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A Study on the Baseline Widal Titre amongst Healthy Individuals in Trichy, India

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ABSTRACT

The interpretation of the single Widal test depends upon the baseline titre which is prevalent amongst healthy individuals in a particular geographical area. Hence the study was undertaken to determine the baseline widal titre, titre of antibodies to the O and H antigens of S. typhi and H antigens of S. paratyphi A and B, amongst apparently healthy blood donors in Trichy, Tamilnadu, India.

Blood samples were collected from healthy blood donors (n=566) who attended our blood bank from August 2010–August 2011 and they were analyzed for the presence of Salmonella antibodies by carrying out the rapid widal slide agglutination test (Tydal-Tulip Diagnostics).

Among the 566 serum specimens which were tested, 302 (53.4%) sera were found to be positive for agglutinins ($1 \ge in 20$) and 264 (46.6%) sera were negative for agglutinins (1 < in 20). Contd...... Based on the above results of our study, it has been recommended that the cut off titre of 1:160 for S.typhi O and H agglutinins, AH agglutinins and BH agglutinins may be considered as diagnostic for enteric fever in Trichy region of Tamilnadu. Key words: Agglutinins, Baseline titre, Enteric fever, Salmonella and widal test.

INTRODUCTION

Enteric fever is an important major public health problem in India. It is a systemic infection caused by Salmonella typhi and Salmonella paratyphi A and B. Exposure of the individuals to contaminated food and water closely correlate with the risk of enteric fever (Sur et al, 2007, Chowta and Chowta, 2005). Typhoid fever is a febrile illness of prolonged duration characterized by step ladder fever, diffuse abdominal pain, frontal headache, delirium, splenomegaly, hepatomegaly and many other systemic manifestations due to septicaemia and bactremia. The definitive diagnosis of enteric fever in patients with the typical clinical picture is made on the basis of isolation of Salmonella from blood, bone marrow, stool or urine and demonstration of 4 fold rise in the antibody titre to both O and H antigens of Salmonella between the acute and convalescent phase. For diagnosing a case of enteric fever, widal test is the second most commonly used test after blood culture. In developing countries, facilities for culture isolation are not always available and diagnosis relies upon detection of agglutinins in a single serum taken on admission to see significant antibody levels (Collier et al., 1998). In endemic countries like India, the interpretation of Widal test depends upon the baseline titre which is prevalent amongst the healthy individuals in a particular geographical area. The baseline titre among the healthy populations of different areas differs substantially and this depends upon the endemicity of the typhoid in each area which has been changing over time (Pang and Puthucherry, 1983, Punia et al., 2003 and Peshattiwar, 2012).

Hence this study was undertaken to determine the baseline widal titre and to define the significant titre for the diagnosis of enteric fever in single serum, in Trichy region, Tamilnadu, India.

MATERIAL AND METHODS

The cross sectional study was carried out in Department of Microbiology, Chennai Medical College Hospital and Research Centre, Trichy. After obtaining informed consent verbally, non repetitive blood samples were collected from healthy blood donors (n=566) of both sexes, in the age group of 20-50 years ,who attended our blood bank during August 2010 to August 2011. Amongst whom, 524 (92.6%) were males and 42 (7.4%) were females. Those who were vaccinated for enteric fever in the preceding 3 years and those with the recent history of fever were excluded from the study.

Sera were first screened for agglutination with Salmonella antigens. All the positive sera were semi quantitatively analyzed for the determination of antibody titre. The Widal test kits containing O, H, AH and BH antigens were supplied by Tulip Diagnostics (P) Ltd. Goa. Positive and negative controls were included in each batch of tests. The results were analyzed and interpreted as per the standard guidelines. The titre was taken as the highest dilution of serum with a visible agglutination.

RESULTS

A total of 566 healthy blood donors were screened for agglutinins against *Salmonella typhi*, paratyphi A and B by the rapid Widal slide agglutination test. Table -1, shows the results of widal test. Out of 566 sera, 302 (53.4%) serum samples were positive for one or more type of agglutinins ($1 \ge$ in 20) and 264 (46.6%) were negative (1< in 20) for agglutinins.

Antigen	No. Of positive samples (%)	Titre (1:20)	Titre (1:40)	Titre (1:80)	Titre (1:160)	Titre 1:320)
S. typhi O	302(53.4)	44(7.8)	84(14.8)	126(22.3)	36(6.4)	12(2.1)
S. typhi H	250(44.2)	8(1.4)	40(7.1)	122(21.6)	64(11.3)	16(2.8)
S. paratyphi AH	46(8.1)	4(0.7)	10(1.8)	22(3.9)	8(1.4)	2(0.4)
<i>S. paratyphi</i> BH	12(2.1)	-	4(0.7)	8(1.4)	-	-

Table.1 Number and percentage of sera with end titres in healthy blood donors.

Table-2.Comparative analysis of baseline titre of O and H agglutinins in different regions of India and Nepal.

Authors	Place	Year	Baseline titre			
			ТО	TH	AH	BH
Shukla et al. 1997	Central India	1997	1:80	1:80	0	0
Punia et al. 2003	Chandigarh	2003	1:80	1:160	1:20	1:20
Patil et al. 2007 Karnataka		2007	1:80	1:80	1:40	1:40
Sneha, 2011 Pondicherry		2011	1:80	1:80	1:40	1:40
Peshattiwar,	Amalpuram (Andhra	2011	1:40	1:40	1:20	0
2012 Pradesh)						
Pal, 2013 Garhwal (Uttarakand)		2011	1:40	1:80	1:20	1:20
Pandey, 2013 Nepal		2012	>1:80	>1:80	>1:80	>1:80
Bahadur, 2013	Raichur (Karnataka)	2013	1:160	1:160	1:20	1:80
Gunjal, 2013	Ahmednagar (Maharashtra)	2012	1:40	1:40	1:80	1:80
Saxena et al. 2013	Hadoti Region (Rajasthan)	2012	40	40	20	20
Present study	Trichy	2011	1:80	1:80	1:80	1:80

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The agglutinins to *S. typhi* were the most prevalent among the sera of various dilutions (53.35% for O antigen and 44.2% for H antigen) which were tested. The levels of agglutinins for *Salmonella paratyphi* AH and BH were very low (only 8.1% and 2.1% for the AH and BH antigens respectively).

Table-1 depicts that the distribution of 302 samples(53.35%) with anti O agglutinins, 44 (7.8%) had a titre of 1:20, 84 (14.8%) had a titre of 1:40, 126(22.3%) had a titre of 1:80. 36 (6.4%) had a titre of 1:160 and 12 (2.1%) had the highest titre of 1:320.Similarly, among the 250 (44.2%) sera, which showed *S. typhi* H agglutinins, 8(1.4%) had a titre of 1:20, 40 (7.1%) had a titre of 1:40, 122 (21.6%) had a titre of 1:80, 64(11.3%) had a titre of 1:160 and 16(2.8%) had the highest titre of 1:320.

Out of the 46 (8.1%) sera which showed *S. paratyphi* AH agglutinins, titre of \geq 1:20 was seen in 4(0.7%) sera, a titre of 1:40 was seen in 10(1.8%), titre of 1:80 was seen in 22(3.9%). The highest titre of 1:160 and 1:320 were found in 8(1.4%) and 2(0.4%) sera respectively.

Among the 12 (2.1%) sera which showed *S. paratyphi* BH agglutinins, 4 (0.7%) had a titre of 1:40. The highest titre of 1:80 was seen in 8(1.4%) sera.

DISCUSSION

Hence enteric fever is highly endemic in our country; it is the prime suspect cause of PUO. The isolation of Salmonella species from blood is the gold standard for diagnosis of acute infection. Lack of feasibility of the culture isolation, make the 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 such a rise is not always possible and the patient cannot wait for long. Lack of proper timing of the sample collection and the initiation of antibiotic therapy before diagnosis is made, could be one of the major reasons for the poor isolation rates (Madhusudhan and Manjunath, 2012 and Pal et al., 2013) and the blunted antibody response (Schroeder, 1968). Analysis of this study showed that the sera of significant proportion of healthy individuals in this area contained Salmonella agglutinins which were capable of reacting to the variable titres in Widal test. In acute enteric fever, a rise in O agglutinin titre followed by a slow increase of H agglutinin occurs. The H agglutinin response persists longer than O agglutinin response. In our study; we found that 53.4% sera were reactive for O agglutinins and 44.2% for H agglutinins of *S. typhi*. The highest level of titre was found to be 1:320 for the O (2.1%) and H agglutinins (2.8%) of S. typhi. However highest percentage of samples (22.3% for the O agglutinins, 22.6% for H, 3.9% for AH, 1, 4% for BH agglutinins of Salmonella serotype typhi) showed a titre of 1:80. Thus the baseline titre for the O, H, AH and BH Salmonella agglutinins was assumed to be 1:80.

From Table-2, it has been evident that the baseline titre is subject to variations, depending upon the geographical area and hygienic conditions of the region. Hence the baseline titre of a particular area must be known and updated with time (Pang and Puthucherry, 1983). The most likely reason for the very high titre in our study could be poor health and sanitary conditions.

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CONCLUSION

Any titre above the baseline titre can be taken as a diagnostic titre for the diagnosis of enteric fever. Based on our study results, it has been recommended that the significant titre of the O agglutinins and H agglutinins of *Salmonella typhi, Salmonella paratyphi* AH and paratyphi BH was \geq 1: 160.

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