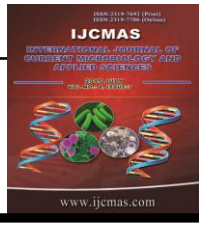
Int.J.Curr.Microbiol.App.Sci (2015) 4(7): 775-778

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 7 (2015) pp. 775-778**
http://www.ijcmas.com



Original Research Article

Study of baseline Widal titre amongst healthy individuals in and around Indore, India

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Keywords

Baseline Widal titre, Widal test, Enteric fever

A B S T R A C T

Interpretation of Widal test depends upon the baseline titre of agglutinins to O and H antigens of *S. typhi* and H antigens of *S. paratyphi A and B* prevalent amongst healthy individual in particular geographical area. Hence study was undertaken to determine the base line Widal titre (Titre of agglutinins to O and H antigens of *S. typhi* and H antigens of *S. paratyphi A and B*) amongst apparently healthy individuals in and around Indore. Blood samples were collected from healthy blood donors (n= 591) attending our blood bank. The Widal tube agglutination test was carried out by Reckon Diagnostic. Serum was serially diluted in 0.9% normal saline starting at a dilution of 1 in 20. For H and O agglutinins 0.5 ml of serially diluted serum was added to glass tubes followed by equal volume of antigenic suspension (Total volume of 1 ml) all tubes were incubated overnight at 37^{O}C before reading. Among The 591 serum specimens which were tested 294 (49.72%) were found to be positive for agglutinins(> 1:20) and 297 (48.56%) were negative (<1:20) for O' agglutinins of *Salmonella enterica serotype typhi*. For 'H' agglutinins of *Salmonella enterica serotype typhi* 282 samples were negative (<1:20), while 309 (52.26) sample were positive for agglutinins(> 1:20). Based upon the results of our study it is recommended that the significant titre of 'H' agglutinins and 'O' agglutinins of *Salmonella enterica serotype typhi* is 1 in 160 and 1 in 40 respectively. While the significant titre of 'H' agglutinins of *Salmonella enterica serotype paratyphi A* is 1 in 40 and the significant titre of 'H' agglutinins of *Salmonella enterica serotype paratyphi B* is 1 in 40.

Introduction

Enteric fever is endemic in India and continues to be one of the major health problems (Punia et al., 2004). Widal test developed, by Ferdinand Widal in 1896 is a tube agglutination test mostly commonly used for diagnosis for enteric fever. (Shreenath K et al., 2014). This test detects agglutinins against O and H antigens of *Salmonella typhi* and H antigens of *Salmonella paratyphi A and B*. Interpretation of Widal test depends upon the baseline titre of these agglutinins to O

and H antigens of *S. typhi* and H antigens of *S. paratyphi A and B* prevalent amongst healthy individual in particular geographic area. Base line titre of agglutinins vary from place to place and it depends upon the endemicity of enteric fever and it keeps on changing from time to time.(Acharya T et al. 2013, Olopoenia et al, 2007).Hence the following study was undertaken to determine the base line Widal titre amongst apparently healthy individuals in and around Indore (Madhya Pradesh).

Materials and Methods

The study was conducted at the department of Microbiology, Index medical college, Hospital and research centre, Indore. 591 non-repetitive blood samples were collected from healthy blood donors of age group 20-50 years of both sexes from blood bank. All the donors who were vaccinated for enteric fever in past or blood samples submitted for Widal test or individuals with history of fever of unknown origin or found to be positive for *Malaria, Microfilaria, HBsAg, HIV, HCV, Treponema pallidum* were excluded from the study. Commercially available antigens containing *Salmonella enterica serovar typhi* O and H antigens and *Salmonella enterica serovar paratyphi AH* antigen and *paratyphi BH* antigen were used (Reckon diagnostics Ltd). The tube agglutination test was carried out. Serum was serially diluted in 0.9% normal saline starting at a dilution of 1 in 20. For H and O agglutinins 0.5 ml of serially diluted serum was added to glass tubes followed by equal volume of antigenic suspension (Total volume of 1 ml) all tubes were incubated overnight at $37^{O}C$ before reading. A negative control was included in each batch of test. Result were interpreted and analysed as per standards. Baseline titre for Widal anti O (TO), anti H (TH AH and BH) agglutinins was the highest dilution of serum with visible agglutination shown by any of the study serum samples.

Results and Discussion

Total 591 serum samples were tested by Widal tube agglutination test. 512 of these were male donors and rest (79) were female donors. Table 1 shows results of Widal test. Distribution of 591 samples with anti- O titre against *Salmonella enterica subsp. enterica serotype typhi* was as follows, agglutinating titre for TO - 1 in 20 in 118 samples (19.96 %), for TO - 1 in 40 in 171 samples (28.93 %), for TO - 1 in 80 in 04 samples (0.67 %). Distribution of 591 samples with anti – 'H' titre ≥ 1: 20 against *Salmonella enterica serotype typhi* was as follows, agglutinating titre for TH – 1 in 20 in 104 samples (17.59 %), for TH – in 1 in 40 in 45 samples (7.61 %), for TH – 1 in 80 in 158 samples (26.73 %), for TH – 1 in 160 in 2 samples (0.33 %). Distribution of 591 samples with anti – AH titre 1: 20 was seen in 19 samples (3.21 %) and anti – AH titre ≥ 1: 40 was seen in 03 samples (0.50 %). Distribution of 591 samples with anti – BH titre 1: 20 were seen in 09 samples (1.52 %).

The purpose of this study was to develop baseline titers for the interpretation of Widal test results in our geographic area. The isolation of salmonella species from blood is the gold standard for the diagnosis of enteric fever. Lack of easy availability of culture isolation make widal test as the alternative laboratory procedure for the diagnosis of enteric fever. Classically a fourfold rise of antibody titre in paired sera, 10- 14 days apart is considered diagnostic of enteric fever. However this is not always possible. Lack of improper timing of sample collection and initiation of antibiotic therapy before diagnosis is made could be one of the major reasons for poor isolation rates

(Madhusudan and Manjunath 2012 and Pal et al, 2013). Analysis of this study shows the sera of significant of healthy individuals in this area contain Salmonella agglutinins which were capable of reacting to the variable titres in Widal test. In our study, we found that 49.72% sera were reactive for O agglutinins and 52.26 % sera were reactive for H agglutinins of S. typhi. Our results are in agreement with previous workers (Vazhavandal G et al, 2014). However for S. typhi O agglutinins, highest percentage of samples (28.93%) of were reactive in dilution of 1 in 40 and for S. typhi H agglutinins 26.73 % of samples were reactive in dilution of 1 in 80. Our results were in agreement Pal et al 2013 and Gunjal et al 2013. However Shukla et al 1997 and Patil et al 2007 had shown baseline widal titre of 1:80 for O agglutinins of S.tyhi. [6,7,] For H agglutinins of S. typhi our results were in agreement with Shukla et al 1997, Patil et al 2007,Pal et al 2013.While Punia 2003, Bhadur 2013 had reported baseline titre of 1:160. Highest percentage of samples (3.21%)were reactive in dilution of 1 in 20 for *Salmonella enterica serovar paratyphi A*. Our findings are in agreement with previous reports by Pal et al 2013, Shreenath K et al while Vazhavandal G et al had reported 8.1 %. 0.33% of samples had titre 1:20 for Salmonella enterica serovar paratyphi B 'H' antigen. 1.52% of samples were reactive for *Salmonella enterica serovar paratyphi B* 'H' antigen in dilution of 1:20. Our findings are in agreement with Pandey et al.

Several studies have highlighted the limitations of using the Widal test in the laboratory diagnosis of Salmonella. The worst being non specificity. Despite this fact, considering the low cost and the absence of comparatively cheap tests the widal tube agglutination test is likely to remain the test of choice in many developing countries, as of ours, provided a baseline antibody titre of healthy individual in the population is known. Based upon the results of our study it is recommended that the significant titre of 'O' agglutinins of *Salmonella enterica subsp. enterica serotype typh*i is 1: 80, significant titre of 'H' agglutinins of *Salmonella enterica subsp. enterica serotype typh*i is 1: 160. While the significant titre of 'H' agglutinins of *Salmonella enterica subsp. enterica serotype paratyphi A* is $\geq$ 1: 40 and the significant titre of 'H' agglutinins of Salmonella enterica serotype paratyphi B is $\geq$ 1: 40.

Table.1 Sexwise distribution of blood donors

Sex	Number	Percentage
Male	512	86.63 %
Female	079	13.36 %

Tabel.2 Agewise distribution of Blood donors in both sexes

Age	Number	Percentage
20-30 years	368	62.26
31-40 years	211	35.70
41-50 years	12	02.03

Table.3 Titre of tube widal agglutination test among 591 apparently health blood donors

Agglutinins	Titre (<1:20)	Titre (1:20)	Titre (1:40)	Titre (1:80)	Titre (1:160)	Titre 1: 320
S. typhi O						
Number	297	118	171	04	01	0
Percentage	48.56	19.96	28.93	0.67	0.16	0
S. typhi H						
Number	282	104	45	158	2	0
Percentage	47.71	17.59	7.61	26.73	0.33	0
S. paratyphi AH						
Number	562	26	3	0	0	0
Percentage	95.09	4.39	0.50	0	0	0
S. paratyphi BH						
Number	589	2	0	0	0	0
Percentage	99.66	0.33	0	0	0	0

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