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Original Research Article

Study of the Baseline Widal Titres among Healthy People of Ranchi, Jharkhand, India

Khushboo Kumari[1], Kumari Seema[2], Ashok Kumar Sharma[3], Manoj Kumar[4]

[1]JRA, Department of Microbiology, RIMS, Ranchi, Jharkhand, India

[2] Research Scientist (VRDL), Department of Microbiology, RIMS, Ranchi, Jharkhand, India

[3]Associate Professor, Department of Microbiology, RIMS, Ranchi, Jharkhand, India

[4]Professor, Department of Microbiology, RIMS, Ranchi, Jharkhand, India

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Corresponding Author: Dr. Manoj Kumar
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Abstract

Aim: The aim of the present study was to determine baseline Widal titers of Salmonella agglutinins among healthy people 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 years 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 titer value and 130 had negative (i.e. <20) end titer value. Among 150 females 86 i.e. 57.33% were found to be positive (≥ 20) for the end titer value and 64 i.e. 42.67% were found to be negative (<20) for the end titer values. Among 150 males 84 i.e. 56% were found to be positive (≥ 20) for the end titer values and 66 i.e. 44% were found to be negative (< 20) for the end titer values.

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.

Keywords: Widal titers of Salmonella agglutinins,Endemic healthy people, Ranchi

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Introduction

Enteric fever is endemic in all parts of India. [1] 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. [1] Definitive diagnosis of enteric fever depends on isolation of Salmonella from blood, stool, urine, bone marrow, bile or other body fluids. [2-4] 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. [5]

The test becomes reliable if at least two properly staged tests show about a four-fold rise in antibody levels. [6] 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 [7] and so, a single cutoff value is widely used. [8] Widal test can be used as a diagnostic tool in endemic areas, if we know the baseline titre in a population. Interpretation of a single Widal test result needs to be based on the average baseline titre which is seen among healthy individuals.

The aim of the present study was to determine baseline Widal titers of Salmonella agglutinins among healthy people in Ranchi, Jharkhand State, 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) 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 venepuncture 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 centrifused 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 analysed 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%
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 (≥ 20) and negative (< 20) end titre values among females and male

End Titre values	No. of females	Percentage
Positive	86	57.33%
Negative	64	42.67%
Total	150	100%
End Titre values	**No. of males**	**Percentage**
Positive	84	56%
Negative	66	44%
Total	150	100%

Among 150 females 86 i.e. 57.33% were found to be positive (≥ 20) for the end titre value and 64 i.e. 42.67% were found to be negative (<20) for the end titre values. Among 150 males 84 i.e. 56% were found to be positive (≥ 20) for the end titre values and 66 i.e. 44% were found to be negative (< 20) for the end titre values.

Table 4: 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 titres values (≥20) of S. Typhi O, S.Typhi H, S. paratyphi 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 5: Age distribution of positive samples

Age group	Total no.	Positive	Percentage
18-20 yrs	39	25	64.10%
21-30 yrs	119	61	51.26%
31-40 yrs	72	41	56.94%
41-50 yrs	70	43	61.42%
Total	300	170	56.66%

Maximum proportion of people with positive finding belongs to age group 18-20 yrs (64.10%) and minimum proportion belongs to age group 21-30 yrs (51.26%).

Discussion

Every year, thousands of cases of typhoid and paratyphoid infections are reported worldwide. [9] Enteric fever continues to be a major health problem in developing countries. WHO estimated an annual infection rate of 21.6 million with global fatality rate of 10%. [10] Salmonella enterica serovar typhi (S. typhi) and Salmonella Para typhi A,B,C are the causative agents of enteric fever. Typhoid fever is an acute, life-threatening febrile illness caused by S. typhi which results in about 200,000 deaths worldwide each year largely in developing countries. [11]

In present study total 300 samples were taken. Visible agglutination for any agglutinin of Salmonella Typhi O, H and Salmonella Para typhi AH, BH were seen in 170 (56.66%) samples. This finding is comparable to Prasant et al (55.12%) [12], S. Gunjal et al (50.6%) [13] and Bijapur Gufran Ahmed et al (25.2%) [14] found less agglutination samples than that of present study. The prevalence of Salmonella enterica serotypes Typhi O, Typhi H, Para typhi AH and Para typhi BH agglutinins in healthy people of our study are 45%, 32.67%, 6.67% and 7.67% respectively. With respect to Salmonella Typhi O agglutinins, this study is comparable with findings of Pokhrel et al (54%). [15] With respect to Salmonella Typhi H agglutinins our finding (32.67%) is comparable to Jeyakumari et al (31.5%) [16] and Saxena et al (31.5%). [17] With respect to Salmonella paratyphi AH agglutinins our finding (6.67%) is comparable to Kataria et al (7.3%) [18] and Bijapur Gufran Ahmed et al [14] (6.8%). Higher prevalence were seen in the study of Pokharel et al (12%). [15]

In this study, Salmonella Typhi O agglutinins end titer value upto ≥1:20, ≥1:40, ≥1:80, ≥160 were present in 10.66%, 21.66%, 12% and 0.66% of samples respectively. Which is comparable to the values of Seema Mittal et al (2014) (15) .She found up to ≥1:20, ≥1:40, ≥1:80, ≥1:160 of Typhi O was present in 15%, 19.6%, 8.4% and 0% of samples respectively. Whereas Anupriya et al (2018) [19] showed 24%, 25%, 7.5% and 4.5% of samples corresponds to ≥1:20, ≥1:40, ≥1:80, ≥160 end titre values respectiely. Nidhi Sharma et al (2017) [20] in her study found that end titre values ≥1:20, ≥1:40, ≥1:80, ≥160 were present in 29.11%, 62.94%, 1.17% and 0.29% of samples respectively. Current study has maximum frequency (21.66%) corresponding to end titre value 1:40 ,which simulates with the study done by K shreenath et al[21] and Nidhi Sharma et al. [20]

Conclusion

We concluded that widal test is an accessible, cheap and simple test which has diagnostic significance in endemic areas.In endemic area,Ranchi Widal titre in the community is 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.

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