



A Study of Antibodies against *Salmonella typhi* and *Salmonella Paratyphi* in a Healthy Population

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Abstract

Background: Typhoid fever is present in all parts of the world where water supplies and sanitation are substandard. Widal test is used as a serological technique to aid in the diagnosis of typhoid fever. Interpretation of Widal test depends upon the baseline titre which is prevalent amongst healthy individuals in a particular geographic area.

Aim: To determine the frequency of distribution of *Salmonella* antibodies and basal antibody titre in the community to suggest a minimum diagnostic titre for Widal test.

Materials and Method: Five hundred (500) sera collected from healthy individuals were tested by Widal tube agglutination test over a period of one year.

Results: Antibodies in titres +1/50 and above were seen in 62 (12.4%). Of these 62 samples 52 (10.4%) had antibodies to *Salmonella typhi* 'O' antigen (TO) and 9 samples (1.8%) showed the presence of antibodies to *Salmonella typhi* 'H' antigen and 1 sample (0.2%) showed the presence of antibodies to *Salmonella paratyphi A* 'H' antigen. ST'O" antibody titre of +1/50 (7.6%) seen in 38 samples, +1/100 seen in 12 samples (2.4%) & +1/200 in 2 samples (0.4%). ST'H'+1/50 seen in 7 samples (1.4%) and +1/200 (0.4%) in 2.

Conclusion: Based on this study it seems appropriate to recommend the minimum diagnostic titre as +1/100 for 'O', 1/50 for 'H' antibodies of *Salmonella typhi* and 1/50 for 'H' antibodies to *Salmonella paratyphi A*.

Keywords: Widal test, *Salmonella typhi*, *Salmonella paratyphi A*, *Salmonella paratyphi B*.

Introduction

Typhoid fever is present in all parts of world where water supplies and sanitation are sub standard. In India endemicity is so high that typhoid is suspected in every patient with fever for more than 3-4 days. In India the annual incidence is about 100-1000 cases per 100000 populations and up to 1% population may carry *Salmonella typhi*.¹ Enteric fever is caused by

Salmonella typhi and *Salmonella paratyphi A*, B, and C. In India infections caused by *Salmonella paratyphi B* and C are rare.² Classic typhoid fever is a severe infection with high mortality rate of about 20%.³ The definitive diagnosis of typhoid fever requires the isolation of *Salmonella typhi* or *Salmonella paratyphi* from patient's blood, or bone marrow but it is seldom achieved. The initiation of antibiotic therapy before the diagnosis may be the reason for poor isolation. Further the

cost, the time required and the lack of culture facilities limit the diagnosis through culture. As a result the diagnosis largely relies upon the clinical features and serological tests like Widal test.⁴

Widal test is based on demonstrating the presence of agglutinins in the serum of an infected patient against 'H' & 'O' antigens of *S.typhi* and 'H' antigens of *S.paratyphi* A& B. Agglutinins may also be present on account of prior disease, in apparent infection or immunization. Variability in the preparation of antigens may give negative results. Interpretation of single Widal test result is to be based on the average baseline titre which is seen among healthy individuals. The study was undertaken to determine the baseline Widal titre against 'O' & 'H' antigens of *S.typhi* and *S.partyphi* A&B among apparently healthy individuals.

Materials and Methods

This cross sectional study was conducted in Department of Microbiology Govt. Medical College Kozhikode, Kerala over a period of one year.

Inclusion criteria: The study group consists of healthy people who were not immunized against typhoid and have not suffered from fever or diarrhea within 2 months. A total of five hundred samples were collected from four categories of people for a period of one year. 125 samples from Healthy blood donors, 125 from Staff and students of Govt. Medical College, Kozhikode, 150 from HIV negative patients who attended the ICTC attached to department and 100 from HIV Sentinel surveillance samples from antenatal group.

Exclusion criteria: Those with signs and symptoms of enteric fever and those who were already vaccinated were excluded from the study.

Control group: Samples from cases of suspected enteric fever that were submitted for Widal test were taken as control. A total of 2785 serum samples were used as control.

Screenings of volunteer donors were done by questionnaire. Randomly selected non repetitive

samples were collected from healthy donors who were in the age group of 7-60 years of both genders .Maximum number of samples (218) were obtained from people between 20-29 years.141 samples obtained from 30-39 year age group, 65 from 40-49 years, 61 from 10-19 years, 12 samples from 50 years and above and 3 samples from below 10 years. Of 500 samples 271 (54.2%) samples were from males and 229 (45.8%) from females.

Around 5ml of blood was collected under aseptic precautions, allowed to clot, serum separated and labeled .Serum samples were processed according to the standard tube dilution method of Widal test using TO, TH, AH & BH antigen. In house preparation of TO&TH antigens were done using *S.typhi* 901 'O' & 'H' strains by standard method.⁵ For *Salmonella paratyphi* A 'H' & B'H' antigen, febrile antigen set, Beacon distributors, PVT limited was used. A serial doubling dilutions of serum is done in sterile normal saline ranging from 1:25 to 1:200 dilutions .Equal volumes of 0.3 ml diluted serum and 0.3ml of 'H' and 'O' antigens mixed in Dreyer's and Felix tubes respectively and Incubated in water bath at 37 °C overnight. A known positive controls and negative controls and antigen controls are set up with each batch of test. Readings were taken after overnight incubation. The highest dilution of serum giving agglutination is noted as titer.

Results

A total of 500 samples were tested. 62 samples (12.4%) showed the presence of antibodies in serial dilutions from +1/50 onwards. The antibodies to *Salmonella typhi* 'O' antigen was more frequent (10.4%), when compared to *Salmonella typhi* 'H' antigen (1.8%), and *Salmonella paratyphi* A 'H' antigens (0.2%). B'H' antibody titre in 1/50 or above was not detected in any samples. This study also shows the increase in titre of *Salmonella typhi* 'H' antibody with age.

In the control group of 2785 fever patients, STO+1/50 was seen in 17.7%, 1/100 in 12.91%

and 1/200 seen in 11.05%.STH antibody titre 1/50 was seen in 7.6%,+1/100 in 3.4%,+1/200 in 2.6% and 1/400 in 2.4%. *Salmonella paratyphi* A ‘H’

antibody titre +1/50 was seen in 0.9% and +1/100in1.4%.*Salmonella paratyphi* B ‘H’antibody seen in 0.9%

Table.1. *Salmonella typhi* and *Salmonella paratyphi* A antibody titres in study group

Endpoint titre	<i>Salmonella typhi</i>				<i>Salmonella paratyphi</i>	
	TO	%	TH	%	A‘H’	%
<1/50	448	89.6	491	98.2	499	99.8
+1/50	38	7.6	7	1.4	1	0.2
+100	12	2.4	0	0	0	0
+1/200	2	0.4	2	0.4	0	0

Table.2. *Salmonella typhi* and *Salmonella paratyphi* antibodies in control group

End point titre	<i>Salmonella Typhi</i>				<i>Salmonella paratyphi</i>		<i>Salmonella Paratyphi</i>	
	TO	%	TH	%	A‘H’	%	B‘H’	%
<1/50	1625	58.34	2339	84	2719	97.7	466	99.1
+1/50	492	17.7	211	7.6	27	0.9	4	0.9
+1/100	360	12.91	94	3.4	39	1.4	0	0
+1/200	308	11.05	73	2.6	0	0	0	0
+1/400	0	0	68	2.4	0	0	0	0
Total	2785	100	2785	100	2785	100	470	100

Table.3 Distribution of *Salmonella typhi* & *S.paratyphi* in the study group-Age wise

Age group	No	STO				STH				A‘H’			
		<1/50	+1/50	+1/100	+1/200	<1/50	+1/50	+1/100	+1/200	<1/50	+1/50	+1/100	+1/200
Upto 9	3	2	1	0	0	3	0	0	0	1	0	0	0
10-19	61	50	10	1	0	60	0	0	1	61	0	0	0
20-29	218	197	13	8	0	217	1	0	0	218	0	0	0
30-39	141	129	9	1	2	136	4	0	1	141	0	0	0
40-49	65	59	5	1	0	63	2	0	0	64	1	0	0
50& above	12	11	0	1	0	12	0	0	0	12	0	0	0
Total	500	448	38	12	2	491	7	0	2	499	1	0	0

Discussion

In India Typhoid endemicity is so high that it is suspected in every person with fever for more than 3 to 4 days duration. Of the 500 samples from healthy individuals studied under categories mentioned, a total of 62 samples (12.4%) showed the presence of antibodies in serial dilutions from 1/50 onwards. In a study conducted at Trivandrum Medical College during 1998-2001 by Dr. Sajini⁶ 75.8% of samples in general population showed presence of antibodies when dilution from 1/20 was taken. In the present study *Salmonella typhi* antibodies are more frequent (10.4%).A titer

of+1/50 (7.6%),1/100(2.4%) and 1/200(0.4%) were seen in *Salmonella typhi* ‘O’antibody .1.8% showed the STH antibody-a titre of +1/50 (1.4%), 1/100 (0%)1/200(0.4%). *Salmonella paratyphi* A‘H’antibody titre of 1/50 was seen in 0.2%.B‘H’antibody titre in 1/50 or above was not detected in any samples.

In this study the level of antibodies against *Salmonella typhi* (12.2%) was higher than antibody against *Salmonella paratyphi* ‘A’ (0.2%). Study by Balbir Singh⁷ also shows similar frequency of distribution. Antibody titers against *Salmonella* were seen to be more prevalent in

males in the present study (7%), when compared to females (4.2%). Similar higher antibody prevalence in males had been reported by Joshi.PJ et al 1998 at Jamnagar.⁸

In this study *Salmonella typhi* 'H' antibody titre increases with age. *Salmonella typhi* 'H' antibodies was 3.08% in the age group 40-49, while the prevalence in the other age group were 2.84% in 30-39, 1.64% in 10-19 and 0.5 in both 20-29 and 0-9 age group. Myron M Levine *et al* at Peru in 1978 also shows an increase in *Salmonella typhi* 'H' antibody titre⁹ with age. In a study by Parry C.M. et al. 1999 in Vietnam, a cut off titre off >1:200 for O and >1/100 for H antibody was recommended.¹⁰ A low titre of 1:50 for 'O' and 'H' antibodies was recommended by Chow C.D et al 1987 in Hong Kong¹¹ and Hoffmann .et.al in 1986 at Jakarta.¹²

Though the rise of widal titre in paired serum samples is generally indicative of enteric fever, a single elevated *Salmonella typhi* 'O and 'H' antibody titres were helpful in making a presumptive diagnosis of typhoid fever in an individual person⁸. It is suggested that in children <10 years of age the 'H' antibody titre alone is of little value in diagnosing typhoid fever as it can arise as a non specific response to other infections.

Conclusion

For practical purpose, any titre that occurs in more than 5% of normal population- that is, one that might be found by chance in 1/20 normal sera, is considered as not being a significant indication of active infection.^{13,14} This arbitrary rule has the advantage of giving statistical significance of P <0.05 to any positive results. Hence forth based on this study it seems appropriate to recommend the minimum diagnostic titre as 1/100 for 'O', 1/50 for 'H' antibodies of *Salmonella typhi* and 1/50 for 'H' antibodies to *Salmonella paratyphi* A.

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