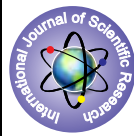


Baseline Antibody Titer Against *Salmonella Enterica* in Healthy Population of Gujarat, India.



Medical Science

KEYWORDS : Baseline titre, Enteric fever, *Salmonella*, Widal test.

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ABSTRACT

Background: Enteric fever is a systemic infection that is caused by a salmonella serotypes. Widal test is widely used laboratory test for diagnosis of enteric fever especially where culture facilities are not available.

*Aims and Objectives: To establish the presumptive and diagnostically significant titre for the diagnosis of enteric fever. Material and method: Study was conducted on 250 healthy individuals. Widal titre was estimated by tube agglutination test for O, H, AH and BH antibodies. Result: From 250 sera tested, 209 sera showed presence of agglutinins to one or more antigens while remaining 41 sera didn't show the presence of agglutinins. Conclusion: In Jamnagar city area Widal agglutination titre cut off level for the presumptive diagnosis of enteric fever against *S. typhi* is > 1:80 for anti "O" and "H", *S. paratyphi A* is > 1:30 for anti "AH", *S. paratyphi B* is > 1:20 for anti "BH".*

INTRODUCTION:

Salmonella enterica serotypes *typhi* and *Salmonella enterica* serotype *Paratyphi A* are common causative organisms for typhoid and paratyphoid fevers, respectively, whereas serotype *Paratyphi B* is rare in our country[1-3]. Definitive diagnosis of enteric fever depends on isolation of salmonellae from blood, stool, urine, bone marrow, bile or other body fluids[4-6]. However, it is a relatively costly method and is not always available. Widal agglutination test is widely used for diagnosis of enteric fever in these settings. The test is based on demonstration of the presence of agglutinin (antibody) in the serum of an infected patient, against the H (flagellar) and O (somatic) antigens of *salmonella enterica* serotype *typhi*, *paratyphi A* and *paratyphi B* during the acute and convalescent period of infection[7].

Antibody titre may be high in healthy individuals in the presence of cross reacting antigens, such as malaria, brucellosis, dengue fever, healthy carrier state, chronic liver disease, endocarditis or other enterobacteriaceae infections[8]. There are more than 40 cross-reacting antigens between *S. typhi* and other enterobacteriaceae[9]. Persons who had past enteric infection or vaccinated with the old typhoid vaccine (TAB) may develop transient anamnestic reaction during an unrelated febrile illnesses, such as malaria[10]. This study was designed to determine the baseline antibody titre in healthy individuals against various serotypes of *S. enterica* using the Widal test.

Material & Method:

Total 250 randomly collected blood samples from apparently healthy blood donors of blood bank, female attending antenatal clinic of G.G.H., Jamnagar in the period of December 2008 to April 2009 were tested for presence of antibodies against *salmonella enterica* group of organisms. Health screening of the volunteer donors was done using survey questionnaires.

Collection and preservation of blood samples:

5 ml of venous blood collected, were centrifuged at a high speed for 5 min in order to separate the serum from the blood cells and later serum was collected in sterile vials.

Reagents:

SPAN's Stained salmonella antigens test kit for slide and tube test, containing *Salmonella typhi* "H", *Salmonella typhi* "O", *Salmonella Paratyphi* "AH" and *Salmonella paratyphi* "BH" antigens were used.

Procedure:

Total 4 rows were set for each serum sample to be tested. Each row consisting of 8 test tubes of (3"x0.5") size. Each sample was serially diluted with normal saline i.e.1:10, 1:20, 1:30, 1:40, 1:50, 1:60, 1:70, 1:80 in all the rows of test tubes. Each test tube contains 1 ml of diluted serum. In 1st row and 2nd row, *Salmonella typhi* - O and H antigen were added. In 3rd and 4th row,

one drop of *Salmonella typhi*- O antigen *Salmonella paratyphi*- AH and BH suspensions were added. Each of the batches of test was accompanied by a saline control for each of the antigens used. Result were interpreted after overnight incubation at 37 °C and observed for the presence of agglutination. The highest dilution showing definite agglutination was noted as the titre of that particular serum.

RESULTS:

Out of 250 sera tested, 208(83%) were from male and 42(17%) were from female. From 250 sera tested, 209(83.6%) showed presence of agglutinins to one or more antigens used while remaining 41(16.4) did not show the presence of agglutinins to any of the antigens. In these 209 positive, frequency distribution among male and female is 173(69.2) and 36(14.4).

Table-1:- 194 sera have agglutinin titre against 'O' antigen of *salmonella enteric* serotype *typhi* in the range of 1:10 to 1:80. Only one sample has titre of >1:80. 150 sera shows antibody titre against 'H' antigen of *salmonella enterica* serotype *typhi* in the range of 1:10 to 1:80. Only two samples have titre of >1:80.78 sera shows antibody titre against 'H' antigen of *salmonella enterica* serotype *paratyphi - A* in the range of 1:10 to 1:40. No sera shows titre against 'H' antigen of *salmonella enterica* serotype *paratyphi A* >40. 43 sera shows antibody titre against 'H' antigen of *salmonella enterica* serotype *paratyphi - B* is in the range of 1:10 to 1:30. No samples shows titre against 'H' antigen of *salmonella enterica* serotype *paratyphi B* >30.

Table-2:- Shows that highest incidence is found in age group 20-29 and least with age group 50-59.

DISCUSSION:

In a situation where second sample collection is not feasible, knowledge of the agglutinin levels in the sera of normal subjects from the patients community can form the baseline on which a diagnosis can be made[11]. This study was designed to determine the average baseline antibody titre in healthy individuals against various serotypes of *S. enterica* using the Widal test.

In present study, out of 250 sera 209 sera, i.e. 83.6% showed presence of agglutinins to one or more antigens used. Enteric fever is water borne disease predominantly where the environmental conditions are favorable for their survival in water and sewage. Hence, in such unhygienic conditions, improper sanitation and sewage disposal the communities are persistently in contact with the infecting agent so it gives rise to high normal agglutinin titre and wide range of various types of agglutinins against different salmonella group of organisms. In our study we found that 92 - 95% serum samples show agglutinin titre ≤1:60 against "O" and "H" antigens, 31.2% showed presence of anti "H" agglutinin against *S. paratyphi A* and 18.8% showed presence of anti "H" agglutinin against *paratyphi B*. This sug-

gests that *paratyphi A* and *paratyphi B* infection is less prevalent than that with *S. typhi*.

The cut off titre in a particular population depends on the background level of the typhoid antibodies and the level of the typhoid vaccination, which may vary with time[12]. According to these results, cut off value should be $\geq 1:80$ for anti-"O" and anti-"H", $\geq 1:30$ for *S. paratyphi A* and $\geq 1:20$ *S. paratyphi B* for Jamnagar city. Baseline titre for the diagnosis of typhoid fever in the Garhwal region is 1:40 for the anti-"O" and 1:80 for the anti-"H" agglutinins, 1:20 for paratyphoid A and B[13]. According to Pokhrel BM significant titers $\geq 1:80$ for anti-O, $\geq 1:160$ for anti-H[13]. Study in Vietnam found that using Widal test cut off titers of $\geq 1:200$ for O and $\geq 1:100$ for H agglutinin[14].

Proper hygiene and sanitation are the keys to a low prevalence of enteric fever in the developed countries, which can result in a low antibody titre. A study in Singapore showed that all the

patients with non-typhoid fever had an anti-O agglutinin titer of less than 1:40, while 82% had an anti-H agglutinin of less than 1:40[15]. *E. Kaarsalo* Suggested cut off value 1:20 and 1:40 for "O" and "H" antigen[16]. We found large diversity in results from different places and countries too. Hence, the baseline titre of a particular area should be known.

CONCLUSION:

In Jamnagar city area Widal agglutination titre cut off level for the presumptive diagnosis of enteric fever against;

- *S. enterica serotype typhi* is $\geq 1:80$ for anti "O" and anti "H".
- *S. enterica serotype paratyphi A* is $\geq 1:30$ for anti "AH".
- *S. enterica serotype paratyphi B* is $\geq 1:20$ for anti "BH".

Time to time evaluation of normal baseline titre should be made, especially in endemic area to determine Salmonella agglutinin titre in the apparently healthy population of local community for better diagnostic yield.

Table-1: Number and percentage of sera with end titres.

Salmonellae	No. Positive (%)	End titre								
		1:10	1:20	1:30	1:40	1:50	1:60	1:70	1:80	>80
Salmonellae Typhi - O	194 (77.6%)	43 (17.2)	66 (26.4)	36 (14.4)	18 (7.2)	11 (4.4)	10 (4.0)	05 (2.0)	04 (1.6)	01 (0.4)
Salmonellae Typhi - H	150 (60.0%)	57 (22.8)	32 (12.8)	24 (9.6)	12 (4.8)	08 (3.2)	06 (2.4)	05 (2.0)	04 (1.6)	02 (0.8)
Salmonellae Paratyphi - AH	78 (31.2)	46 (18.4)	18 (7.2)	10 (4.0)	04 (1.6)	-	-	-	-	-
Salmonellae Paratyphi - BH	43 (17.2)	27 (10.8)	14 (5.6)	02 (0.8)	-	-	-	-	-	-

Table-2: Incidence of positive agglutinin titres in relation to age.

Age in years	No. of Cases	Agglutinin TH % (No. of cases)	Agglutinin TO % (No. of cases)	Agglutinin AH % (No. of cases)	Agglutinin BH % (No. of cases)
10 - 19	30	8.4 (21)	9.6 (24.0)	6 (15.0)	4 (10.0)
20 - 29	95	24 (60.0)	30.8 (77.0)	10.1 (25.0)	7.6 (19.0)
30 - 39	75	13.6 (34.0)	20.8 (52.0)	8 (20.0)	3.6 (9.0)
40 - 49	33	10 (25.0)	10.4 (26.0)	5.2 (13.0)	3.2 (8.0)
50 - 59	17	4 (10.0)	6 (15.0)	2 (5.0)	0.4 (1.0)
Total	250	60 (150.0)	77.6 (194.0)	31.2 (78.0)	18.8 (47.0)

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