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Research article

STUDY OF THE BASELINE WIDAL TITRES AMONG HEALTHY INDIVIDUALS OF RURAL POPULATION IN PUDUCHERRY

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ABSTRACT

Background: Enteric fever is endemic in developing countries like India. Widal test in a single serum sample is often the only test relied upon for laboratory diagnosis. The test is considered positive if the antibody titres are higher than the cut - off value in a single test or a rising titre in paired sera. But normal baseline titres in healthy population and cutoff values have not been established in our area. So the aim of our study was to determine the base line titres of antibodies among apparently healthy populations and to define the significant titres of widal test. **Materials and Methods:** Samples were initially screened by Widal slide agglutination test and further confirmed by the Quantitative tube agglutination test. **Results:** Among the 500 samples from apparently healthy individuals, 260 samples were positive for agglutinins. 141 were positive for 'O' agglutinin and 163 were positive for 'H' agglutinin of Salmonella typhi. Among 141 samples, 30 showed agglutination up to 1in 20 titre, 100 up to 1in 40 and 25 (4.8%) up to 1in 80. Among 163 samples, 18 showed a titre of 1 in 20, 120 showed a titre of 1 in 40 and 21(4.2%) up to 1 in 80. **Conclusion:** When a single widal titre is used for the diagnosis of enteric fever in our locality, it will be more appropriate to change the currently used cutoff levels against S.typhi to ≥ 1 in 160 for anti-O and 'H' agglutinins remains the same of ≥ 1 in 160.

Keywords: Baseline titer, Widal test, Healthy population

INTRODUCTION

Enteric fever continues to be a global health problem, especially in tropics and subtropics [1] and enteric fever 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] Enteric fever is caused by Salmonella enterica subspecies enterica serotype Typhi and Salmonella enterica subspecies enteric serotype Paratyphi A and Paratyphi B. In contrast to other Salmonella serotypes, these enteric fever serotypes have no known hosts other than humans. The mode of transmission is by close contact with the patients or carriers. Since the clinical presentation of enteric fever is non-descript, laboratory tests are essential for diagnosis. The gold standard and definitive diagnosis is by isolation of Salmonella enterica serotype Typhi, ParatyphiA and Paratyphi B from blood, bone marrow, stool or urine etc. which is about 90% in the first week of illness and decreases to about 50% by the third week from blood sample. Culture facilities are not available in the rural set up where Widal agglutination test has a greater scope for diagnosis of enteric fever in developing countries. Widal test though in vogue for more than hundred years is still

plagued with controversy about its usefulness in diagnosis of enteric fever. [4] The use of Widal test in the diagnosis of typhoid fever during the acute phase of the illness has largely been abandoned in developed countries. [5] Due to practical difficulties in the management of a patient the results of a single test performed at the end of the first week is often the only test relied upon for laboratory diagnosis. The significant titres of antibodies to 'O' and 'H' antigens varies from place to place and with time since antibodies that react with Salmonella 'O' and 'H' antigens appear in a variety of other conditions like malaria, dengue, other gram negative infections and in healthy persons in endemic areas. [6, 7] In endemic areas the use and interpretation of single Widal test depends on result of the baseline titres among the healthy population. It is therefore necessary to establish the baseline titres periodically in each region to define the significant titres of 'O' and 'H' antibodies for proper interpretation of the results.

Objectives

1. The objective of the present study is to determine the base line titres of antibodies for each of the 'O' and 'H' antigens of Salmonella enterica serotype Typhi, Paratyphi 'A' & Paratyphi'B' among apparently healthy blood donors and patients attending the Microbiology laboratory for various investigations other than for enteric fever.
2. To define the significant titres of widal test for diagnosis of enteric fever in a single serum sample.

MATERIAL AND METHODS

Type of study: Cross sectional

Subjects: Total samples (n = 500) were collected in which, Serum samples of Healthy blood donors (n = 300) who voluntarily donated blood to our hospital blood bank during the study period.

Inclusion criteria: Donors who had no history of any illness suggestive of enteric fever in the preceding six months. Negative for all screening tests routinely done in donors includes Malaria parasite antigen, HBsAg, and antibodies to HIV 1 and 2, HCV and Treponema pallidum. No history of typhoid vaccination in the preceding three years was included. Serum samples (n=200) received in the Microbiology laboratory for various serological tests accept widal. These samples were from the seemingly healthy individuals who came for Medical checkup and Antenatal visits.

Only samples of serum with negative results for the requested serological tests were included in the study. Patients without fever or gastroenteritis or any illness suggestive of enteric fever in the preceding six months and not vaccinated for typhoid in the preceding three years were included in the study.

Exclusion criteria: Subjects who did not meet the above criteria were excluded.

Methods: Informed consent was obtained from all the donors and patients and approval of the institutional ethics committee was obtained. Questionnaires were given to evaluate the present and past clinical conditions. About 5ml of blood was collected in a clean dry test tube from each of the subjects and allowed to clot. Serum was separated and stored in the refrigerator at 2-8º C for no more than seven days. All samples were initially screened by rapid slide agglutination test using the standard colored Salmonella antigen supplied by 'SPAN DIAGNOSTICS', Surat, India. Samples showing agglutination with undiluted serum were retested by the standard tube agglutination test of Widal for antibodies against all the four antigens of Salmonella typhi 'O' and 'H', Salmonella paratyphi 'AH'and 'BH' as per the standard procedure. Serial dilutions of serum were done starting from 1/20 to 1/640 and one drop of the appropriate antigen suspension was added. Incubate 'H' agglutinations for 4 hours at 37ºC in a water bath and read after standing on the bench for half an hour and 'O' agglutinations for 4 hours at 37ºC and read after overnight refrigeration at 4ºC. The highest dilution of serum showing visible agglutination was taken as the endpoint and titre expressed as reciprocal of the dilution.[8] For quality control a known positive and negative control sera was also included with each run. The results were analyzed for age, sex and base line titre taken was the highest titre shown by any of the study samples. Statistical analysis by using SPSS – version 20 was also carried out. In which one sample T-test was done to compare the current titre value with the observed titre values. All samples of blood donors (n=300) were screened by Immunochromatography strip for Malaria parasite, HBsAg, antibodies to HIV (Tri dot ELISA), HCV (Dot ELISA), and Treponema pallidum (RPR). Samples of donors negative for all the above cited infections alone were included in the

study. In the patients group all serum samples (n=200) other than those requested for Widal test and which were negative for the respective tests were included in the study.

RESULTS

Among the total of 500 blood samples, 300 samples were collected from healthy blood donors who donated blood to our hospital blood bank and 200 samples from patients who reported to the Microbiology laboratory for various serological tests except for Widal. Demographic distribution of individuals according to age group and sex is given in the [**Table 1]**

Table: 1. Demographic distribution of individuals according to age group and sex.

Total Participants		**Frequency**	**%**
		500	100
Sex	Male	380	76
	Female	120	24
Age groups	16- 20 years	55	11
	21- 30years	270	54
	31- 40years	150	30
	41-50 years	25	5

Samples were analyzed by using SPSS. Among the 500 samples, 260 samples were positive and 240 were negative for agglutinins to Salmonella serotypes typhi and paratyphi A & B. In these positive samples, 143 (28.7%) were positive for 'O' agglutinin and 157 (31.5%) were found positive for 'H' agglutinin of Salmonella typhi. Only 14 (2.8%) and 11 (2.2%) samples were positive for agglutinins to paratyphi AH & BH respectively. Amongst 143 samples positive for 'O' agglutinins, 30 (6%) showed agglutination up to 1in 20 titre, 93 (18.7%) up to 1in 40 and 18 (3.6%) up to 1in 80 and in 157 samples positive for 'H' agglutinins, 18 (3.6%) showed a titre of 1 in 20, 120 (24.1%) showed a titre of 1 in 40 and 15 (3.0%) up to 1 in 80. Among 14 samples (2.8%) were positive for anti 'AH' in the titre of 1in 20 and 7 (1.4%) were in 1 in 40 whereas among 11 samples positive for anti 'BH', 8 (1.6%) were in the titre of 1 in 20 and 3 (0.6%) were in 1 in 40. **(Table 2)**

Table: 2. Number and percentage of samples positive for agglutinins with end titres against different serotypes of Salmonella enterica.

Serotype	**Antibody type**	**No and % of positive samples**	**Dilution (1 in 20)**	**Dilution (1 in 40)**	**Dilution (1 in 80)**	**Dilution (1 in 160)**	**Dilution (1 in 320)**
S. typhi	Anti- TO	143 (28.7)	30 (6)	93 (18.7)	18 (3.6)	2 (0.4)	NIL
S. typhi	Anti-TH	157 (31.5)	18 (3.6)	120 (24.1)	15 (3.0)	3 (0.6)	1 (0.2)
S. paratyphi A	Anti-AH	14 (2.8)	7 (1.4)	6 (1.2)	NIL	NIL	NIL
S. paratyphi B	Anti-BH	11 (2.2)	7 (1.4)	4 (0.8)	NIL	NIL	NIL

The one sample T test showed that the observed value is different from the present recommended value and it is statistically significant (P value < 0.01). **(Table 3)**

Table: 3. one sample T – test

Name of the agglutinins	**N**	**Mean**	**Std. Deviation**	**Sig - (2 tailed) 'P' value**
Salmonella 'O'ab	500	11.66	22.300	< 0.01
Salmonella 'H'ab	500	12.72	20.722	< 0.01
Salmonella 'AH'ab	500	.76	4.926	< 0.01
Salmonella 'BH'ab	500	.60	4.252	< 0.01

DISCUSSION

Bacterial culture remains the gold standard for definitive diagnosis of enteric fever but lack of the facility and cost limits its use in the developing countries. [2] The widal test which detects agglutinating antibodies to Salmonella enterica subspecies enteric serotype Typhi 'O' and 'H' antigens and 'H' antigens of Salmonella enterica subspecies enteric serotype Paratyphi A and B is widely used in the developing countries due to the ease of performing the test, low cost and relatively rapid results. Widal test is also useful for diagnosis of

patients already on antibiotics which may inhibit the growth in culture.

The present study showed that frequency of agglutinins to Salmonella typhi 'O' and 'H' is more than that of Salmonella paratyphi AH and BH. This indicates that exposure to Salmonella paratyphi A & B is less than Salmonella typhi among the population in Puducherry which is similar to that of other regions in our country. [9, 10]

In the present study the highest dilution of agglutination against 'O' antigen of S. typhi was 1:160 in two out of 500 samples (0.4%) tested ie) less than 1% while it was 3.6% at a titre of 1 in 80 which hitherto has been assumed as the significant titre for 'O' in this region. The highest titre for Salmonella typhi 'H' was 1in 320 in 1 out of 500 (0.2%) of the study population and 3 out of 500 (0.6%) showed a titre of 1in 160 which is the presumed significant titre in this region. The study shows that a titre of ≥ 1 in 160 for 'O' and 'H'antibodies of Salmonella typhi holds good as the percentage of positivity of these titres is significantly less (<1%) among the population screened. Nevertheless the majority of the screened population were having a titre of 1 in 40 (18 – 24%) for both 'O' and 'H' antibodies of Salmonella typhi, the titre ≥ 1 in 80 should also be considered as a significant titre with clinical correlations. Also the highest dilution against 'AH' antigen was 1in 40 in 6 out of 500 samples (1.2%) and 1in 20 in 7 out of 500 samples (1.4%) against BH antigen.

Recent study done by Aruni et al and Sreenath etal [11,12] showed the significant titres should be greater than 1: 80 for anti – O and greater 1: 160 for anti – H for a presumptive diagnosis of typhoid fever. In the study done by Senewiratne B etal [13] a titre of ≥ 1 in 160 against 'H' and 'O' antigens of Salmonella typhi was seen in only 1% of patient with non typhoidal fever. In the study by ' Frimporg and others at Ghana [14] the anti 'O' titre was ≥ 1 in 160 in 1% of the 307 healthy food handlers while anti 'H' titre of ≥ 1 in 320 for S. typhi was 2.6%. In a study at Jordan by Shehabi AA,[15] during an outbreak of typhoid fever, 92% of patients with positive blood culture developed agglutinins against TO and TH in titres of 1 in 80 or more indicating the utility of a single widal test in the acute stage of the disease.

In the study by Zailani SB etal, [16] the base line titre for Salmonella typhi and paratyphi for both 'O' and 'H' antibodies was 1 in 80 among the healthy individuals of the community at Ile - Ife, Nigeria. In the study by El-Shafie S [17] 10.5% of healthy individuals in Sudan showed a titre of 1 in 320 against S. typhi 'O' and 4.3% and 5.3% showed a titre of 1in 160 for S.paratyphi 'A' and 'B' respectively. In the study by Ibadin MO [18] at Benin City, Nigeria 1.1% of the healthy school children tested showed a titre of 1 in 160 for either antigens of S.typhi.

Studies conducted in various regions of the world at different periods of time and even within the same region showed a variable baseline titre. It is therefore imperative to estimate the baseline titres among the healthy population in every region at regular intervals and interpretation of single Widal test result must take into account of the baseline titres in their respective areas. In the present study the baseline titres in the majority was less than 1 in 160 for Salmonella typhi'O' (99.6%) and 'H' (99.4%) antibodies. The significant titre for 'O' and 'H' antigens of S.typhi could therefore be assumed to be ≥ 1 in 160 in this part of the country. None of them had the baseline titre of ≥ 1in 80 (100%) for S. paratyphi 'AH' and paratyphi 'BH'. The baseline titre for anti 'AH' was found to be 1 in 40 (1.4%) and for anti 'BH' was 1in 20(1.6%). Hence the significant titre could be presumed to be ≥ 1in 80 for 'AH' and ≥ 1in 40 for 'BH' antibodies of our region.

CONCLUSION

More than fifty percent of healthy participants (52%) were positive to agglutinins for serotypes of Salmonella. This indicates that enteric fever is strongly endemic in our region which would be the sanitation index of a country. Our study disclosed that periodic evaluation of baseline titres of Salmonella serotypes agglutinins, particularly in endemic areas is necessary to avoid false positive results. Based on the results of our study it is recommended to change the currently used cut - off value, that is ≥ 1 in 80 for 'O' agglutinins of Salmonella typhi to ≥ 1 in 160. The significant titre for 'H' agglutinins of Salmonella typhi remains the same, which is ≥ 1 in 160. The significant titre for 'AH' of Salmonella paratyphi A is considered to be of ≥ 1in 80 and for Paratyphi B is ≥ 1in 40. The baseline titre for 'TO' is same in most of the regions while the titres against 'TH' is variable in the different studies.

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Conflict of Interest: Nil

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