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Original Research Article

Study of the Titres of Salmonella Enterica Serotypes Typhi and Paratyphi A and B Agglutinins among Apparently Healthy Individuals using Tube (Serial Dilution) Method

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Abstract

Aim: The aim of the present study was to determine the titers of salmonella enterica serotypes typhi and paratyphoid A and B agglutinins among apparently healthy individuals using tube (serial dilution) method.

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. Salmonella end titres (≥20) for any agglutinins. Among 300 (100%) samples positive end titres values (≥20) of S. Typhi O, S. Typhi H, S. paratyphoid AH, S. Para typhi BH were 135 (45%), 98 (32.67%), 20 (6.67%), 23 (7.67%) respectively.

Conclusion: The average titer for antibodies to "O" and "H" antigens of Salmonella enterica serotype typhi was 1:40 and hence, based on the above results, it could be recommended to use a cutoff level of ≥1:80 for a single antibody test titer. Similarly, average titre for antibody to H antigen of Salmonella enterica serotype Para typhi A and Para typhi B was 1:80 and the cutoff level was ≥1:160 for a single antibody test titre.

Keywords: titers, serotypes typhi, Para typhi A and B agglutinins; healthy people, serial dilution method.

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. 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. Often the case remain undiagnosed. If titration is done then almost every person shows some titre value . this study is done to determine the titre vailue of apparently healthy people for different Serotype of Salmonella Typhi and Paratyphi B causing enteric fever.

The gold standard and definitive diagnosis is by isolation of Salmonella enterica serotype Typhi, Paratyphoid, 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 aim of the present study was to determine the average titre value of salmonella enterica serotypes typhi and Para typhi A and B agglutinins among apparently healthy individuals using tube (serial dilution) method.

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 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 2o- 8o C 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 titer.

Results

Table 1: Titration 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%
Level of Education		
Illiterate(I)	60	20%
Primary(P)	90	30%
Secondary(S)	90	30%
Tertiary(T)	60	20%
Source of drinking water		
Purified Filtered Water(PFW)	40	13.33%
Personal Motor(PM)	68	22.67%
Hand Pump(HP)	40	13.33%
Tap Water(TW) municipality supply	127	42.33%
Well(W)	25	8.33%
Food born disease awareness		
Yes	158	52.67%
No	142	47.33%

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 titre value and 130 had negative (i.e. <20) end titre value. Salmonella end titres (≥20) for any agglutinins i.e. S. Typhi O, S. Typhi H, S. Paratyphi AH, S. Paratyphi BH were taken as positive and end titres (< 20) were taken as negative.

Table 3: Distribution of positive end titre values of S. Typhi O, S. Typhi H, S. Para typhi AH, and S. Para typhi BH among people under the study.

Agglutinins	Positive	Negative	Total
S. Typhi O	135 (45%)	165 (55%)	300 (100%)
S. Typhi H	98 (32.67%)	202 (67.33%)	300 (100%)
S. Para typhi AH	20 (6.67%)	280 (93.33%)	300 (100%)
S. Para typhi BH	23(7.67%)	277 (92.33%)	300 (100%)

Among 300 (100%) samples positive end titers values (≥20) of S. Typhi O, Typhi H, S. Para typhi AH, S. Para typhi BH were 135 (45%), 98 (32.67%), 20 (6.67%), 23 (7.67%) respectively. Among 300 (100%) samples negative end titres values (<20) of S. Typhi O, Typhi H, S. Para typhi AH, S. Para typhi BH were 165 (55%), 202 (67.33%), 280 (93.33%), 277 (92.33%) respectively.

Table 4: Age distribution of Salmonella Typhi O agglutinin end titre values in percentage

Age group	< 20	20	40	80	160	≥20 Total
18-20 yrs	58.97%	7.7%	17.95%	12.82%	2.56%	41%
21-30 yrs	55.46%	12.61%	19.33%	11.77%	0.84%	44.53%
31-40 yrs	54.17%	11.11%	20.83%	13.88%	0%	45.83%
41-50 yrs	52.86%	8.57%	28.57%	10%	0%	47.14%
Total	55%	10.66%	21.66%	12%	0.66%	45%

Maximum proportion i.e. 47.14% of people with positive end titre values of Salmonella Typhi O agglutinin belongs to age group 41-50 years, followed by age group 31-40 years (45.83%), 21-30 years (44.53%) and lastly of 18-20 years (41%).

Table 5: Age distribution of Salmonella Typhi H agglutinins end titre values

Age group	< 20	20	40	80	160	≥20 Total
18-20 yrs	56.41%	12.82%	10.25%	20.51%	0%	43.59%
21-30 yrs	71.42%	10.08%	7.56%	10.92%	0%	28.57%
31-40 yrs	63.89%	11.11%	6.94%	16.67%	1.38%	36.11%
41-50 yrs	70%	12.86%	5.71%	11.42%	0%	30%
Total	67.33%	11.33%	27.33%	13.66%	0.33%	32.66%

Maximum proportion i.e. 43.59% of people with positive end titer value of Salmonella Typhi H agglutinins belongs to age group 18-20 yrs, followed by age group 31-40 yrs (36.11%), then 41-50 yrs (30%) and lastly of 21-30 yrs (28.57%).

Table 6: 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 no. of females (33) having positive end titres value at 40.

Table 7: 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 titre value at 80 and highest no. (20) of males having positive end titre value at 80.

Discussion

Salmonella enterica subsp. enterica serotype Typhi is the etiological agent of typhoid fever. In India, the disease is endemic with an incidence which ranges from 102 to 2219 per 100,000 populations. [7] It results in considerable morbidity, absenteeism and resource utilization. [8] Enteric fever afflicts the local community and the travelers to the endemic areas, the incidence being on upsurge during the rainy season due to water logging and the contamination of the water with faecal material. [9] The social factors that add to the enigma are the pollution of the drinking water supplies due to open air defecation, urination, sub-standard food, personal hygiene habits and health ignorance. The definitive diagnosis of enteric fever in the patients with a compatible clinical picture are the isolation of the Salmonellae from blood, bone marrow, stool or urine [10] and the demonstration of the 4 fold rise in the antibody titre to both the O and the H antigens of the organism between the acute and the convalescent phase sera. [11]

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. 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. Among 300 (100%) samples positive end titres values (≥20) of S. Typhi O, Typhi H, S. Para typhi AH, S. Para typhi BH were 135 (45%), 98 (32.67%), 20 (6.67%), 23 (7.67%) respectively. Among 300 (100%) samples negative end titres values (<20) of S. Typhi O, Typhi H, S. Para typhi AH, S. Para typhi BH were 165 (55%), 202 (67.33%), 280 (93.33%), 277 (92.33%) respectively. Maximum proportion i.e. 47.14% of people with positive end titre values of Salmonella Typhi O agglutinin belongs to age group 41-50 years, followed by age group 31-40 years (45.83%), 21-30 years (44.53%) and lastly of 18-20 years (41%). The highest no. of females (33) having positive end titres value at 40. Recent study done by Sreenath et al [12] 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 [13] 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 [14] in North Kerala, 25.2% were found positive for TO antigen and for TH antigen, 15.2% of total samples were positive.

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

Conclusion

The average titre for antibodies to “O” and “H” antigens of Salmonella enterica serotype typhi was 1:40 and hence, based on the above results, it could be recommended to use a cutoff level of ≥1:80 for a single antibody test titer. Similarly, average titre for antibody to H antigen of Salmonella enterica serotype Para typhi A and Para typhi B was 1:80 and the cutoff level was ≥1:160 for a single antibody test titre.

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