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Study of the Baseline Widal titre among apparently healthy individuals attending Saveetha Medical College and Hospital Blood Bank.

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ABSTRACT

The Widal test is used as an alternative to the microbial culture for the diagnosis of enteric fever. The widal titres among the healthy populations of different areas differ substantially depending upon the endemicity of typhoid in each area. Updating the baseline wida titre is a must for the proper interpretation of the Widal test. Hence, the following study is undertaken to determine the average baseline widal titre. The descriptive study was done with non-repetitive blood samples from apparently healthy blood donors (n=600) who attended our blood bank from June 2013 to December 2013. Widal titres were analysed by performing standard tube agglutination test. Out of 600 donors, 30.84% donors were from Thiruvallur district. 98.7% and 99.5% showed ≤1:40 and ≤1:80 for O and H titres of *Salmonella enterica* serovar Typhi respectively. 99.4% and 99.7% showed 1:20 titre for H antigen of *Salmonella enterica* serovar Paratyphi A and Paratyphi B respectively. The baseline titre for the TO and TH was found to be 1:40 and 1:80 respectively. The baseline titre for AH and BH was found to be 1:20. Hence 1:80 and 1:160 for TO and TH respectively and 1:40 for AH and BH was considered as diagnostic widal titre.

Keywords: baseline, widal titre, endemicity, enteric fever.

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INTRODUCTION

Enteric fever is endemic in India and it continues to be one of the major health problems. *Salmonella enterica* serovar Typhi, Paratyphi A and Paratyphi B is the aetiological agent of typhoid fever. In India, the disease is endemic with an incidence which ranges from 102 to 2219 per 100,000 populations [1]. The definitive diagnosis of enteric fever in the patients with a compatible clinical picture are the isolation of the Salmonellae from blood, bone marrow, stool or urine and the demonstration of the 4 fold rise in the antibody titre to both the O and the H antigens of the organism between the acute and the convalescent phase sera [2].

The gold standard for its diagnosis rests on the recovery and identification of the causal organisms, from blood during the first few days of the illness, or from faeces during the second and third weeks of the illness or from urine during the third and fourth week[3]. The serological test, Widal test, is a well known test, used as an indirect test to detect the "shadows" "footprints" of *Salmonella* groups. The possibility of a quick serodiagnostic test for typhoid fever has engaged the attention of scientists in the last few years. Haemagglutination, coagulation, fluorescent antibody, enzyme linked immunosorbent assay (ELISA) and counter immuno electrophoresis (CIEP) have all been used for the serological diagnosis of typhoid fever [4].

In these settings, the Widal test, a serological test which was developed by Georges Fernand Isidore Widal in 1896, is an alternative to the microbial culture, which is commonly used for the diagnosis of enteric fever ever since its introduction 100 years back [1,3]. Though fought with many problems still Widal test is used widely for the serodiagnosis of enteric fever in many peripheral centers, as it is the most economical test available for the diagnosis of enteric fever [5]. To provide its aid in the diagnosis of typhoid fever, the widal test utilizes a suspension of killed *Salmonella enterica* subsp*. enterica* serovar Typhi as the antigen to detect the antibodies against the O and H antigens of *Salmonella enterica* serovar Typhi and against the H antigens of *Salmonella enterica* serovar Paratyphi A and B [2].

The interpretation of the Widal test depends upon the baseline titre which is prevalent among the healthy individuals 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. Updating the baseline widal titre is a must for the proper interpretation of the Widal test [6,7,8]. Hence, the following study is undertaken to determine the baseline widal titre (titre of the antibodies to the O and H Antigens of *Salmonella typhi* and to the H Antigens of *S. Paratyphi A* and *B*) among apparently healthy individuals in our set up.

MATERIALS AND METHODOLOGY

The descriptive study to analyze the baseline widal titre among apparently healthy individuals was conducted in the Department of Microbiology at Saveetha Medical College and Hospital after obtaining the ethical committee's clearance and the university scientific review board's approval. The details of age, sex, history of fever etc., were collected from the donors. Non-repetitive blood samples of 600 donors were collected from apparently healthy donors who attended the blood bank at Saveetha Medical College and Hospital. Those individuals who were not willing for the study, those who had persistent fever for >7 days in past 6 months, those individuals found to be positive for Malaria, HBsAg and antibodies to HIV, HCV and *Treponema pallidum*, those who suspected typhoid fever by a doctor in the past 6 months and vaccinated individual in the last 1 month were excluded from the study. The samples are processed by standard tube agglutination test [9]. Commercially available antigens which contain the *Salmonella enterica* serovar Typhi O and H antigens, the *Salmonella enterica* serovar Paratyphi AH antigen and the Paratyphi BH were used.

For each serum, starting dilution in saline, e.g., a 1-in-10 dilution was prepared by pipetting 0.1 mL serum into 0.9mL saline. For each bacterial antigen, 1-6 tubes for six doubling serum dilutions and tube no.7 for a control without serum was arranged in a rack. Mix the contents (0.1ml serum and 0.9ml saline) of tube 1 and transfer 0.5mL into tube 2. Repeat the process upto tube 6, from which, after mixing, 0.5mL is discarded. Each tube will then contain 0.5mL fluid, tubes 1-6 containing serum dilutions of 10, 20, 40, 80, 160 and 320. Tube 7 had only saline without serum.

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With a graduated 1mL pipette, 0.5mL of the antigen to each tube was added starting from tube 1-7. The serum dilutions in tubes 1-6 are then 20, 40, 80, 160, 320 and 640. Tube 7 was the negative control. The rack of agglutination tubes was placed in a water-bath immersed so that the surface of the water is level with the mid points of the columns of fluid in the tubes. For H agglutinations tubes were incubated for 2-4 hrs at 37^0C and read after standing on the bench for half an hour. For O agglutinations tubes were incubated for 4-6 hrs at 37^0C and read after overnight refrigeration at 4^0C. The results were read by viewing the tubes under a good light against a dark background and with the aid of a ×2 magnifying lens. The tubes were rotated if necessary, to swirl up granules from the deposit. The large flakes of typical H agglutination were visible with the naked eye. The small granules of O agglutination were visible only with the magnifying lens. The control tube 7 which is without serum was examined to exclude autoagglutination.

RESULTS

A total of 600 apparently healthy donors were screened for the agglutinins against the *Salmonella enterica* serovars, Typhi, Paratyphi A and Paratyphi B by the Widal tube agglutination test. Out of 600 donors, 30.84% of donors were from Thiruvallur district followed by Chennai (25.67%), Kanchipuram district (23.5%) and other districts of Tamil Nadu (20%). The district wise distribution of donor is shown in Table 1. Among 600 donors, 60% were negative for Widal test (i.e., <1:20), only 40% showed positive result. The frequency distribution is shown in Table 2.

Table 1: District wise distribution of donors.

District	Frequency	Percent
Thiruvallur	185	30.84%
Chennai	154	25.67%
Kanchipuram	141	23.5%
Other districts	120	20%
Total	600	100%

Table 2: Results of Widal test of total participants.

Antibody titers	Proportions of samples (Frequency)	Percentage
Negative Agglutinins (≤1:20)	360	60%
Positive Agglutinins (≥1:20)	240	40%
Total Participants	600	100%

Table 3: Results of TO, TH, AH & BH titre of *Salmonella enterica* serovars Typhi, Paratyphi A and B

Antigen	Distribution	Widal titre				
		<1:20	**1:20**	**1:40**	**1:80**	**1:160**
S. typhi O	Frequency	392	123	77	8	-
	Percent	65.3	20.5	12.8	1.3	-
S. typhi H	Frequency	360	111	88	38	3
	Percent	60	18.5	14.7	6.3	0.5
S.paratyphi AH	Frequency	583	13	2	2	-
	Percent	97.2	2.2	0.3	0.3	-
S.paratyphi BH	Frequency	589	9	2	-	-
	Percent	98.2	1.5	0.3	-	-

The distribution of the samples with an antibody titre of ≥ 1: 20 against different serotypes of Salmonella enterica subsp. enterica are as follows: Typhi showed an anti-O titre of ≤1:20 in 123 samples(

20.5%), 77 samples (12.8%) had a titre of 1:40 and eight samples(1.3%) had titre of 1:80. Similarly anti-H titre of ≤1:20 is found in 111(18.5%), 88 samples(14.7%) had a titre of 1:40 and 38 samples(6.3%) had 1:80 titre and the highest antibody titre of 1:160 was found in only in 3 samples (0.5%). 99.4% and 99.7% showed ≤ 1:20 titre for H antigen of *Salmonella enterica* serovar paratyphi A and paratyphi B respectively. Table 3 shows the frequency and percentage of titres of TO, TH, AH & BH titre of *Salmonella enterica* serovars Typhi, Paratyphi A and B.

DISCUSSION

Typhoid and paratyphoid fever continue to be important causes of illness and death, particularly among children and adolescents in south-central and Southeast Asia, where enteric fever is associated with poor sanitation and unsafe food and water. In 2000, typhoid fever caused an estimated 21.7 million illnesses and 217,000 deaths, and paratyphoid fever caused an estimated 5.4 million illnesses worldwide. Infants, children, and adolescents in south-central and Southeastern Asia experience the greatest burden of illness [10].

Blood culture remains the gold standard for definitive diagnosis of enteric fever, however most enteric fever occurs in low-and middle-income countries where blood cultures are often unavailable, unaffordable, or inconsistently applied [11]. This makes widal agglutination test as the most common alternative laboratory procedure for the diagnosis of enteric fever. This test detects 'O' and 'H' antibodies against various Salmonella species. The O antigen is the somatic antigen and antibodies against the O antigen are predominantly IgM which rise early in the illness and disappear early. The H antigens are flagellar antigens of *Salmonella enterica* serovar Typhi, Paratyphi A and Paratyphi B. Antibodies to H antigens are both IgM and IgG which rise late in the illness and persist for a longer time [6,12].

In this study, the highest level of the widal titre was found to be 1:80 for the O antigen and 1: 160 for the H antigen of *Salmonella enterica* serovar Typhi. However, 99% of the samples showed a titre of ≤1:40 to the O antigen and 99.5% samples had a titre which was ≤1:80 to the H antigen of *Salmonella enterica* serovar Typhi. Hence the baseline titre for the O and H antibodies of *Salmonella typhi* was found to be 1:40 and 1:80 respectively. The baseline titre for the 'H' antigen of *Salmonella enterica* serovar Paratyphi A and Paratyphi B was found to be 1:20. Similar views have been expressed by earlier workers [13, 14]. A study by Peshattiwar [7] also showed the significant titre of the 'H' agglutinins and the 'O' agglutinins of *Salmonella enterica* serovar Typhi was 1:80. While the significant titre of the 'H' agglutinins of *Salmonella enterica* serovar Paratyphi A was 1: 40, the significant titre of the 'H' agglutinins of *Salmonella enterica* serovar Paratyphi B was 1:20. Another study by NS Madhusudhan [8] showed highest titre of 1:40 for O, 1:80 for H & 1:40 for AH and BH antigen and considered it as significant titre. Abdul Kaleem Bahadur [15] reported 1:320 for TO and TH and ≥1:40 for AH and ≥1:160 for BH as baseline titre.

According to these studies, the widal titres among the healthy populations of different areas differ substantially and it depends upon the endemicity of typhoid in each area, which has been changing over time. So estimation of titre periodically in an endemic area is recommended for the effective diagnosis of enteric fever.

CONCLUSION

Based upon the results of this study, it is recommended that the baseline titre can be 1:40 and 1:80 respectively for the O and H antigen of *Salmonella enterica* serovar Typhi. 1:40 and 1:20 as the baseline titre for the 'H' antigen of *Salmonella enterica* serovar Paratyphi A and Paratyphi B respectively. Hence 1:80 and 1:160 for TO and TH respectively and 1:40 for AH and BH was considered as diagnostic widal titre in our area.

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