Slide Agglutination Reaction Results Of Widal Test Of Febrile Patients Suspected Of Typhoid Fever

	O antigen		H antigen	
	Frequency	%	Frequency	%

Reactive	361	66.6	314	57.9
Non Reactive	181	33.4	228	42.1
Total	542	100	542	100

Semi Quantitative Tube Agglutination Test: (titration):

The patient sample whose slide agglutination test was reactive were tested for titration (Semi Quantitative tube agglutination).

The number of patients, reactive for O antigen were 361(542) and for H Ag were 314(542).

155 of 361 (42.9%) cases did not show any agglutination for O antigen in the Semi Quantitative tube agglutination test, 134 of 314 (42.6%) did not show any agglutination for H antigen in the Semi Quantitative tube agglutination test.

The cut of value of antibody titer for O Antigen was 1:80, and for H Antigen was 1:160 was taken to detect recent infection with Salmonella typhi.

Taking Anti TO $\geq 1:80$ as a cut of value we found 56(15.5%) patients, had typhoid infection, and Anti TH $\geq 1:160$ as a cut off value, 22 (7.0%) patients had recent typhoid infection.

The total number of patients who had indicative of recent either by O or H Ag is 48, among these the total number of patients who had indicative of recent either both O $\geq 1:80$ and H O $\geq 1:160$ Antigen is 29.

Table 2: The Frequency Distribution Of Semi Quantitative Tube Agglutination Titration Test Of Widal Test In Febrile Patients Suspected Of Typhoid Fever.

Titre	O Antigen			H Antigen		
	Frequency	% (n=361)	% total (n=542)	Frequency	% (n=314)	%total (n=542)
No agglutination	155	43	28.6	134	42.7	24.7
1:20	94	26	17.3	108	34.4	20
1:40	78	21.6	14.4	36	11.4	6.6
1:80	37	4.23	2.76	14	4.5	2.6
1:160	14	3.87	2.58	13	4.1	2.4
1:320	3	0.8	0.56	9	2.9	1.7
1:640	2	0.5	0.37	0	0	0
Total	361	100	66.57	314	100	58

Blood culture : Of the 542 blood cultures, only eleven cases (2. 03%) S. typhi were isolated from the patients while S. paratyphi cases were five cases (0.92%) . The total prevalence of typhoid fever is 2.95%.

Table 3: The Distribution Of Blood Culture Results Of Febrile Patients Suspected Of Typhoid Fever.

Bacteria	Number of isolates (%)	Percentage %
S. typhi	11	2.03%
S. paratyphi	5	0.92%
Other bacteria	130	23.98%
Negative blood culture	396	73.06%
Total	542	

Table 4: Prevalence Of Typhoid Fever

Prevalence	No. of Cases	%
S.typhi	11	2.03%
S.paratyphi	5	0.92%
Prevalence		2.95%

Table 5 : Showing Specificity, Sensitivity, Positive Predictive Value, And Negative Predictive Value Of Tube Agglutination Test.

	Anti TO Antibodies	Anti TH Antibodies
Specificity	70%	40%
Sensitivity	81.25%	96%
Positive Predictive Value	10.93%	18.10%
Negative Predictive Value	96%	98.8

The specificity, sensitivity, Positive Predictive Value, and Negative Predictive Value of the Widal test with Anti TO Antibodies is 70%, 81.25%, 10.93%, and 96%, respectively, and with H Ag is 40%, 96%, 18.1%, 98.8% respectively.

DISCUSSION:

The sensitivity and specificity of Widal titer of anti TO 1:80 and higher in this study were about 70% and 81.25% respectively and 40% and 96% for anti TH titer of 1:160 and higher. The overall sensitivity of titer positive Widal test was about 70%, similar with anti TO titer because there was no only anti TH titer positive culture proven typhoid fever identified. This is similar with the study conducted in the endemic area of Vietnam by Olsen et al. for the evaluation of serodiagnostic assay of acute enteric fever [12]. Another study done in Kenya has shown that Widal testing done on acute phase serum of patients suspected to have typhoid fever had limited diagnostic capability given its low sensitivity in which among all typhoid cases only 26% had diagnostic titer while 53.6% had O and H titer less than 1:40 [13]. This is similar with the study conducted by Andualem et al. St. Paul's Hospital, in Ethiopia.[1]

CONCLUSION:

Widal test has a low Sensitivity, Specificity and Positive Predictive Value, but it has good Negative Predictive Value which indicates that a negative Widal test result has a good predictive value for the absence of the disease but a positive result would have a low predictive value for the presence of typhoid fever.

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