



Study of Baseline Titres for Widal test among Population in Kakinada Region of AP.

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Abstract

Background: Culture is the gold standard for diagnosis of enteric fever. Due to the poor isolations of *Salmonella* in recent times, Widal test is still a useful alternative aid. Good interpretation of the test cannot be made unless we know the correct baseline titres in population of particular area.

Aim and Objectives: To determine the base line titres of antibodies in healthy individuals.

Material and Methods: Blood samples were collected from 100 healthy individuals from August to November 2017. Widal tube agglutination test was performed to know the titres.

Results: In the total samples screened, most frequently recorded titre for O & H agglutinins were 1:40 for *Salmonella Typhi*. Similarly for *Salmonella Paratyphi A* and B, anti H titres were 1:20 each respectively.

Conclusion: It is concluded that the cut-off value of antibodies titres for *Salmonella Typhi* O and H antigens is >1:40, and for *Paratyphi A* & B H antigen is >1:20 in this region. Any titre more than this should be taken as high and interpreted in correlation with clinical findings or titers observed in paired sera.

Keywords: Baseline titre, Widal tube agglutination test, Enteric fever, Healthy population.

Introduction

Enteric fever continues to be a global health problem, especially in tropics and subtropics¹ and is a major endemic health problem in developing countries like India. The global estimation of typhoid fever was about 21.6 million in 2000 and 5412,744 illness were due to paratyphoid fever. These fevers are considered as a major cause of morbidity and mortality in developing countries with more than 90% of cases found in Asia only.^{2,3} The term Enteric fever includes typhoid fever which is caused by *Salmonella enterica* subspecies enterica serotype Typhi and paratyphoid fever caused by serotype Paratyphi A and Paratyphi B. The mode of transmission includes poor personal hygiene, contamination of

food and water. Since the clinical presentation of enteric fever is non-descript, laboratory tests are essential for diagnosis.

Definitive diagnosis of enteric fever depends on isolation of *Salmonella* from blood, stool, urine, bone marrow, bile or other body fluids⁴. However, in countries like India, isolation of organism is often jeopardized by lack of facilities or inadequate and/or improper antibiotic use prior to investigation, less culture isolations in recent times also which is time consuming and expensive. For these reasons, laboratory diagnosis of enteric fever relies heavily on serological tests such as the Widal test⁵. The test becomes reliable if at least two properly staged tests show about a four-fold rise in antibody levels⁶.

In India, most of the patients present late to the hospital and they require an immediate diagnosis and a specific treatment. Often, a single sample has to be relied upon, instead of paired serum samples⁷. For appropriate interpretation of the results, it is necessary to establish the baseline titres periodically. The present study was therefore, undertaken to establish the normal baseline titre in healthy individuals.

Material and Methods

Approval of the institutional ethical committee was obtained and the present community based cross-sectional study was conducted over a period of four months from August to November 2017 in the Department of Microbiology in this Tertiary care hospital. Blood samples were collected from healthy volunteers from both urban and rural areas between the age groups of 11 to 60 yrs which include both sexes. The study protocol and objectives was duly explained and a written consent was taken from the subjects before sample collection.

Inclusion criteria: Apparently healthy participants i.e. who did not have any obvious signs and symptoms of infectious in recent past i.e. < 6 months were included as study subjects.

Exclusion criteria: 1. Individuals who have suffered from fever in last six months. 2. Those who took typhoid vaccine any time in past 3 yrs.

Collection of sample: 5 ml of venous blood sample was collected from each participant and was allowed to clot at room temperature. Sera

were separated by using micropipette. The separated sera were labeled and stored at 2-8 °C for no more than seven days in case of delay in processing.

Widal test

All serum samples were subjected to standard tube agglutination method for the determination of antibodies against the antigens of Salmonella Typhi 'O', 'H' and Salmonella Paratyphi 'AH', 'BH' as per standard guidelines. A commercially available antigens which contained Typhi- O & H, Paratyphi-AH, BH were used, which were procured from Span diagnostic Ltd.

Serial dilutions of serum were done from 1:20 to 1:1280 and a drop of appropriate antigens was added to each tubes. The tubes were than incubated overnight at 37°C in a water bath. The results were interpreted and analyzed as per the standard guidelines. A negative control was included in each batch of the tests.

The Widal anti-O agglutinin (TO) and the anti-H agglutinin (TH) titre was taken as the highest dilution of serum showing visible agglutination.

Fig. no.1 Commercial Antigen kit used in present study



Fig.no.2. Serial double dilution method

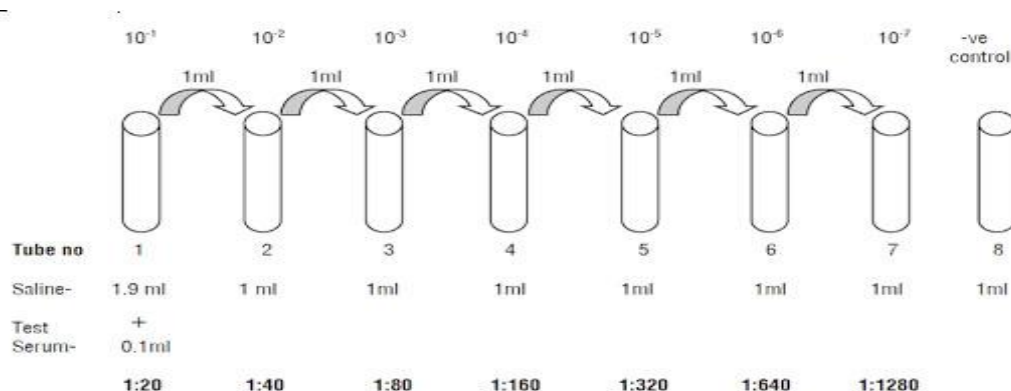
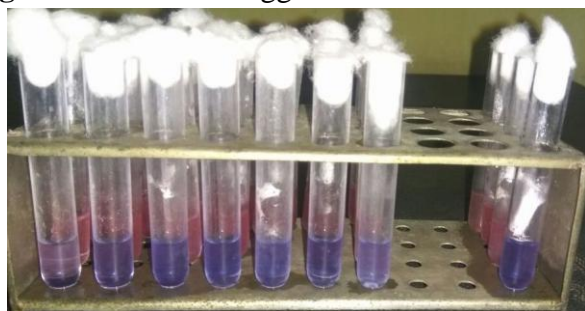


Fig.no.3. Widal tube agglutination test



Results

A total of 100 healthy subjects between age group of 11 to 60 years provided serum samples, for testing the baseline antibody titre against *Salmonella enterica* serotypes causing enteric fever, using Widal tube agglutination test.

The sample in the study includes subjects, distributed equally from both Urban & Rural areas belonging to age groups of 11-60years vide shown in Fig. no 4. Majority come between 11-30 years of age, shown in Fig no.5.

Fig.No.4. Sample distribution in the study

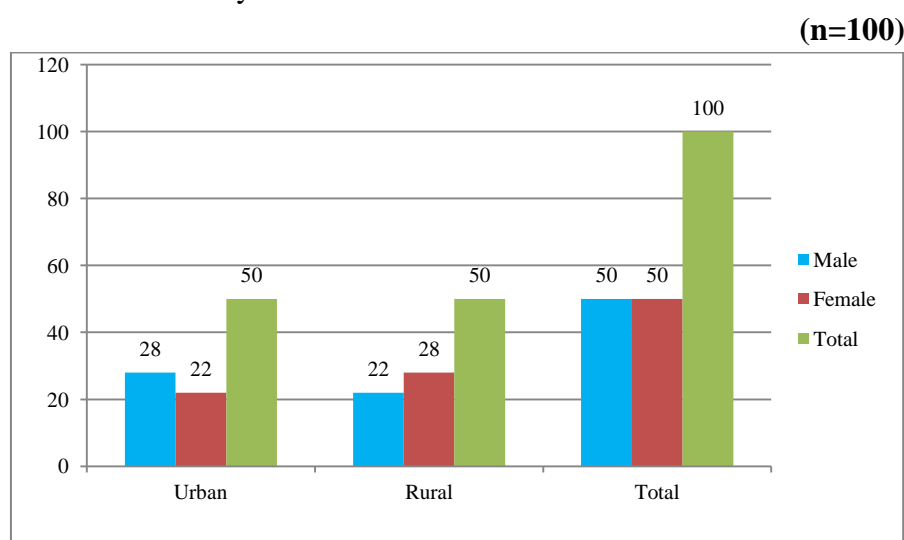


Fig.No.5. Age wise distribution

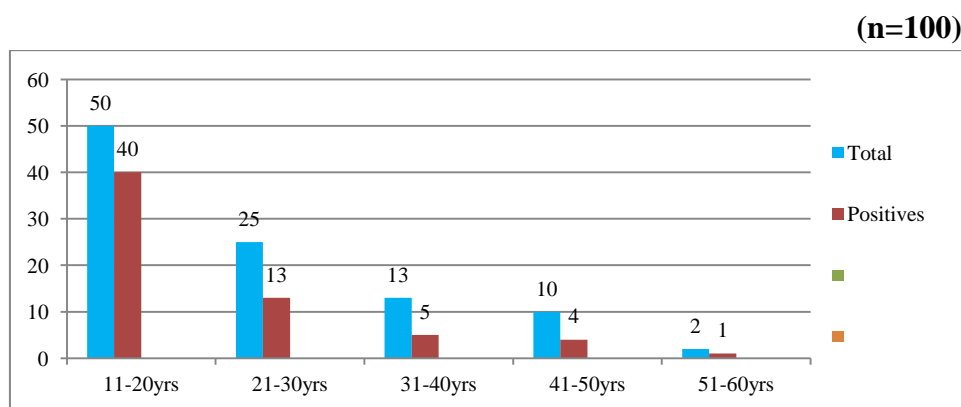


Table no. 1: Titres against different serotypes of *Salmonella enterica* in the study population

(n=100)

Serotype	Ab	Agglutination		Dilutions						
		No. Negative (%)	No. Positive (%)	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
S.Typhi	TO	11 (11%)	89 (89%)	18 (20.2%)	58 (65.1%)	10 (11.2%)	2 (2.2%)	1 (1.1%)	-	-
S.Typhi	TH	34 (34%)	66 (66%)	36 (54.5%)	27 (40.9%)	1 (1.5%)	1 (1.5%)	1 (1.5%)	-	-
S.Para A	AH	97 (97%)	3 (3%)	2 (66.6%)	1 (33.3%)	-	-	-	-	-
S.Para B	BH	98 (98%)	2 (2%)	2 (100%)	-	-	-	-	-	-

Table No.2 Titres of O and H agglutinins of *Salmonella enterica* serotype Typhi viewed in individual subjects

S.Typhi O	S.Typhi H	Total
1:20	1:20	18
1:40	1:20	18
1:40	1:40	27
1:40	1:80	1
1:40	1:160	1
1:40	1:320	1
1:80	1:40	0
1:80	1:80	0

Discussion

Early diagnosis of enteric fever is important for both outpatient & inpatient therapy. Culture isolation remains the gold standard for definitive diagnosis. But in resource constraint setting, it is not feasible to do on routine basis. Widal agglutination test is widely used as an alternative in developing countries due to its low cost, easy to perform and rapid result.

Analyzing the results, the present study showed titres for O antigen which is common to enteric fever causing salmonella, only 89% were reactive. In this 58(65.1%) showed titres of 1:40. In the remaining 31 samples 18 (20.2%) showed 1:20 followed by titres 1:80 in 10(11.2%), 1:160 in 2(2.2%) and 1:320 in 1(1.1%) sample. The best way to interpret H antigen titre can only be made if there is a rise in base line titre of O antigen. Likewise, if we see the results of both O & H titres for S.Typhi together in individual samples

vide Table no 1 highest numbers i.e. 26 showed titre of 1:40 for O with the corresponding H as 1:40. Remaining samples showed low and high variations. These variations could be explained due to anamnestic reactions, duration of exposure to salmonella antigens because of early treatment and individual variations in immune reactivity. As such in the present study the baseline antibody titre for S. Typhi O & H antigens can be fixed as 1:40 & 1:40 respectively since it is seen in majority of subjects.

Similarly with regard to S. Paratyphi A & B the baseline titre for O can be taken as 40 since it is common for all Salmonella serotypes causing enteric fever. When it comes to H antigen of S. Paratyphi A & B, unlike S. Typhi most of the subjects i.e 97-98 % in the study population showed negative titre. Only 5 samples gave positive titre in which 4 showed titres of 1: 20. This indicates that the exposure to Salmonella Paratyphi infections is relatively less when compared to S.Typhi in this area.

Statistical analysis was performed by SPSS (Statistical Package for Social Science) 20.0 software for baseline titres by using one sample student t test to know the statistical significance of the difference in Widal titre obtained in the present study (O & H 1: 40) to that of conventional baseline titre value (O 1:80, H 1:160). Here the result shows p value is <0.01 which indicate significance of the present study.

Table .no.3 One sample T test in the study population

Name of Antibodies	N	Mean	SD	SEM	Significance (2 tailed) Pvalue
Anti-TO ab	89	46.29	37.971	4.025	< 0.01
Anti-TH ab	66	40.00	54.083	6.708	< 0.01

Studies conducted at different parts of our country, for baseline antibody titre for enteric fever causing salmonella vide Table no 4 showed

variations with few values correlated with our results.

Table no 4. Comparison of baseline titres for O & H agglutinins of enteric fever Salmonella in different parts of India

Author	Place	Year	Titre			
			TO	TH	AH	BH
Saxena ¹⁰ et al	Hadoti	2012	≥ 40	≥ 40	≥ 20	≥ 20
Shekar pal ¹¹ et al	Garhwal	2013	≥ 40	≥ 80	≥ 20	≥ 20
Bijapur ¹² et al	Kannur	2014	≥ 40	≥ 40	< 20	< 20
Jeyakumari ¹³ et al	Puducherry	2015	≥ 80	≥ 80	≥ 40	≥ 20
Sunil Hatkar ¹⁴ et al	Badnapur	2016	≥ 40	≥ 80	≥ 40	≥ 20
Nidhi Sharma ¹⁵ et al	Jabalpur	2017	≥ 40	≥ 40	≥ 40	≥ 20
Present study	Kakinada	2017	≥ 40	≥ 40	≥ 20	≥ 20

Because of these variations from place to place and time to time it is mandatory to evaluate the baseline antibody titre among healthy population at regular interval for good interpretation of Widal test.

Conclusion

This study reveals, for interpretation of Widal test in this study population, first we should see the titre of O antigen. If it shows titre of more than 1:40 it indicates early enteric fever infection or it could be an anamnestic reaction. If this rise is associated with more than 1:40 for S.Typhi or more than 1:20 titre for S.Paratyphi A & B H antigens it should be viewed as suspected infection of enteric fever. This value, with a good clinical correlation can help to say as strong suspicion of enteric fever, provided there is only a single sample. However the precise diagnosis can be given only by seeing rising titres in paired sera samples.

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