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Original Research Article

Study of Gender wise Distribution of the Significant Widal Titers of Salmonella Agglutinins among Healthy People in Ranchi, Jharkhand (India)

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Abstract

Aim: The aim of the present study was to determine significant Widal titers of Salmonella agglutinins among healthy people of different genders in Ranchi, Jharkhand (India.)

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 titre value. The highest number of males (32) having positive end titers value at 40. Among 135 Positive end titer values 73, (54.08%) were females and 62 (45.93%) were males. The highest no. (21) of females having positive end titer value at 80 and highest no. (20) of males having positive end titer value at 80.

Conclusion: We concluded that widal test is an accessible, cheap and simple test which has diagnostic significance in endemic areas provided judicious interpretation of the test is made based on the agglutinin levels prevalent in the normal population of the region at a particular time period.

Keywords: Widal titers , Salmonella agglutinins, Genderwise, healthy people .

Introduction

Enteric fever is a serious global public health problem in developing countries including India with global estimate of >21.6 million cases annually. [1] The causative organism is Salmonella typhi transmitted to human beings through feco-oral route resulting in considerable morbidity. It afflicts the local community and travelers to the endemic areas. The incidence rises during the rainy season due to water logging and contamination of water with fecal material. Social factors favoring it, are the pollution of drinking water supplies due to open defecation, urination, personal hygiene habits, and health ignorance. [2]

Clinical diagnosis of typhoid may be difficult because of altered or atypical presentation of patients. In developing countries, often patients visit hospital late in course of illness or they take drugs as self or unauthorized prescription before reporting to doctors. The gold standard and definitive diagnosis is by isolation of Salmonella enterica serotype Typhi, Para typhi A, and Para typhi B from blood, bone marrow, stool, or urine which is about 90% in the 1st week of illness and decreases to about 50% by the 3rd week. [3]

Blood culture has demerits such as unavailability, cost, and relatively long turnaround time (TAT), hence not much utilized test in developing countries. Hence, an alternative is Widal test which is simpler, rapid, and cost-effective. The test becomes reliable if at least two properly staged tests show about a four-fold rise in antibody levels. [4] In India, most of the patients present late to the hospital and they

require an immediate diagnosis and a specific treatment and often, a single sample has to be relied upon, instead of paired serum samples [5] and so, a single cutoff value is widely used. [6]

The test becomes reliable if at least two properly staged tests show about a four-fold rise in antibody levels. [7] In India, most of the patients present late to the hospital and they require an immediate diagnosis and a specific treatment and often, a single sample has to be relied upon, instead of paired serum samples [8] and so, a single cutoff value is widely used. Widal test can be used as a diagnostic tool in endemic areas, if we know the baseline titer in a population. Interpretation of a single Widal test result needs to be based on the average baseline titer which is seen among healthy individuals.

The aim of the present study was to determine genderwie Significant Widal titers of Salmonella agglutinins among healthy people of Ranchi, Jharkhand (India.)

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) 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 colored suspension of-

- Salmonella Typhi O antigen
- Salmonella Typhi H antigen
- Salmonella para typhi A H antigen
- Salmonella para typhi 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%

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 titer 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 tires (< 20) were taken as negative.

Table 3: Gender distribution of Salmonella Typhi O agglutinin end titre values

S.TyphiO end titre	Female	Male	Total
<20	77	88	165
20	16	16	32
40	33	32	65
80	23	13	36
160	1	1	2
Total	150	150	300

The highest number of males (32) having positive end titres value at 40.

Table 4: Gender distribution of positive end titre values for Salmonella Typhi O

Positive End Titre value	Female	Male
≥20 titre (Percentage)	73 (54.07%)	62 (45.93)

Among 135 Positive end titer values 73, (54.08%) were females and 62 (45.93%) were males.

Table 5: Gender distribution of end titer values of Salmonella Typhi H agglutinin

S.TyphiO end titre	Female	Male	Total
<20	99	103	202
20	17	17	34
40	13	9	22
80	21	20	41
160	0	1	1
Total	150	150	300

The highest no. (21) of females having positive end titer value at 80 and highest no. (20) of males having positive end titer value at 80.

Discussion

Enteric fever is endemic in all parts of India.[9] The term, 'enteric fever' includes typhoid fever which is caused by S. typhi and paratyphoid fever which is caused by S. para typhi A, B and C. Definitive diagnosis of enteric fever depends on isolation of Salmonella from blood, stool, urine, bone [10-12] However, in countries like India, isolation of organism is often jeopardized by lack of facilities or inadequate and/or improper antibiotic use prior to

culture and also, culture positive cases are very less, time consuming and expensive. For these reasons, laboratory diagnosis of enteric fever relies heavily on serological tests such as the Widal test. [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. Among 300 samples under the study, 170 had positive (i.e. ≥ 20) end titer value and 130 had negative (i.e. <20) end titer value. The highest number of males (32) having positive end titres value at 40. Among 135 Positive end titer values 73, (54.08%) were females and 62 (45.93%) were males. The highest no.(21) of females having positive end titer value at 80 and highest no. (20) of males having positive end titer value at 80. The highest no. of females (33) having positive end titers value at 40. Recent study done by Sreenath et al [14] showed the significant titers should be >1:80 for anti–TO and >1:160 for anti–TH for a presumptive diagnosis of typhoid fever. In study conducted by Shrikant Kogekar et al [15] at Indore, the positivity for TO antigen was comparable with the present study (49.72%), while for TH antigen, it was relatively very high to the tune of 52.26% of samples. In another study conducted by Bijapur et al [16] in North Kerala, 25.2% were found positive for TO antigen and for TH antigen, 15.2% of total samples were positive.

Conclusion

Widal test is an accessible, cheap and simple test which has diagnostic significance in endemic areas. Judicious interpretation of the test is made based on the agglutinin levels prevalent in the normal population of the region at a particular time period.

During study it was found that female population shows higher titre value than male population. Highest no. of females (21) having positive end titre value at 80 And highest no. of males (20) having positive end titre at 80.There is need of time to time determination of baseline titers of different community.

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