Original Research Article

Study of the Cut off Titres and Determination of the Significant Titres for the Widal Agglutination Test for the Diagnosis of Enteric Fever by a Single Serum Test in a Tertiary Care Hospital, Rims, Ranchi (Jharkhand)

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Abstract

Aim: The aim of the present study was to establish the cut off titers and to determine the significant titers for the Widal agglutination test for the diagnosis of enteric fever by a single serum test in RIMS, Ranchi.

Methods: The study was carried out in the Serology section of Dept of Microbiology, Rajendra Institute of Medical Sciences, Ranchi, Jharkhand(India)for a period of 12 months. Volunteers of both the sexes of 18-50 years of age group of different community of Ranchi who live here for more than 5 years and coming to our microbiology and biochemistry department for different routine test were included. 300 patients were selected in the study.

Results: There were equal number of male and females. People of 18-50 years of age group were included. Among them maximum no. of people were from 21-30 yrs age- group and minimum no. of people were from 18-20 yrs. 20% were illiterate and 42.33% had tap water form municipal as source of water. 52.67% had food born disease awareness. Among 300 samples under the study, 170 had positive (i.e. ≥ 20) end titre value and 130 had negative (i.e. <20) end titer value. Highest frequency (7.69%) of samples with overall positive end titer values for Salmonella Para typhi AH agglutinin belongs to age group 18-20 years, followed by age group 41-50 years (7.1%), then 31-40 years (6.9%) and lastly of 21-30 years (5.88%) respectively. Overall positive end titer values for Salmonella Para typhi BH shown by 7.67% samples only.

Conclusion: Overall, the level of H agglutinins was found to be more helpful than the level of O agglutinins. When the O-agglutinin titer is ≥400 or the H-agglutinin titer is ≥200, typhoid can be diagnosed with reasonable confidence.

Keywords: cut off titers, significant titers, Widal agglutination, enteric fever

Introduction

Salmonella enterica serotype Typhi (S. typhi), the causative agent of typhoid fever, was calculated to have caused approximately 200,000 deaths globally in 2000. [1] The clinical picture of typhoid fever is nonspecific; confirmed diagnosis through blood or bone-marrow culture requires expensive and labor-intensive isolation and identification of the organism, which may take up to seven days. A cheap and rapid alternative laboratory test is desirable, especially for developing country settings where typhoid fever is a major public health burden.

Various agglutination tests have been developed[2] of which the Widal method is the oldest and remains the most widely used. The test was first introduced by F. Widal in 1896 [2] and is based on a macroscopically visible serum - mediated agglutination reaction between S. typhi somatic lipopolysaccharide O antigens (TO) and flagellar H antigens (TH). Laboratories in industrialized countries have stopped performing the assay. In Africa the Widal test is still widely used because typhoid fever is perceived to be endemic in the area [3] and the Widal test is the only rapid diagnostic assay that is available and affordable. The Widal test is commonly performed when children and adults present with fever to treatment centers, as few centers have the capacity to perform micro-bacterial culture. [4]

The signs and symptoms of uncomplicated typhoid fever are nonspecific, and an accurate diagnosis on

clinical grounds alone is difficult. [5] Although a definitive diagnosis can be made by isolation of Salmonella typhi from blood or bone marrow [6], in areas of endemicity, such as Vietnam, bacterial culture facilities are often unavailable and the Widal test is the only specific diagnostic investigation tool available. The Widal test has been in use for more than a century as an aid in the diagnosis of typhoid fever. [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 value of the test for the diagnosis of typhoid fever has been debated for as many years as it has been available. [9,10] There is no consensus concerning diagnostic criteria for interpreting the test. Serological diagnosis relies classically on the demonstration of a rising titer of antibodies in paired samples 10 to 14 days apart. In typhoid fever, however, such a rise is not always demonstrable, even in blood culture-confirmed cases. This situation may occur because the acute-phase sample was obtained late in the natural history of the disease, because of high levels of background antibodies in a region of endemicity, or because in some individuals the antibody response is blunted by the early administration of an antibiotic. [10]

The aim of the present study was to establish the cut off titers and to determine the significant titres for the Widal agglutination test for the diagnosis of enteric fever by a single serum test in Ranchi.

Materials and Methods

The study was carried out in the Serology section of Dept of Microbiology, Rajendra Institute of Medical Sciences, Ranchi Jharkhand, India for a period of 12 months. Volunteers of both the sexes of 18-50 years of age group of different community of Ranchi who live here for more than 5 years and coming to our microbiology and biochemistry department for different routine test were included. 300 patients were selected in the study.

Inclusion Criteria-

1) Apparently healthy volunteers (18-50 years) who has been living in Ranchi since 5 years and above.
2) Volunteers who had not taken antibiotic within past 1 month.

Exclusion Criteria-

1) Persons who were with active infection, febrile or recently been treated for malaria, microfilaria, typhoid fever, tuberculosis, hepatitis, syphilis or AIDS.
2) Individuals who had been vaccinated for typhoid in the past three years.
3) History of fever of unknown origin.
4) Individuals of less than 18 years and more than 50 years of age.

Informed consent of volunteers for this study was also obtained.

Confidentiality of subjects and their data were maintained.

Name of the test- Widal agglutination test by tube (serial dilution) method.

Reagents-

Killed coloured suspension of-

• Salmonella Typhi O antigen

• Salmonella Typhi H antigen

• Salmonella paratyphi A H antigen

• Salmonella paratyphi B H antigen

Note: - Two type of reagents were used

1.Reagent extracted in CRI ,Kasauli(India) .

2.Reagent of Patho zyme diagnostic

Sample collection and processing-

Five (5) milliliters of blood samples were collected by venipuncture from consenting volunteers in a plain vial. The blood was allowed to be clotted for half an hour and then transported to the laboratory and centrifuged at 3000 rpm for 3 minutes in order to separate the serum from blood cells.

All sera were stored at 2- 8 degree Celcius in refrigerator and analyzed within 2-3 days. 0.4 ml of 2-fold serially diluted sera (dilutions from 1:20 to 1:320) in normal saline were tested by adding an equal amount of antigen.

A negative control is included in each batch of test.

Procedures-

1) For each serum, prepared a starting dilution in saline eg;- 1 in 10 dilution by pipetting 0.1 ml serum into 0.9 ml normal saline.
2) For each bacterial antigen, arranged in a rack, a row of a mixing tubes, eg;- tubes no. 1-6 for six doubling serum dilution and tube no. 7for a control without serum.
3) With a fresh graduated 1 ml pipette, placed 0.4ml normal saline in each of tubes 2-7 .Then pipetted 0.4ml of the starting serum, dilution into the empty tube 1, and another 0.4ml into saline, containing tube2.
4) Mixed the fluid in tube 2 by pipetting up and down several times. Then transferred 0.4ml from tube2 into tube3.
5) With the same pipette mix the contain of tube 3and transfer0.4 ml into tube 4. Repeated the process up to tube no.6 from which after mixing discarded 0.4 ml. Now each tube is containing 0.4ml fluid, tube no. 1-6 containing serum dilutions of 10,20,40,80,160,320 and tube no.7 only saline.

6) With a fresh graduated 1ml pipette, added 0.4 ml of the bacterial suspension to each tube, starting at tube 7 and working back from tube 6 to tube 1.the serum dilution in tube 1 to tube 6 are now 20, 40, 80,160,320 and 640.

7) Place the rack of agglutination tubes in water bath (maintained at 370C) for overnight (18 -24 hrs).

8) Read the result by viewing the tube under good light with the aid f a magnifying lens. '0'

agglutination were visible as a small granules and 'H' agglutination were visible as large flakes of wool.

9) Highest dilutions of serum anti-O, anti-H, anti-AH and anti-BH agglutinin showing visible agglutination were taken as end point titre.

Results

Table 1: Baseline characteristics

Gender	No. of people	Percentage
Male	150	50%
Female	150	50%
Age – group		
18-20 yrs	39	13%
21-30 yrs	119	39.66%
31-40 yrs	72	24%
41- 50 yrs	70	23.33%

There were equal number of male and females. People of 18-50 years of age group were included. Among them maximum no. of people were from 21-30 yrs age- group and minimum no. of people were from 18-20 yrs. 20% were illiterate and 42.33% had tap water form municipal as source of water. 52.67% had food born disease awareness.

Table 2: Distribution of positive (≥ 20) and negative (<20) end titre values

End Titre Values	No. Of samples	Percentage
Positive	170	56.66%
Negative	130	43.33%
Total	300	100%

Among 300 samples under the study, 170 had positive (i.e. ≥ 20) end titer value and 130 had negative (i.e. <20) end titre value. Salmonella end titers (≥20) for any agglutinins i.e. S. Typhi O, S. Typhi H, S. Para typhi AH, S. Para typhi BH were taken as positive and end titers (< 20) were taken as negative.

Table 3: Age distribution of Salmonella Para typhi AH agglutinin end titer values in percentage

Age group	< 20	20	40	80	160	≥20 Total
18-20 yrs	92.3%	5.12%	2.56%	0%	0%	7.69%
21-30 yrs	94.11%	3.36%	1.68%	0.84%	0%	5.88%
31-40 yrs	93%	4.16%	2.77%	0%	0%	6.9%
41-50 yrs	92.85%	5.7%	1.42%	0%	0%	7.1%
Total	93.33%	4.33%	2%	0.33%	0%	6.67%

The overall positive end titre value for Salmonella paratyphi AH by 6.67% samples only. Highest frequency (7.69%) of samples with overall positive end titer values for Salmonella Para typhi AH agglutinin belongs to age group 18-20 years, followed by age group 41-50 years (7.1%), then 31-40 years (6.9%) and lastly of 21-30 years (5.88%) respectively. Highest frequency of samples (4.33%) corresponds to most common end titer value 20.

Table 4: Age distribution of Salmonella Para typhi BH agglutinin end titre values

Age group	< 20	20	40	80	160	≥20 Total
18-20 yrs	89.74%	10.25%	0%	0%	0%	10.25%
21-30 yrs	94.11%	3.36%	2.52%	0%	0%	5.88%
31-40 yrs	91.66%	4.16%	1.38%	2.77%	0%	8.33%
41-50 yrs	91.4%	5.7%	2.87%	0%	0%	8.57%
Total	92.33%	5%	2%	0.67%	0%	7.67%

Overall positive end titre values for Salmonella Para typhi BH shown by 7.67% samples only. The highest frequency (10.25%) of people with overall positive end titer value for Salmonella Para typhi BH agglutinin belongs to age group 18-20 years, followed by age group 41-50 years (8.57%), 31-40 years (8.33%) and of 21-30 years (5.88%) respectively.

Discussion

Enteric fever is endemic in the Indian subcontinent, and population-based surveillance studies in selected regions add considerably in estimating the global burden of enteric fever. The incidence rate of typhoid is more than 100 cases per 100000 people per year. [11] Enteric fever caused by Salmonella typhi is called as typhoid fever and Salmonella paratyphi causes the paratyphoid fever. Typhoid is characterized by malaise, fever, abdominal discomfort, relative bradycardia, transient rash, and organomegaly and may end up in complications such as intestinal perforation, hemorrhage, and perinephric abscess. [12,13]

There were equal number of male and females. People of 18-50 years of age group were included. Among them maximum no. of people were from 21-30 yrs age- group and minimum no. of people were from 18-20 yrs. 20% were illiterate and 42.33% had tap water form municipal as source of water. 52.67% had food born disease awareness. Among 300 samples under the study, 170 had positive (i.e. ≥ 20) end titre value and 130 had negative (i.e. <20) end titer value. Salmonella end titres (≥20) for any agglutinins i.e. S. Typhi O, S. Typhi H, S. Para typhi AH, S. Para typhi BH were taken as positive and end titres (< 20) were taken as negative. The overall positive end titre value for Salmonella paratyphi AH by 6.67% samples only. The baseline titer determined was 40 for anti-O and anti-H antibodies and <20 for anti-AH and anti-BH antibodies in another study done in North Kerala where public health facilities and hygienic conditions are better. [14] Another study done in Raichur recommended TO and TH titer of ≥320 as diagnostic of typhoid fever, and for AH and BH titers, it is ≥40 and ≥160, respectively. [15] Signiicant Widal titer study done in rural Pondicherry recommended ≥160 for O and H agglutinins while for AH to be ≥80 and BH to be ≥40 to be considered a significant titer in diagnosing typhoid. [16] A study done in Dehradun on apparently healthy population found significat agglutination titer for TO ≥20 in 28%, TO ≥40 in 24.6%, TO ≥80 in 10.3%, TH ≥80 in 7.6%, AH ≥20 in 6.6%, and BH ≥20 in 4.6%. [17]

Highest frequency (7.69%) of samples with overall positive end titre values for Salmonella paratyphi AH agglutinin belongs to age group 18-20 years, followed by age group 41-50 years (7.1%), then 31-40 years (6.9%) and lastly of 21-30 years (5.88%) respectively. Highest frequency of samples (4.33%) corresponds to most common end titre value 20. Overall positive end titre values for Salmonella Para typhi BH shown by 7.67% samples only. The highest frequency (10.25%) of people with overall positive end titre value for Salmonella Para typhi BH agglutinin belongs to age group 18-20 years, followed by age group 41-50 years (8.57%), 31-40 years (8.33%) and of 21-30 years (5.88%) respectively. 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 anti-TO titer of 1:80 in Kolkata, India was 58% and 85%. [18] There is also the possibility of cross-reactivity with non-bacterial infections such as malaria, dengue, hepatitis A, and infectious mononucleosis. [20]

Conclusion

Overall, the level of H agglutinins was found to be more helpful than the level of O agglutinins. When the O-agglutinin titer is ≥400 or the H-agglutinin titer is ≥200, typhoid can be diagnosed with reasonable confidence.

Based on results of our study, for the population of Ranchi, we recommend significant cut off levels for single antibody titre against-

Salmonella Typhi O as ≥1:160 ; Salmonella Typhi H as ≥ 1:160; Salmonella paratyphi AH ≥1:40, Salmonella paratyphi BH ≥1:40 .

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