

## THE BASELINE WIDAL TITRE AGAINST SALMONELLA ENTERICA IN HEALTHY POPULATION OF GWALIOR AND CHAMBAL DIVISION OF MADHYA PRADESH

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### Abstract:

**Background:** Enteric fever is a major endemic health problem in India. 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 participants in a particular geographical area.

**Aim:** The present study was under taken to establish the normal baseline titre in apparently healthy blood donors and also to determine cutoff values for the Widal test in apparently healthy population.

**Material and Methods:** Blood samples were collected from healthy blood donors over the period from July 2019 to October 2020 and they were analyzed for the presence of the Salmonella antibodies by carrying out the Widal tube agglutination test.

**Results:** Out of the 500 participants, which were tested, 297 (59.4%) sera were found to be positive for Widal test and 203 (40.6%) were negative. The most frequently recorded baseline titre of the reactive sera was 1:20 for the anti-O antibodies and it was 1:40 for the anti-H antibodies and this was the baseline titre for this region. The frequency rate was higher in male and the most frequent blood group was noted maximum in B.

**Conclusion:** Baseline titre for O, H, AH and BH antigen is 1:20, 1:40, 1:10 and 1:10 respectively. It has been recommended that the significant titre of the O agglutinins and H agglutinins of *Salmonella* Typhi, *Salmonella* Paratyphi AH and Paratyphi BH is 1:80, 1:160, 1:40 and 1:40 respectively for area in and around Gwalior and Chambal region. Therefore, recommend changing currently used baseline titre of widal test in this region.

**Key words:** Baseline Widal titres; Enteric fever, Widal test

### Introduction

Enteric fever is a major endemic health problem in India. It is a serious bloodstream infection caused by the human restricted bacterial pathogens *Salmonella enterica* subspecies *enterica* serotype Typhi and paratyphoid fever caused by *Salmonella enterica* subspecies *enterica* serotypes

Paratyphi A, Paratyphi B, and Paratyphi C.<sup>1</sup>The transmission occurs faeco-orally through contaminated water and food. An estimated 14.3 million infections and more than 135,000 deaths are caused by enteric fever worldwide each year.<sup>2</sup>The criterion standard for diagnosis of enteric fever has long been culture isolation of the organism.<sup>3</sup>

Among these, the isolation of bacteria from blood or bone marrow is considered the gold standard. However, in developing countries, culture facilities are not easily available or accessible and serological tests such as Widal test remain the mainstay of diagnosis. The Widal test detects the presence of antibodies against antigens of *Salmonella enterica* in the blood, provide a more convenient alternative. In many countries as in India, the method of choice is widal test which is almost a century old laboratory technique. The interpretation of the Widal test depends upon the baseline titre which is prevalent amongst the healthy participants 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.<sup>4,5</sup> Updating the baseline Widal titre is mandatory for the proper interpretation of the Widal test. Regular updating of the baseline titer is a must for the proper interpretation and utilization of the Widal test in diagnosis of enteric fever.<sup>6</sup>

In the view of above, the present study was carried out to evaluate the baseline antibody titre for O, H antigens of

*Salmonella enterica* subspecies *enteric* serotype Typhi and H antigens of Paratyphi A and Paratyphi B among apparently healthy population of Gwalior and Chambal division of Madhya Pradesh, India taking into consideration the fact that enteric fever is a major public health problem in Gwalior and Chambal division of Madhya Pradesh.

### Materials and Methods

The study was conducted at Department of microbiology, Gajra Raja Medical College and J.A. Group of Hospitals, Gwalior, Madhya Pradesh, which includes population of Gwalior and Chambal Division of Madhya Pradesh comprises of seven districts i.e. Gwalior, Sheopur, Shivpuri, Guna, Ashok nagar, Morena, Bhind and Datia. The major sources of water for this population are public supply water, boreholes and rivers. Approval of the institutional ethical committee was obtained for the carrying out this study.

The subjects included 500 healthy blood donors. Blood samples were collected after obtaining written informed consent from all of them (male and female sexes) aged 16 to 55 years, who attended the blood bank of our institution during the period of July 2019 to October 2020.

**Table-1: District wise distribution of participants in the study**

District	Number
Gwalior	163
Bhind	73
Morena	74
Sheopur	34
Shivpuri	58
Datia	40
Ashoknagar	24
Guna	34
Total	500

The blood donors were screened using a survey questionnaire. Blood samples were

collected from apparently healthy donors, who had neither been vaccinated with

TAB (Typhoid and Paratyphoid A and B) vaccine or oral typhoid vaccine/Vi vaccine nor had suffered from enteric fever in the past. Individuals with any active or recent infections including hepatitis B, hepatitis C, malaria, Dengue fever, and HIV/AIDS and any type of fever in the last 6 months were excluded from this study. The Widal tube agglutination test was done on all sera using commercially available antigens containing *S. enterica* subsp. *enterica* ser. Typhi O and H antigens and *S. enterica* subsp. *enterica* ser. Paratyphi A 'H' antigen (SPAN diagnostic private limited). 0.4 ml of two fold serially diluted patients sera (dilution from 1:20 to 1:320) in 0.9% normal saline was tested by adding an equal volume of antigen. A negative control was included in each batch of the test.

**Statistical Analysis:** Data was analyzed in an excel sheet and the variables were summarized using frequency count, percentage, mean and standard deviation.

**Results:** A total of 500 healthy

participants from different districts [Table-1] were screened for the agglutinins against the *Salmonella enterica* subspecies *enterica* serotypes, Typhi, Paratyphi A and Paratyphi B by the Widal tube agglutination test. We found the relationship between participants and sex. The frequency rate was higher in male, 473 (94.6%) patients compared with females 27 (5.4%).

A total of 297 (59.4%) samples were positive for the agglutinins ( $\geq 1$  in 20) whereas 203 (40.6%) samples did not show agglutinins ( $\leq 1$  in 10) [Table-2]. Table 3 shows the frequency of participants according to their age group among different age groups. The highest frequency (24.5%) was observed in the group aged 21-25 years followed by the groups aged 26-30 (21.8%), 31-35 (17%), 36-40 (15.2%), 41-45 (11.6%), 46-50 (6%), 16-20 (2.8%) and 51-55 (1.6%) respectively. The lowest frequency (1.6%) was observed in the group aged 51-55 years.

**Table-2: Distribution of samples for agglutination in Widal Test**

Widal status	Frequency	Percentage
Positive Agglutinins ( $\geq 1:20$ )	297	59.4
Negative Agglutinins ( $\leq 1:10$ )	154	40.6
Total	500	100

**Table 3: Frequency of participants according to their age group**

Age groups (Year)	Number of participants (Percentage)
16-20	14 (2.8)
21-25	121 (24.2)
26-30	109 (21.8)
31-35	84 (17)
36-40	76 (15.2)
41-45	58 (11.6)
46-50	30 (6)
51-55	08 (1.6)

The distribution of the samples with an antibody titre of  $\geq 1:10$  against different serotypes of *Salmonella enterica* subspecies *enterica* showed an antibody to the anti 'O' antigen in 236

(47.2%) samples, an antibody to the anti ‘H’ antigen in 297 (59.4%), an antibody to the anti AH antigen in 69 (13.8%) samples and an antibody to the anti BH antigen in 36 (7.2%) (Table 4). Among the 236 (47.2%) participants, who were found to be positive against O antigen of *S. Typhi*, 92 (18.4%) participants had a titre of 1:10, 142 (28.4%) participants had a titre of 1:20, 02 (0.1%) participants had a titre of 1 : 40 and 1 (0.2%) participants had a titre of 1 : 80. Similarly, among the *S. Typhi*, 297 (59.4%) participants, who were found to be positive against H antigen of *S. Typhi*, 75 (15%) participants had a titre of 1:10, 87 (17.4%) participants had a titre of 1 : 20 and 134 (26.8%) participants had a titre of 1 : 40.

**Table 4: Distribution of samples with antibody titre>1:20 against different serotypes of *Salmonella enterica subspecies enterica***

Serotype	Antibody type	Frequency	Percentage
Typhi	Anti O antigen	236	47.2
Typhi	Anti H antigen	297	59.4
Paratyphi A	Anti AH antigen	69	13.8
Paratyphi B	Anti BH antigen	36	7.2

Out of the 69 (13.8%) participants who tested positive against H antigen of *S. Paratyphi A*, 26 (5.2%) participants had a titre of 1 : 10, 38 (7.6%) participants had a titre of 1 : 20 and 5 (1%) participants had a titre of 1 : 40. It can be seen that comparatively less participants (13.8%) were positive for H antigen of *S. Paratyphi A*. Among 36 (7.2%) participants positive against H antigen of *S. Paratyphi B*, 09 (1.8%) participants had a titre of 1 : 10 and 27 (5.4%) participants had a titre of 1 : 20. (Table 5)

**Table 5: Comparison of frequency and percentage of baseline antibody titres against different serotypes of *Salmonella enterica***

Antigen	Total	Dilution end titres (N = 400)					
		(1:10)	(1:20)	(1:40)	(1:80)	(1:160)	(1:320)
<i>S. Typhi</i> “O”	236 (47.2%)	92 (18.4%)	142 (28.4%)	02 (0.1%)	01 (0.2%)	-	-
<i>S. Typhi</i> “H”	297 (59.4%)	75 (15%)	87 (17.4%)	134 (26.8%)	-	-	-
<i>S. Paratyphi</i> AH”	69 (13.8%)	26 (5.2%)	38 (7.6%)	5 (1%)	-	-	-
<i>S. Paratyphi</i> BH”	36 (7.2%)	09 (1.8%)	27 (5.4%)	-	-	-	-

The most frequent blood group in relation to the frequency of participants was noted maximum in group B (42%) and followed by O (38.6%), AB (17.4%) and A (14.5%) respectively [Table 6].

**Table 6: Frequency of participants according to their blood group**

Blood Group	Number(percentage)
A (+/-)	74 (14.5)
B (+/-)	210 (42)
AB (+/-)	87 (17.4)
O (+/-)	129 (38.6)

**Discussion:** Enteric fever afflicts the local community and the other factors such as incidence being on upsurge during the rainy season due to water logging and the contamination of the water with faecal material. The social factors that add to enigma are the pollution of the drinking water supplies due to open air defecation, urination, substandard food, personal hygiene habits and health ignorance.<sup>7,8</sup>

The isolation of the various strains of *Salmonella enterica subspecies enteric* from blood remains the gold standard for the diagnosis of enteric fever. The Widal agglutination test has always been the preferred test for diagnosis of enteric fever in many developing countries, including India, where it is extensively practiced. When interpreting the Widal test it is of utmost important that the test be interpreted against the background of normal titre of the population in a particular area. These titres may also vary among the endemic areas and with time.

So each country, or region should have the baseline titres of their healthy population which should be updated with time.<sup>9</sup>

This study was the first ever study done in Gwalior and Chambal division of Madhya Pradesh, to the best of our knowledge, which was conducted for the estimation of baseline antibody titre in healthy participants against serotypes Typhi and Paratyphi A and Paratyphi B of *Salmonella enterica*. Our study has shown that disparate titres of antibody, against the different serotypes of *Salmonella enterica*, exist in healthy participants. The results of our study established to develop the baseline titers in healthy participants for the interpretation of widal test results in our geographic area. After the analysis of this study we found that the sera of significant of healthy participants in this area contain *Salmonella* agglutinins which were capable of reacting to the variable titres in Widal test.

**Table 7: Comparative analysis of baseline titre of ‘O’ and ‘H’ agglutinins in different regions of India**

Authors	Place	Year	Percentage of Positive Samples	Baseline Titre	
				TO	TH
Gunjal S <i>et al</i> <sup>10</sup>	Ahmednagar (Maharashtra)	2012	81.55	1:40	1:40
Saxena N <i>et al</i> <sup>11</sup>	Hadoti region (Rajasthan)	2012	54	1:40	1:40
Sharma S <i>et al</i> <sup>12</sup>	Jaipur (Rajasthan)	2013	42.6	1:40	1:80
Mittal G <i>et al</i> <sup>13</sup>	Uttarakhand	2014	51	1:40	1:80
Vazhavandal G <i>et al</i> <sup>14</sup>	Trichy (Kerala)	2014	53.5	1:80	1:40
Jeyakumari <i>et al</i> <sup>15</sup>	Puducherry	2015	56.8	1:80	1:80
Vijaylaxmi <i>et al</i> <sup>16</sup>	Jodhpur (Rajasthan)	2017	56	1:20	1:40
Our study	Gwalior (Madhya Pradesh)	2020	59.4	1:20	1:40

The level of titre detectable in healthy population of different area vary considerably. Frequency of antibodies in normal population reported by various workers from different parts of India ranges from 1:20 to 1:160. The highest

level of titre for TO, TH, AH and BH was found to be 1:80, 1:40, 1: 40 and 1: 20 respectively. Our findings were in almost similar with those of Saxena N *et al*.<sup>13</sup> Some of the authors has reported high titre of antibodies, which may be caused by

infections with cross reacting organisms other than *Salmonella*. For example, cross reaction between a normally harmless gut bacterium, *Citrobacterfreundii*, can confuse the diagnosis of typhoid bacilli which share antigenic properties.

In the present study, the baseline titre for the TO, TH, AH and BH was found to be 1:20, 1:40, 1: 20 and 1: 20 respectively. Our findings were in accordance with those of Vijay laxmi *et al.*<sup>16</sup> Some of authors<sup>10,12,13</sup> has reported comparatively higher titre than our study, which may have contributed to this discrepancy, because the differences in the antibody response may be due to the poorly standardized antigen preparation and the sharing of the antigen determinants with other *Salmonella* and the variations in the titres could have been caused by differences in endemicity of typhoid in each area. 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 participants in the population is known. In India most of the cases of typhoid fever occur in 5-39 age group. In our study only adults are included and the youngest volunteer was 17-year-old. Maximum incidence is noted in the age group 21-25 years. Both sexes are equally susceptible, but in our study 473 (94.6%) male and 27 (5.4%) females participants were present. Males are more because for blood donation males are preferred due to his good built. There is no seasonal variation in the countries where the disease is non-endemic. Peak incidence was noted during the period from June to August, November to January and the cases continue to occur throughout the year.

**Conclusion:** Based on the results of our study, it has been recommended that Widal test remains one of the best, easily accessible, cheap and simple method for the diagnosis of enteric fever. Any titre above the baseline titre can be taken as a significant titre for the diagnosis of enteric fever. In our study, baseline titre for O, H, AH and BH antigen is 1:20, 1:40, 1:10 and 1:10 respectively. It has been recommended that the significant titre of the O agglutinins and H agglutinins of *Salmonella* Typhi, *Salmonella* Paratyphi AH and Paratyphi BH is 1:80, 1:160, 1:40 and 1:40 respectively for area in and around Gwalior and Chambal region. Therefore, recommend changing currently used baseline titre of widal test in this region.

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