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Determination of Base Line Widal Titre value among the Healthy Individuals of Pondicherry, India

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Abstract
Background: *Dense population and poor sanitation makes enteric fever is a endemic disease in developing countries like India. Widal agglutination test is the popular test and it remains a cornerstone in diagnosing enteric fever. In endemic regions the normal healthy individual contains antibodies which will react with variable titre in the Widal test, due to a past exposure and give false positive results. Determining the base line titre value is very important and the present study aim to determine the average baseline titre of the apparently healthy population in the region of Pondicherry, India.*
Materials & Methods: *Blood samples were collected from healthy individuals (n=500) and analysed for the presence of antibodies using Widal test.*
Results: *Out of 152 sera which showed positive to S.typhi 'O' agglutinins, titre of >1:20 was seen in 22(4.4%) sera, a titre of 1:40 was seen in 128(25.6%), titre of 1:80 was seen in 2(0.4%). Amongst 164 sera positive for S.typhi 'H' agglutinins, 14 (2.8%) showed agglutination up to 1in 20 titre, 37 (7.4%) up to 1in 40 and 113 (22.6%) up to 1in 80. The agglutinating titre of 1:40 for the AH titre against Salmonella serotype Paratyphi A was seen in 8(1.6%) samples, a titre of 1:40 was seen in 4(0.8%) samples. Among 9 samples 5 (1.0%) samples were positive for anti 'BH' of Salmonella serotype Paratyphi B in the titre of 1in 20 and 4 (0.8%) samples were in 1 in 40 titre.*
Keywords: *Widal test, enteric fever, pondicherry*

Introduction

Enteric fever is an endemic disease which is prevalent around the globe mainly occurs in areas with poor quality potable water and sanitation. In India, the morbidity rate of typhoid is 102 to 2219 per 100,000 population[(1)]. According to WHO, the annual infectious rate for enteric fever is 21.6 million with the appropriate death rate of 600 000 and highest percentage is from Asia and Africa[(2)]. In developed countries the incidence of typhoid fever is very less due to proper sanitation but antibiotic resistant *Salmonella sp* pose a grave problem[(3)]. Enteric fever is classified as typhoid which is caused by *Salmonella typhi* and the paratyphoid is caused by *S. paratyphi* A, B and C. Both the organism are gram negative, motile and non flagellate variant occurs and are non capsulated. The

toxicity of this organism depends upon the presence of lipopolysaccharides in the outer membrane[2]. Enteric fever is severely prevalent in local community and travelers to these areas are affected mainly during the rainy season due to water logging and fecal contamination in water. The fever spread through faeco-oral route by food or water contaminated with urine and faeces of a patient or from a carrier. Circulating bacteria in blood stream and intestinal tract from patient infected with typhoid can able to easily spread the disease by contaminating the food and water.

Diagnosis of enteric fever is difficult due to multi drug resistance, change in antigen presentation and intake of inadequate dose of antibiotics during the virulent stage of infection and time consuming[4]. Conclusive diagnosis of enteric fever can be made by isolating the bacterium from different samples like blood, stool, urine, bone marrow, bile or other body fluids. However, this method is costly and not always available in developing countries[5]. Therefore, a pertinent alternative is the use of the serological technique called Widal test[6]. Widal test detects agglutinins against O and H antigens of *Salmonella typhi* and H antigens of *Salmonella paratyphi* A and B. Interpretation depends upon the baseline titre of this agglutinins to O and H antigens of *Salmonella typhi* and H antigens of *S.paratyphi* [7]. In Widal test, suspension of killed *Salmonella* was used as antigen which agglutinin with the antibody present in the patient sample with suspected enteric fever. The agglutinins of base line titre value vary according to the place which depends upon the prevalence of the fever and it differs from period to period[5]. Updating the baseline titre value is compulsory for the proper diagnosis of enteric fever. The present study focuses on the determination of base line Widal titre among the healthy individuals in and around Pondicherry.

Materials and Methods

The study was performed in the department of Microbiology, Sri venkateshwara medical college hospital and research centre, Ariyur, Pondicherry. The study protocol and objectives were clearly explained and informed consent was acquired from all the donors. 500 non-repetitive blood samples were collected from healthy individual of both gender during the period of December 2015 to May 2016. About 5 ml of the venous blood was collected in a plain tube and was allowed to clot at room temperature for about 30 minutes. The samples were centrifuged at 3000 rpm for 10 minutes to separate the serum from the blood. The serum was collected, transferred to storage vials and stored at -20^{o}c.

Stained *Salmonella* antigen test kit for tube test was procured from Arkray health care pvt Ltd. The test kit contains smooth suspensions of antigens "O" and "H" of *Salmonella* serotype Typhi, "H" antigen of *Salmonella* serotype Paratyphi A, and "H" antigen of *Salmonella* serotype Paratyphi B. The experiment was conducted by bringing the sera and kit reagent to room temperature. Widal titre was estimated by confirmatory quantitative tube agglutination test according to the manufacturer instructions. The result was elucidated and analyzed according to the standard guidelines and negative control was included.

Results

A total of 500 healthy volunteers were screened for agglutinins against Salmonella enterica subspecies enterica serotypes, Salmonella typhi, paratyphi A and B by the rapid Widal tube agglutination test. Out of 500 sera, 266(53.2%) serum samples were positive and 234(46.8%) serum samples were negative for Salmonella serotypes typhi and paratyphi A & B. In this positive samples, 152 (30.4%) were positive for 'O' agglutinin and 164 (32.8%) were found positive for 'H' agglutinin of Salmonella typhi. Only 12 (2.4%) and 9 (18%) samples were positive for agglutinins to paratyphi AH & BH respectively.

Out of 152 sera which showed positive to S.typhi 'O' agglutinins, titre of >1:20 was seen in 22(4.4%) sera, a titre of 1:40 was seen in 128(25.6%), titre of 1:80 was seen in 2(0.4%). Amongst 164 sera positive for S.typhi 'H' agglutinins, 14 (2.8%) showed agglutination up to 1in 20 titre, 37 (7.4%) up to 1in 40 and 113 (22.6%) up to 1in 80. The agglutinating

titre of 1:40 for the AH titre against Salmonella serotype Paratyphi A was seen in 8(1.6%) samples, a titre of 1:40 was seen in 4(0.8%) samples. Among 9 samples 5 (1.0%) samples were positive for anti 'BH' of Salmonella serotype Paratyphi B in the titre of 1in 20 and 4 (0.8%) samples were in 1 in 40 titre (Table 1).Comparative analysis of Baseline titre of O and H agglutinins in different regions of India was presented in Table 2.

Table 1: Number & percentage of sera with end titres in healthy volunteers

Antigen	No. of positive sample (%)	Dilution 1:20	Dilution 1:40	Dilution 1:80
S. typhi O	152 (30.4%)	22 (4.4%)	128 (25.6%)	2 (0.4%)
S. typhi H	164 (32.8%)	14 (2.8%)	37 (7.4%)	113 (22.6%)
S. paratyphi AH	12 (2.4%)	8 (1.6%)	4 (0.8%)	-
S. paratyphi BH	9 (1.8%)	5 (1.0%)	4 (0.8%)	-

Table 2: Comparative analysis of Baseline titre of O and H agglutinins in different regions of India

Author	**Place**	**Year**	**Baseline titre**			
			TO	**TH**	**AH**	**BH**
Shukla et al.	Central India	1997	1:80	1:80	0	0
Punia et al.	Chandighar	2003	1:80	1:160	1:20	1:20
Patil et al.	Karnataka	2007	1:80	1:80	1:40	1:40
Sneha et al.	Pondicherry	2011	1:80	1:80	1:40	1:40
Peshattlwar et al.	Andhrapredesh	2011	1:40	1:40	1:20	0
Pal et al.	Uttarakand	2011	1:40	1:80	1:20	1:20
Bahadur	Karnataka	2013	1:160	1:160	1:20	1:80
Gunjal	Maharashtra	2012	1:40	1:40	1:80	1:80
Saxena	Rajasthan	2012	1:40	1:40	1:20	1:20
Vazhavandal G et al.	Trichy	2010	1:80	1:80	1:80	1:80
Shekar Pal et al.	Garhwal	2013	1:40	1:80	1:20	1:20
Kataria et al.	Dehradun	2013	1:80	1:80	1:20	1:20
Bijapur et al.	Kannur	2014	1:40	1:40	0	0
Rucha Patki	Mumbai	2014	1:40	1:80	1:80	1:80
Jeyakumari et al.	Puducherry	2015	1:80	1:80	1:40	1:20
Shetty jeevan	Uttar Pradesh	2016	1:80	1:80	1:40	1:40
Nidhi Sharma et al.	Jabalpu	2017	01:40	01:40	01:40	01:20
Present study			01:40	01:40	01:20	01:20

Discussion

In India the prevalence of enteric fever is increased due to increase of anti microbial resistance. In 2010 global burden of typhoid fever was approximately 12 million. Most of the cases were treated effectively using antibiotics but mortality rate remains 1% which shows 130,000 deaths occurs annually[8]. Early diagnosis of enteric fever is the essential for treatment. The gold standard for the diagnosis of enteric fever depends on the isolation of various strains of *Salmonella enterica subspecies enterica* from the blood sample[9]. However, in the current scenario improper use of broad spectrum antibiotics, self medication practice and improper timing of sample collection results in the reduced productivity of blood culture technique[4]. The cut off value differ among various population which is based on the level of the typhoid antibodies and typhoid vaccination. The variation depends on the prevalence of typhoid in endemic area and the value may change over time. Therefore updating the base line titre value among the healthy individuals over the period of time is mandatory. The preeminent method to observe H antigen titre is through rise in base line titre of O antigen. The present study were performed in Pondicherry among the healthy individuals which showed 30.4% persons were positive for *S. typhi* O, 32.8% showed positive for *S. typhi* H and 2.4% for *S. paratyphi AH*. The result implies that healthy individuals showed positive value for atleast one antigen therefore the baseline titre value against O antigen and H antigen of *Salmonella enterica* serotype Typhi were found to be 1 : 40 and 1 : 80 respectively. This is concordant with the previous studies conducted in other places of India there by the disease is endemic as shown in the table 2. This study helps to screen the suspicious infection of fever by seeing titre values in paired sera sample.

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