



## Baseline Widal Titres among Healthy Volunteers ANUPRIYA

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**Abstract :** Objective Enteric fever is an endemic problem in India. The objective of this study was to determine average base-line widal titres among healthy volunteers in and around Tirunelveli. Materials and Methods Cross-sectional study was done on 200 voluntary blood donors. Widal titres were measured using Standard quantitative tube agglutination test. Results Among the 200 blood samples, 70 were male 130 were female. A total of 122 individuals had titres greater than 20 for O agglutinins 136 individuals had titres greater than 20 for H agglutinins. Among the total population, 53 individuals had only H agglutinins positive, while 39 individuals had only O agglutinins positive. Since positive sera with titres greater than 80 occurred in more than 5 percentage of population, this could be taken as presumptive diagnosis of enteric fever in study area. Conclusion Thus the titres of greater than 180 for O agglutinins and greater than 1160 for H agglutinins are base line widal titres, when obtaining second serum sample is impractical.

**Keyword :** Agglutinins, Salmonella typhi, Salmonella paratyphi, Typhoid fever, widal test

### Introduction

Enteric fever continues to be a major health problem in developing countries. WHO estimated an annual infection rate of 21.6 million with global fatality rate of 10%.<sup>1</sup> The clinical picture of enteric fever is non-specific. Definitive diagnosis depends on isolation of Salmonellae from blood, bone marrow and body fluids which may take up to 7 days. Widal agglutination test is an alternative laboratory test used for serological diagnosis of enteric fever. Up to 70% of adults, show an early rise of antibody titre in first week of infection, which forms the basis for widal test.<sup>2,3</sup> Several factors have contributed to uncertainty of the test which includes poorly standardized antigens, sharing of antigenic determinants with other Salmonellae, and previous immunization with TAB vaccine. Epidemiology of cross-reacting antigens determines the baseline of widal test, as antibodies produced against these antigens may cross react with Salmonella antigens.<sup>4</sup> Therefore a fourfold rise in antibody titre between acute and convalescent phase is considered to be of diagnostic value. This is not practically helpful in establishing diagnosis of acute illness, so a single cut off value is used. In a given population, interpretation of single widal test needs to be based on average base line titre among healthy individuals. This project was designed to determine the baseline antibody titre among healthy volunteers in Tirunelveli.

### Materials and Methods

This cross sectional study was conducted at the Department of Microbiology, Government Medical College, Tamil Nadu from June to September 2011. Sample size was 200 which included healthy students and voluntary blood donors with no history of fever for three months and no history of vaccination for enteric fever. Informed consent was obtained from all participants involved in the study. This study was approved by the Institutional Ethical Committee. Tube agglutination test was done using commercially available antigens *Salmonella typhi* O and H antigen and *Salmonella*

*paratyphi* AH and *Salmonella paratyphi* BH antigen (Febrile antigen set for WIDAL (tube) test SPAN diagnostics limited). Sera was diluted from 1:20 to 1:320 as per manufacturer's instruction. For quality control, the positive polyspecific control was also processed in the same dilutions as test sample. Similarly, normal saline was used for negative control. The tubes were incubated overnight at 37°C. In positive reactions with H antigens, deposits of agglutinated bacteria should appear fluffy or floccular and with the O antigen, finely granular.

### Results

A total of 200 patients analysed, 130(65%) were females & 70(35%) were males (Table 1).

Of 200 sera examined 122 (61%) agglutinated 'O' antigens & 138(68%) agglutinated 'H' antigens. Of the positive samples which agglutinated 'O' antigens 48(24%), 50(25%), 15 (7.5%), 9(4.5%) had titres of 1:20, 1:40, 1:80, 1:160 respectively. With 'H' agglutinins 54(27%), 44(22%), 23 (11.5%), 14(7%), 1(0.5%) had titres of 1:20, 1:40, 1:80, 1:160, 1:320 respectively. 2(1%), 1(0.5%) had titres of 1:40, 1:80 for 'AH' agglutinins respectively. 'BH' agglutinins were negative for all sera samples examined (Table 2).

Among 200 healthy volunteers, 53 persons agglutinated with only 'H' antigens, while 39 persons agglutinated with only 'O' antigens (Table 3).

Of the 70 males and 130 females 61(87%) males and 115 (88.5%) females showed titres of less than 80 for "O" agglutinins and 9(13%) males and 15(11%) females showed titres more than 80. For "H" agglutinins 60(88.5%) males and 102 (78.5%) females showed titres less than 80 and 10(11.5%) males and 28(21.5%) females showed titres more than 80 (Table 4).

## Discussion

The incidence of enteric fever in India has been reported to be as high as 7.6/1000 per year with an estimated 6-8million cases/yr which pose a serious threat to public health.<sup>5</sup> In acute febrile illness in an endemic typhoid region where the clinical picture is ambiguous, a simple, alternative test should be used to differentiate typhoid from non-typhoidal febrile illnesses.

In the present study agglutinins to "O" & "H" were present in significant proportions in Tirunelveli district. The IgM somatic antibody appears first & represents initial serologic response which has more clinical relevance as it indicates recent infection while IgG flagellar H antibody once formed are more enduring in circulation and persist longer. Only 3 persons had titres >1:40 for "AH" agglutinins while none were positive for "BH" agglutinins. It is estimated that there is one case of paratyphoid fever for every 4 cases of typhoid fever.<sup>6</sup>

The results of the present study shows that 53(26.5%) were positive only for 'H' agglutinins and 39 (19.5%) were only positive for 'O' agglutinins. 'O' antibody appears 6-8 days after onset of fever and disappears earlier whereas 'H' antibody appears 10-12 days after infection and persist for long time.<sup>7</sup>

In the present study, there was no significant difference in levels of titres between males and females except in titres of greater than 80 where the prevalence of 'H' agglutinins is higher 28 (21.5%) among females when compared to males 10(11.5%). This is probably due to sub clinical infections and delayed health seeking attitude among females, hence infections may go undiagnosed.

For practical purposes, titres occurring in more than 5% of subjects under study were not diagnostically significant and should be regarded as normal in that population.<sup>8,9,10</sup> According to which titres >80 for "O" agglutinins, in 15(7.5%) samples of the study group and titres >160 for "H" agglutinins in 14(7%) samples of the study population, could be taken as a cut-off for presumptive diagnosis of enteric fever in the study area. Similar titres were reported by Bharat *et al* in Nepal and Okonko *et al* in Nigeria.<sup>4,10</sup>

Certain factors have to be borne in mind before the interpretation of widal test results:

Possibility of cross reactions with a variety of other diseases caused by non-salmonella organisms which includes malaria, dengue, miliary tuberculosis, endocarditis, chronic liver disease and brucellosis<sup>11,12</sup> has to be considered.

Immunoglobulins found in certain immunologically abnormal states may also cause cross-reactions which includes rheumatoid arthritis, rheumatic fever, nephrotic syndrome and multiple myeloma.<sup>7</sup>

Strain variation in *S.typhi* could alter the immunological response. It is probable that in endemic areas where population is permanently 'immunologically sensitised' due to constant exposure, the response to infections is more rapid reaching higher levels of agglutinins.<sup>12</sup>

Other factors including poorly standardized antigen preparation, technical difference, the sharing of antigenic determinants with other Salmonellae, effects of treatment with antibiotics and previous immunization with TAB vaccine may contribute.<sup>13,14</sup> to cross reactions.

Thus number of aspects should be kept in mind as possible causes of error or confusion in the interpretation of widal test. Over 100 years (developed by Fernanderz Widal in 1896) since the introduction of widal test as a means of detecting enteric fever it is still extensively used by laboratories to detect typhoidal illness.

## Conclusion

Thus it could be concluded that in endemic areas, widal test is still of significant diagnostic value provided judicious interpretation of the test is made against a background of pertinent information, especially by co-relating the baseline titre levels in normal individuals in that geographical area.

In endemic areas a single widal test can be of diagnostic value in the early stage of disease, thereby reducing morbidity and mortality from typhoid. For better diagnosis of enteric fever, an ideal test which is rapid, accurate & sensitive is needed in the present scenario.

## RESULTS-TABLES

TABLE 1: Age-Sex Distribution of participants in study

Age in years	Male	Female
11-20	29	70
21-30	22	43
31-40	12	10
41-50	7	7
Total	70(35%)	130(65%)

TABLE 2: Titre-wise distribution of agglutinine

Titre	'O' agglutinine	'H' agglutinine	'AH' agglutinine	'BH' agglutinine
<1:20	78(39%)	64(32%)	197(98.5%)	-
1:20	48(24%)	54(27%)	-	-
1:40	50(25%)	44(22%)	2(1%)	-
1:80	15(7.5%)	23(11.5%)	1(0.5%)	-
1:160	9(4.5%)	14(7%)	-	-
1:320	0	1(0.5%)	-	-
Total	200	200	200	

TABLE 3: Distribution of 'H' agglutinine alone

Titre	'H' agglutinine positive
1:20	20(10%)
1:40	16(8%)
1:80	11(5.5%)
1:160	6 (3%)
Total	53(26.5%)

TABLE 3: Distribution of 'O' agglutinine alone

Titre	'O' Agglutinine positive
1:20	12(6%)
1:40	21(10.5%)
1:80	3(1.5%)
1:160	3(1.5%)
Total	39(19.5%)

TABLE 4: Sex-wise distribution of titre

Titre	Typhi 'O' Male	Typhi 'O' female	Typhi 'H' male	Typhi 'H' Female
<1:80	61(87%)	115(88.5%)	60(88.5%)	102(78.5%)
>1:80	9(13%)	15(11.5%)	10(11.5%)	28(21.5%)
TOTAL	70	130	70	130

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