

Determination of Baseline Widal Titres Amongst Apparently Healthy Blood Donors in Ahmednagar, Maharashtra, India

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ABSTRACT

Background: Enteric fever is endemic in all parts of India. The Widal agglutination test is widely used for its diagnosis. But the interpretation of Widal test depends upon the baseline titre which is prevalent amongst healthy individuals in a particular geographical area.

Aim: Present study was undertaken to establish the normal baseline titre in apparently healthy blood donors and also to determine cutoff values for the Widal test.

Material and Methods: One hundred and three (103) sera were tested by the Widal tube agglutination test from January 2012 to February 2013.

Results: Eighty four (81.55%) sera were positive for agglutinins (titre \geq 1:20) and the remaining 19 were negative. 31.64% sera were positive for agglutinins of 'O' antigen of *Salmonella typhi* (TO) at a titre of 1:40, 33.87% sera were positive for 'H' antigen of *Salmonella typhi* (TH) at a titre of 1:40 and 38.46% and 36.84% sera were positive for 'H' antigen of *Salmonella paratyphi A* (AH) and *Salmonella paratyphi B* (BH) respectively, both at a titre of 1:80.

Conclusion: Therefore, the baseline titre for TO and TH was noted to be 1:40 and that for AH and BH was noted to be 1:80 and therefore, the cutoff level for TO and TH was \geq 1:80 and that for AH and BH was \geq 1:160.

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

INTRODUCTION

Enteric fever is endemic in all parts of India [1]. The term, 'enteric fever' includes typhoid fever which is caused by *S. typhi* and paratyphoid fever which is caused by *S. paratyphi A*, B and C [1]. Definitive diagnosis of enteric fever depends on isolation of *Salmonella* from blood, stool, urine, bone marrow, bile or other body fluids [2-4]. However, in countries like India, isolation of organism is often jeopardized by lack of facilities or inadequate and/or improper antibiotic use prior to culture and also, culture positive cases are very less, time consuming and expensive. For these reasons, laboratory diagnosis of enteric fever relies heavily on serological tests such as the Widal test [5].

The test becomes reliable if at least two properly staged tests show about a four-fold rise in antibody levels [6]. In India, most of the patients present late to the hospital and they require an immediate diagnosis and a specific treatment and often, a single sample has to be relied upon, instead of paired serum samples [7]. and so, a single cutoff value is widely used [8].

Widal test can be used as a diagnostic tool in endemic areas, if we know the baseline titre in a population. Interpretation of a single Widal test result needs to be based on the average baseline titre which is seen among healthy individuals. The present study was therefore, undertaken to establish the normal baseline titre in apparently healthy blood donors and also, to determine cutoff values for the Widal test.

MATERIAL AND METHODS

This cross sectional study was conducted in the Department of Microbiology, Dr. Vikhe Patil Medical College and Hospital, which is a tertiary care hospital with 700 beds, which is located at Ahmednagar, Maharashtra, India. Approval of the institutional ethical committee was obtained for carrying out this study.

Inclusion criteria

Informed written consent were taken from the donors for the study.

Participants who were apparently healthy were only included as

study subjects. All participants were given questionnaires to fill. All the donors who did not have any obvious signs and symptoms of infectious diseases were included in the study.

Exclusion criteria

Those with obvious signs and symptoms were excluded.

Participants who were already vaccinated for *Salmonella* were excluded from study.

The following study was undertaken to determine the baseline Widal titre (titre of the antibodies to the 'O' and 'H' antigens of *Salmonella typhi* and the 'H' antigens of *Salmonella paratyphi A* and B) amongst apparently healthy individuals in and around Ahmednagar. It 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.

Health screening of the volunteer donors, was done by using a questionnaire. Randomly selected, non-repetitive 103(n=103) blood samples were collected from healthy blood donors who were in the age group of 18 to 60 years, of both the genders, who attended our blood bank from January 2012 to February, 2013.

About 2ml of blood was taken from the tube of each bag, that was not diluted with CPDA-1 which was present inside the blood bag. Serum was separated immediately, labeled and stored at 4°C for further processing. The serum samples were processed according to standard tube dilution method of the Widal test. Commercially available antigens which contained the "O" and "H" antigens of *S. typhi* and "H" antigens of *S. paratyphi A* and B were used, which were procured from Span Diagnostics Ltd. The test used two fold serially diluted sera of patients, dilution being from 1:20 to 1:320. All serum samples were first diluted in a 1:20 ratio with isotonic normal saline (NaCl 8.5gm./liter), in such a way that final volume contained a total of 1ml. For each sample, further 4 dilutions of 1:40, 1:80, 1:160 and 1:320 were made. Then, one drop (0.03ml) each of the appropriate antigen suspensions was added to the corresponding tubes. A known positive control, negative control

Serotype	Agglutinins	End Titres : Frequency (Percentage)				
		1:20	1:40	1:80	1:160	1:320
<i>S. typhi</i>	Anti TO	11 (13.92%)	25 (31.64%)	21 (26.58%)	13 (16.45%)	09 (11.39%)
<i>S. typhi</i>	Anti TH	13 (20.96%)	21 (33.87%)	12 (19.35%)	08 (12.90%)	08 (12.90%)
<i>S. paratyphi A</i>	Anti AH	04 (30.76%)	02 (15.38%)	05 (38.46%)	0 (0%)	02 (15.38%)
<i>S. paratyphi B</i>	Anti BH	04 (21.05%)	07 (36.84%)	07 (36.84%)	0 (0%)	01 (5.2%)

[Table/Fig-1]: Number and percentage of sera with corresponding end titres for agglutinins against *S. typhi*, *S. paratyphi A* and *S. paratyphi B*

Author	year	Percentage of positive samples
Achary et al., [9]	2013	50.6%
Shekhar Pal et al., [10]	2013	42.60%
Prashant Peshattiwar [11]	2012	55.12%
Bharat et al., [8]	2009	62%
Present study	2012	81.55%

[Table/Fig-2]: Comparative analysis of samples positive for agglutinins for *Salmonella* serotypes.

Author	Year	Baseline Titre			
		TO	TH	AH	BH
Shekhar Pal et al., [10]	2013	1:40	1:80	1:20	1:20
Prashant Peshattiwar [11]	2012	1:40	1:80	1:40	NR
Madhusudhan et al., [12]	2012	1:40	1:40	≤1:40	≥1:20
Present Study	2012	1:40	1:40	1:80	1:80

[Table/Fig-3]: Comparative analysis of baseline titre of "O" & "H" agglutinins. NR – Not Reported.

and antigen control were also set up with each batch of the test. All the tubes were mixed well and they were incubated at 37°C overnight (16-20 hours.). After completion of incubation period, the tubes were examined for agglutination. When antigen suspensions were mixed and incubated, anti-*Salmonella* antibodies which were present in the serum reacted with the corresponding antigens to give agglutination. The 'O' antigen being a somatic antigen, brought about a coarse, compact, granular agglutination, whereas 'H' antigen being a flagellar antigen, brought about a large, loose, fluffy, agglutination. The antibody titre was reported as the highest dilution of serum which showed distinct agglutination.

RESULTS

The purpose of this study was to find out the baseline antibody titre in the population and to develop a local recommendation for interpretation of the Widal test results. This was the first study of its type which was done at Ahmednagar, to the best of our knowledge. A total of 103 sera were tested. Among these, 84 (81.55%) showed agglutinations at a titre of ≥1:20 for the "O" and "H" antibodies against *Salmonella typhi*, *Salmonella paratyphi A* or *Salmonella paratyphi B*. The rest of the 19 did not show agglutination. The number and percentage of sera with corresponding end titres for agglutinins against *Salmonella typhi*, *Salmonella paratyphi A* and *Salmonella paratyphi B*, has been presented in [Table/Fig-1].

DISCUSSION

Eighty four (81.55%) of the sera were found to be positive for agglutinins for the *Salmonella* serotypes. Our findings were quite higher than those which were reported by other investigators. A comparative analysis of samples which were positive for agglutinins for *Salmonella* serotypes has been presented in [Table/Fig-2].

The highest level of titre for TO, TH, AH and BH was found to be 1:320. Our findings were in accordance with those of Madhusudhan NS [12], who observed that the highest level of titres for O, H and AH was 1:320. High titre of antibodies in our study may be caused by infections with cross reacting organisms other than *Salmonella*. For example, cross reaction between a normally harmless gut bacterium, *Citrobacter freundii*, can confuse the diagnosis of typhoid bacilli which share antigenic properties [13].

Sera of 31.64% and 33.87% samples showed titres of ≥ 1:40 for TO and TH respectively, whereas highest number of samples i.e. 38.46% and 36.84% respectively, showed titres of ≥ 1:80 for AH and BH. Therefore, as is evident from the above findings, the baseline titre for "O" and "H" antibodies of *Salmonella typhi* was found to be 1:40. Shekhar Pal et al., [10] and Madhusudhan NS [12] also reported a baseline titre of 1:40 for TO, but of 1:80 for TH. A comparative analysis of baseline titres of O and H agglutinins has been presented in [Table/Fig-3].

The cutoff level for a single antibody titre in the present study was ≥1:80 for TO and TH, which was in accordance with the finding of Prashant Peshattiwar [11] but which varied from that of Bahadur et al., [14], who reported that the cutoff level for TO and TH was ≥1:320. The cutoff level for AH and BH was ≥1: 160. The variations in the titres could have been caused by differences in endemicity of typhoid in each area.

CONCLUSION

The baseline titre for antibodies to "O" and "H" antigens of *Salmonella enterica* serotype *typhi* was 1:40 and hence, based on the above results, it could be recommended to use a cutoff level of ≥1:80 for a single antibody test titre. Similarly, baseline titre for antibody to H antigen of *Salmonella enterica* serotype *paratyphi A* and *paratyphi B* was 1:80 and the cutoff level was ≥1:160 for a single antibody test titre.

It is clear that *Salmonella* agglutinins are common among apparently healthy people and as endemicity of typhoid in an area may change over time, more studies should be carried out to determine *Salmonella* agglutinin titre in apparently healthy populations, so that a better judgment which is based on the prevailing agglutinin titres can be made.

REFERENCES

- Ananthanarayan R, Paniker CKJ. Textbook of Microbiology. 8th ed. Hyderabad: Orient Longman Enterobacteriaceae III :*Salmonella*; 2011; Chapter 32: 288-301.
- Manson-Bahr PEC, Bell DR. Manson's tropical diseases London, *Bailliere-Tindall*: 1987;194-206.
- Gilman RH, Termine M, Levine MM, Hernandez-Mendoza P, Hornick R. Comparison of relative efficacy of blood, stool, urine, bone marrow and rose spot cultures for recovery of *Salmonella typhi* in typhoid fever. *Lancet*.1975;1:1211-15.
- Gaddes AM. Imported infections. Unexplained fever. *BMJ*. 1974;4:397-98.
- Patil AM, Kulkarni ML, Kulkarni AM. Baseline Widal Titres in Healthy Children. *Indian J. of Paediatr*. 2007;74:1081-83.
- Gilles RR. *Salmonella* : The Widal test. In Cruickshank R, Dugid JP, Marmion BP, Swain RH. eds. *Medical Microbiology* : 12th edition, AP314, Edinburg, Churchill Livingstone.
- Punia JN, Joshi RM, Gupta V, Arora RK. Determination of the baseline Widal titres from Chandigarh. *Indian J. Med. Microbiol*. 2003;21(2):144.
- Bharat MP, Rajendra K, Shyam KM, Janak K. Distribution of antibody titre against *Salmonella enterica* among healthy individuals in Nepal. *Annals of Clin Microbiol anti-microbial*. 2009;8:112-18.
- Acharya T, Tiwari BR, Pokhrel BM. Baseline Widal Agglutination Titre in Apparently Healthy Nepalese Blood Donors. *JHAS*. 2013;3(1):27-30.
- Pal S, Prakash R, Juyal D, Sharma N, Rana A, Negi S. The Baseline Widal Titre Among the Healthy Individuals of the Hilly Areas in the Garhwal Region of Uttarakhand, India. *Journal of Clinical and Diagnostic Research*. 2013 March,7(3):437-40.
- Peshattiwar P. Study of the Baseline Widal titre amongst healthy individuals in Amalapuram, India. *Journal of Clinical and Diagnostic Research*. 2012 (May) Suppl-1, 6(3):416-17.
- Madhusudhan NS, Manjunath AH. Determination of Baseline Widal Titre Among Healthy Population. *International Journal of Biomedical Research*. 2012,3(12):437-38.

- [13] Onunkwo AU, Nwankwo CH, Umolu DN. Stochastic Appraisal of the Routine serodiagnostic method for Enteric fever in Nigeria. *J. Sci. Eng. Technol.* 2001;8(1):2964-73.
- [14] Bahadur AK, Peerapur BV. Baseline Titre of Widal Amongst Healthy Blood Donors in Raichur, Karnataka. *Journal of Krishna Institute of Medical Sciences University.* 2013 July-Dec;2(2):30-36.

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