

Study of the Baseline Widal Titre Amongst Healthy Individuals in Amlapuram, India

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ABSTRACT

Purpose: The interpretation of the Widal test depends upon the baseline titre which is prevalent amongst healthy individuals in a particular geographical area. Hence, this study was undertaken to determine the baseline Widal titre (titre of the antibodies to the O and H antigens of *S. typhi* and the H antigens of *S. paratyphi A and B*) amongst apparently healthy individuals in Amlapuram, Andhra Pradesh, India.

Materials and Methods: Blood samples were collected from healthy blood donors (n= 490) who attended our blood bank from September 2011 to Nov 2011. The Widal tube agglutination test was carried out. 0.4 ml of two fold serially diluted patients' sera (dilutions from 1:20 to 1:320) in 0.9% normal saline was tested by adding an equal amount of antigen.

Results: Of the 490 serum samples which were tested, 270 serum samples were positive for agglutinins ($1 \geq$ in 20) and 220 serum

samples were negative for agglutinins ($1 \leq$ in 20). However, 96% samples showed a titre which was equal to or less than 1 in 40 to the O antigen and 91% samples had a titre which was equal to or less than 1 in 40 to the H antigen of *Salmonella enterica* serovar typhi. The baseline titre for the O and H antibodies of *S. typhi* was found to be 1: 40. The baseline titre for the *Salmonella enterica* serovar *paratyphi A* 'H' antigen was found to be \leq 1:40 in all the 15 samples.

Conclusion: Based upon the results of our study, it has been recommended that the significant titre of the 'H' agglutinins and the 'O' agglutinins of *Salmonella enterica serotype typhi* was \geq 1: 80. While the significant titre of the 'H' agglutinins of *Salmonella enterica serotype paratyphi A* was \geq 1: 40, the significant titre of the 'H' agglutinins of *Salmonella enterica serotype paratyphi B* was \geq 1: 20.

Key Words: Baseline Widal titre, Widal test, Enteric fever

INTRODUCTION

Enteric fever is endemic in India and it continues to be one of the major health problems here [1]. The Widal test was developed by F. Widal in 1896. The Widal agglutination test is the diagnostic test which is mostly commonly used for the diagnosis of enteric fever, ever since its introduction 100 years back [2]. This test detects the antibodies against the O and H antigens of *Salmonella typhi* and against the H antigens of *Salmonella paratyphi A and B*. The interpretation of the Widal test depends upon the baseline titre which is prevalent amongst the healthy individuals in a particular geographical area. The Widal titres among the healthy populations of different areas differ substantially and this depends upon the endemicity of typhoid in each area, which has been changing over time. Updating the baseline Widal titre is a must for the proper interpretation of the Widal test [3,4,5].

Hence, the following study was undertaken to determine the baseline Widal titre (titre of the antibodies to the O and H antigens of *S. typhi* and to the H antigens of *S. paratyphi A and B*) amongst apparently healthy individuals in Amlapuram (Andhra Pradesh). It was also aimed to define the significant titre for the Widal agglutination test for the diagnosis of enteric fever in an endemic area in a single serum test.

MATERIALS AND METHODS

This cross sectional study was conducted in the Department of Microbiology, Konaseema Institute of Medical Sciences and Research Foundation, Amlapuram, India. After obtaining their

informed consent verbally, non-repetitive blood samples were collected from healthy blood donors (n= 490) of the age group of 20-50 years, of both the sexes, who attended our blood bank from September 2011 to Nov 2011. All the donors who were found to be positive for the following screening tests like those for *Malaria*, *Microfilaria*, *HBsAg* and antibodies to *HIV*, *HCV* and *Treponema pallidum*, those who were vaccinated for enteric fever in the preceding three years, those whose blood samples which were submitted for the Widal test or individuals with the history of fever of unknown origin were excluded from the study. Commercially available antigens which contained the *Salmonella enterica* serovar *typhi* O and H antigens, the *Salmonella enterica* serovar *paratyphi AH* antigen and the *paratyphi BH* antigen were used (Span diagnostics Ltd). The tube agglutination test was carried out. 0.4 ml of two fold serially diluted patients' sera (dilutions from 1:20 to 1:320) in 0.9% normal saline was tested by adding an equal amount of antigen. A negative control was included in each batch of the tests. The results were interpreted after overnight incubation of the samples at 37°C. The results were analyzed. The baseline titre for the O, H, AH and the BH agglutinins was the highest titre which was shown by any of the study samples.

RESULTS

A total of 490 serum samples were screened by using the Widal slide agglutination test and later, the results were confirmed by the Widal tube agglutination. [Table/Fig-1] shows the results of the Widal test. Out of 490 serum samples, 270 serum samples were

Widal status	Frequency	Percentage
Positive for agglutinins ($\geq 1:20$)	270	55.12%
Negative for agglutinins ($1 \leq$ in 20)	220	44.89%
Total participants	490	100%

[Table/Fig-1]: Results of Widal test

positive for agglutinins ($1 \geq$ in 20) and 220 serum samples were negative for agglutinins ($1 \leq$ in 20). The distribution of the samples with an antibody titre of $\geq 1:20$ against different serotypes of *Salmonella enterica subsp. enterica* showed an antibody to the anti-O antigen in 144 samples (53.3%), an antibody to the anti-H antigen in 168 samples (62.2%), an antibody to the anti-AH antigen in 15 samples (5.5%) and an antibody to the anti-BH antigen in 12 samples (4.4%). The distribution of the 144 samples with an anti-O titre of $\geq 1:20$ against *Salmonella enterica subsp. enterica serotype typhi* was as follows; an agglutinating titre for TO - 1 in 20 of 33 samples (22.9%), for TO - 1 in 40 of 105 samples (72.9%) and for TO - 1 in 80 of 6 samples (4.1%). The distribution of the 168 samples with an anti - 'H' titre of $\geq 1:20$ against *Salmonella enterica serotype typhi* was as follows; an agglutinating titre for TH - 1 in 20 of 20 samples (11.9%), for TH - 1 in 40 of 132 samples (78.5%), for TH - 1 in 80 of 13 samples (7.7%) and for TH - 1 in 160 of 3 samples (1.78%). The distribution of 15 samples with an anti - AH titre of $\geq 1:20$ was seen in 5 samples (33.33%) and with an anti - AH titre of $\geq 1:40$ was seen in 10 samples (66.66%). The distribution of 12 samples with an anti - BH titre of $\leq 1:20$ was seen in 11 samples (91.6%) and with an anti - BH titre of $\geq 1:40$ was seen in 1 sample (8.3%).

DISCUSSION

The specific purpose of this study was to develop a local recommendation for the interpretation of the Widal test results. Although the Widal test is widely used in our region for the diagnosis of typhoid fever, no previous attempts were made to know the baseline Widal titre amongst the local population, as per our knowledge.

Among the 490 blood samples of healthy blood donors who were tested, 270 (55.12%) were positive for agglutinins for the *Salmonella serotypes*. The highest level of the Widal titre was found to be 1:80 for the O antigen and it was 1:160 for the H antigen of *Salmonella enterica serovar typhi*. However, 96% of the samples showed a titre which was equal to or less than 1 in 40 to the O antigen and 91% samples had a titre which was equal to or less than 1 in 40 to the H antigen of *Salmonella enterica serovar typhi*. The baseline titre for the O and H antibodies of *S. typhi* was found to be 1:40. Similar views have been expressed by earlier workers [6,7]. The baseline titre for the 'H' antigen of *Salmonella enterica serovar paratyphi A* was found to be $\leq 1:40$ in all the 15 samples. Our findings were in agreement with the reports of previous studies

which were done by Patil AM which were conducted in a similar environment as ours [8]. 15 samples (92%) had a titre of $\leq 1:20$ for the *Salmonella enterica serovar paratyphi B 'H'* antigen. The baseline titre for the *Salmonella enterica serovar paratyphi B 'H'* antigen was $\geq 1:20$.

The results which were obtained in the present study were in agreement with the results of previous studies which were done by Punia JN and with those of few other studies which were conducted in similar environments [1,9,10].

CONCLUSION

Any titre above the baseline titre can be taken as a diagnostic titre for the diagnosis of enteric fever. Based upon the results of our study, it has been recommended that the significant titre of the 'H' agglutinins and the 'O' agglutinins of *Salmonella enterica subsp. enterica serotype typhi* was $\geq 1:80$. While the significant titre of the 'H' agglutinins of *Salmonella enterica subsp. enterica serotype paratyphi A* was $\geq 1:40$, the significant titre of the 'H' agglutinins of *Salmonella enterica serotype paratyphi B* was $\geq 1:20$.

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