

Determination of Baseline Widal Titre among Apparently Healthy Individuals of South India

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Abstract:

Background: Enteric fever is now uncommon in industrialized countries as a result of advancements in food management, water or sewage treatment and introduction of antibiotics. *Salmonella typhi*, *S. paratyphi* A, and *S. paratyphi* B are the causes of enteric fever. Widal test is one of the oldest, most widely used and gold standard serological tests for diagnosis of enteric fever.

Methods: The main objective of this study was to determine the baseline Widal titre among apparently healthy individuals of rural and urban locality around the teaching hospital of Tiruchirapalli, South India. Both genders aged between 18 to 63 years were included and people who tested positive for Widal during the last 6 months were excluded. Three ml of blood samples were collected, thereby screened by rapid slide agglutination test followed by tube agglutination test using *Salmonella* antigens available in the Widal test kit. The observation of agglutinins in different patterns was recorded.

Results: Comparatively, the tube agglutination test showed high sensitive than slide test. Out of 300 samples, 173 and 158 showed positive reaction towards tube and slide test respectively indicated, performing the slide test alone may miss the positive cases. Monoantigenic agglutination was found in two sets including $\geq 1:20$ in O antigen (152 samples) and H antigen (68 samples); Diantigenic responses were recorded with 9 and 3 samples in $\geq 1:20$ of O and AH, and O and BH respectively.

Conclusion: Interestingly, the baseline titre of *Salmonella* antigen was found among healthy individuals indicated their frequent or repeated exposure to the infectious agent.

Keywords: *Salmonella*, WIDAL, seroprevalence, baseline titre.

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Introduction

Typhoid is an infectious disease caused by *Salmonella* species which has widespread global determinations.[1] In developing countries like India, the illness continues to pose a significant public health issue.[2] The disease may occur sporadically, endemically or epidemically.[3] Enteric fever is now uncommon in industrialized countries as a result of advancements in food management, water/ sewage treatment and introduction of antibiotics.

Salmonella typhi, *Salmonella paratyphi* A, and *Salmonella paratyphi* B are the causes of enteric fever.[4] Options for the diagnosis of typhoid fever are clinical signs and symptoms, serological markers, bacterial culture, antigen detection and DNA amplification.[5,6] Blood, bone marrow and stool culture are the most reliable diagnostic methods but they are expensive techniques and some bacterial culture facilities are often unavailable. The test involves the use of antigens of the etiological agents to find the rise in antibody

titre of the patients and interpretation of baseline titre in areas periodically to determine the endemic regions and for definitive diagnosis. Clinical diagnosis of typhoid by Widal is not an easy piece of work as patients take self-medication during the initial period of illness and visit the hospital only during the late stages. Assessing the baseline titres previously would enable the health care facility to keep a constant check at the endemic population and henceforth prevent *Salmonella* infections. This study would benefit all individuals of the community. The people who are detected with high antibody titre will be given treatment accordingly.

Though blood culture of *Salmonella* species is the gold standard, it is not performed in developing countries like India because it is highly expensive and has longer turnaround time (TAT). While confirmatory diagnosis for typhoid, the tube agglutination method of Widal is preferred. As population study to be concerned, Widal test is very much useful in diagnosing in endemic areas in

order determine the baseline titre. Various studies which have been carried out in different states of India have shown that the baseline titre is different in different geographical locations.[7,8,9] Hence, the baseline titre of the study area is determined apparently among healthy individuals.

Methods

This is a cross sectional observational study which includes the population who are apparently healthy individuals residing in rural and urban areas of study area aged between 18 to 63 years and both genders. Around 300 healthy individuals recruited and included. The study was conducted for the period of three months between October and December 2022.

Healthy individuals who come for health checkup, individuals checked on community field visits and workers in a tertiary health care hospital (attenders and janitors) were included in this study and people who tested positive for Widal during the last 6 months were excluded. Written informed consent was to be obtained from all patients and this study was approved by institutional ethics committee (696/ TSRMMCH&RC/ME-1/2022-IEC No: 123 dated 09.07.2022).

Around 3 ml 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 2-8°C for no more than seven days. All the samples were initially screened by rapid slide agglutination test by following standard procedure by including O, H, AH and BH antigens. The results were observed by agglutination macroscopically within one minute.

Further, tube agglutination procedure was also followed and the methodology was performed by seri-

ally diluting the patient's serum in normal saline from 1 in 10 to 1 in 640 dilutions. The test antigenic suspensions (O, H, AH, BH from The King Institute of Preventive Medicine and Research, Chennai) were added. Tubes were incubated in water bath at 37°C overnight.

The interpretation was made as O agglutination which appears as compact granular chalky clumps (disk-like pattern), with clear supernatant fluid; H agglutination appears as large loose fluffy cotton woolly clumps, with clear supernatant fluid; no agglutination/ button formation occurs due to deposition of antigens and the supernatant fluid remains hazy and O agglutinins appear early and disappear early and indicate recent infection. Interestingly, H agglutinins appear late and disappear late was also recorded. O antibodies are serotype nonspecific that is raised in all infections, i.e. *S. typhi*, *S. paratyphi* A and B. H antibodies are specific. H, AH and BH antibodies are raised in *S. typhi*, *S. paratyphi* A and B infections respectively.

Results

The age distribution of healthy individuals included in this study was analyzed thereby the seropositivity was found more (52%) among 18 to 37 years.

The seropositivity is found increased in tube agglutination (173; 57.7%) method than slide agglutination (158; 52.7%). Observationally, 15 false negative observed in slide agglutination method.

The detailed description of age wise assessment of slide and tube methods of seropositivity is depicted in table 1. Gender assessment defined that the female population were found little more and seropositivity were found higher in females (n=103; 34.3%) (Figure 1).

Table 1: Age groups versus Widal test results

Age group (in years)	Participants (n=300)	Slide Agglutination		Tube Agglutination	
		Positive (n=158)	Negative (n=142)	Positive (n=173)	Negative (n=127)
18 - 37	159 (53)	80 (50.6)	79 (55.6)	90 (52)	69 (54.3)
38 - 57	79 (26.3)	41 (26)	38 (26.8)	41 (23.7)	38 (29.9)
58 - 77	62 (20.7)	37 (23.4)	25 (17.6)	42 (24.3)	20 (15.8)

[Figure in parenthesis denoted percentages]

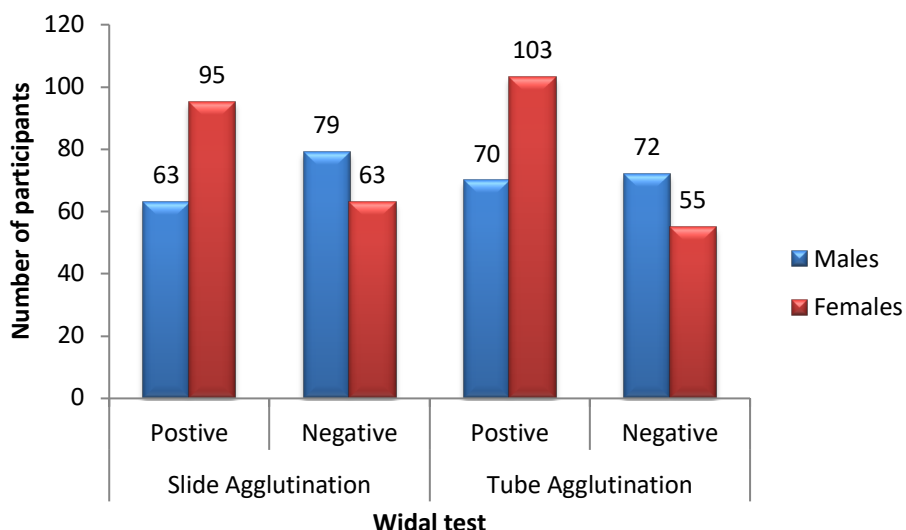


Figure 1: Gender description of Widal seropositivity

While determining monoantigenic reactions in Widal, only O and H antigens showed reactive to the patient’s serum. Dilutions of 1:20 showed maximum reactions compared to higher dilutions. The detailed monantigenic determination was depicted in figure 2.

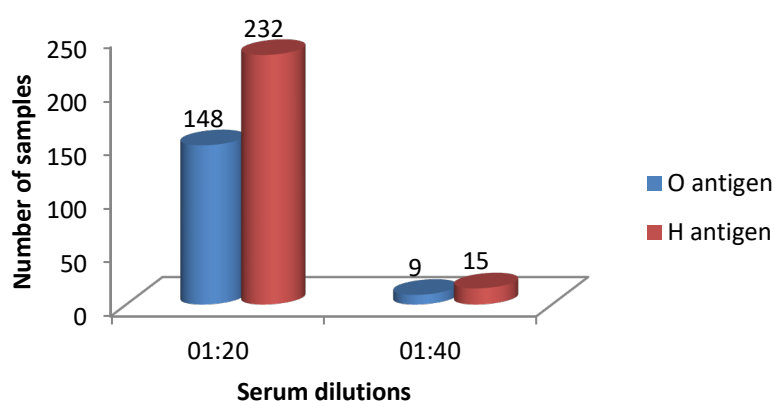


Figure 2: Monoantigenic Widal determination (n=173)

Combinational assessment of four different antigens with their sero positivity were observed thereby O antigen with AH, BH and H whose serotitre was 1:20 among 126 individuals each. Varying titers in the antigenic combinations were also recorded (Table 2).

Table 2: Antigenic combinations with varying titers (n=300)

S.No.	Antigenic Combinations	Number of individuals
1	1:20 titer of O and H antigen	126 (42)
2	1:20 titer of O and AH antigen	126 (42)
3	1:20 titer of O and BH antigen	126 (42)
4	1:40 titer of O and H antigen	01 (0.3)
5	1:20 titer of O and 1:40 of H	5 (1.6)
6	1:20 titer of O and 1:80 of H	5 (1.6)
7	1:20 titer of O and 1:160 of H	4 (1.3)
8	1:40 titer of O and 1:20 of H	5 (1.6)
9	1:40 titer of O and 1:20 of AH	7 (2.3)
10	1:40 titer of O and 1:20 of BH	7 (2.3)
11	1:80 titer of O and 1:20 of H	12 (4)

Discussion

Typhoid fever is identified as a major endemic fecal-oral infectious disease in developing countries like India, with a documented notifiable evidence of increasing trend in every year. While processing the blood and related samples for diagnosis, the isolation of bacteria is superior.

Due to the overuse, abuse and misuse of antibiotics which is also available in counter the sale resulted in negative culture results. Culturing the etiological agent provide smart diagnostic outcome but facilities to perform such methods are not possible

outside the teaching or referral hospitals. In rural or urban or mobile health care delivery centres, there is no such laboratory set up available thus, substituting the diagnosis with Widal test is routine.[10] One hundred and seventy three (57.7%) of the sera were found to be positive for agglutinins for the *Salmonella* serotypes and it were found to be neutral while comparing with other investigations. Review and Discussion revealed a comparative analysis of positive serology for *Salmonella* serotypes agglutinins and were presented in table 3.

Table 3: Review and Discussion of serological positivity

Research study and Place	Year of study	Percentage of serological positivity
Mittal et al., and Uttarkhand-India[11]	2014	51
Shrikant et al., and Indore-India[12]	2015	49.7
Ramesh et al., and Karnataka-India[13]	2016	52.7
Subhajit et al., and Bhubaneswar-India[14]	2017	68
Chandrasekar and Sudeepkumar, Karnataka-India[15]	2018	42
Nimisha et al., and Gujarat-India[16]	2019	22.5
Swati et al., and Uttarpradesh-India[17]	2020	38.6
Bahl et al., and Jammu[18]	2021	45.7
Our study and Tiruchirappalli- India	2022	57.7

Blood culture remains the gold standard for diagnosing the enteric or typhoid fever; but availability of doing culturing and trained technicians are scarce. Thus, performing Widal tube agglutination test is considered as the most common laboratory procedure for diagnosing typhoid fever. In order to determine the baseline serology of *Salmonella* species among healthy individuals, this study is fine tune the statusco of such serological marker to understand the species and subspecies predominance.[16]The positive reactivity of Widal agglutination has been observed in individuals with previous infections by Enterobacteriaceae, malaria and Dengue, TAB vaccine against *Salmonella* infection, and also due to poor standardization and variations of commercially available antigens.[19] Also the false positivity can also be recorded due to cross reaction with nontyphoidal *Salmonella* species.[20]Currently, serotyping has been considered as the core monitoring system for public health assessment in order to determine the *Salmonella* infection. The baseline titre of *Salmonella* antibodies is normally raised among healthy individuals in various endemic areas of developing countries. This determining factor may be very useful for considering before interpretation of Widal results. The antibody titre value of >1:20 is measured as the baseline against *Salmonella* species in healthy individuals.[21]

Conclusion

The agglutinin titers of O and H whose values >1:20 are considered of diagnostic significance in

various endemic areas. Widal screening is inexpensive, noninvasive and test of easy to perform; but have to assess the false positive results carefully. The performance of Widal test gave the most-reliable serological titre determination with high sensitivity and specificity.

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