Evaluation of the Baseline Widal titre among apparently healthy individuals in Tumkuru, Karnataka, India

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Abstract

Background & Objectives: Enteric fever is a major public health problem in India. Widal test is a cheap, affordable and easily available and the most commonly used test for the serodiagnosis of enteric fever. The purpose of the present study is to evaluate the baseline titre for O and H antigens of *Salmonella enterica subspecies enteric serotype Typhi* and H antigens of *Salmonella enteric subspecies enterica serotype Paratyphi A and Paratyphi B* among normal healthy adult population in Tumkuru, Karnataka, India.

Methods: Blood samples were collected from healthy blood donors (n= 493) who attended blood bank of this institution from January 2016-March 2016. All the samples were tested for the presence of Salmonella antibody by Widal tube agglutination test.

Results: Of the 493 samples which were tested, 199(40.13%)) serum samples were positive for one or more agglutinin(≥1:20) and 294(59.63%) serum samples were negative for agglutinins(≤1:20). Out of these 340(68.96%) and 306(62.06%) samples showed a titer of >1:20 to the O and H antigen of *Salmonella enterica serovar Typhi* respectively. An agglutinin titre of >1:20 against H antigen of *Salmonella enterica serovar Paratyphi A* and *Paratyphi B* were found in 85(17.24%) & 119(24.13%) respectively.

Conclusion: Baseline titre of 1:80 for O and H antigens of *Salmonella Typhi* and 1:40 for H antigens of *Salmonella Paratyphi A and B* were observed in the present study.

Keywords: Baseline titre, Widal test, Endemic area, Enteric fever, Healthy individuals.

Introduction

Enteric fever is a systemic infection caused by the human adapted pathogens *Salmonella enterica serotype* Typhi(S. Typhi) and *Salmonella enterica* serotype Paratyphi(S.Paratyphi) A, B & C[1]. It continues to be a global health problem with over 21.6 million cases and at least 2,50,000 deaths occurring annually. Almost 80% of the cases and deaths are in Asia, the rest occur mainly in Africa and Latin America. In developing countries such as India, the disease occurring with an incidence ranging from 102 to 2,2190/100,000 of the population[2]. The diagnosis of typhoid fever on clinical grounds is difficult, as the presenting symptoms are diverse and similar to those observed with other febrile illnesses[3].

The Widal test, a serological test which was developed by George Fernand Isidore Widal in 1896, is an alternative to the microbial culture, which is commonly used for the diagnosis of enteric fever since its introduction 100year back[4]. The interpretation of the Widal test depends upon the baseline titre which is prevalent amongst 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.

Regular updating of the baseline titer is a must for the proper interpretation and utilization of the Widal test in diagnosis of enteric fever[5,6].

The present study has been undertaken to evaluate the baseline antibody titre for O, H antigens of *Salmenella enterica subspecies enterica serotype Typhi* and H antigens of *Paratyphi A* and *Paratyphi B* among apparently healthy individuals in Tumkuru, Karnataka, India.

Materials and Methods

This prospective cross-sectional study was conducted in the department of microbiology at Shridevi Institute of Medical Sciences and Research Hospital, which is a tertiary care hospital located in Tumkuru, Karnataka, India. After obtaining their informed consent verbally, non-repetitive blood samples were collected from healthy blood donors(n=493) who attended blood bank of this institution from Jan 2016 to March 2016. All the blood donors were male from the age group of 18-50years.

Health screening of the blood donors was done using survey questionnaire. Individual with any active, cardiac, lung or kidney diseases were excluded. Blood samples were collected from apparently healthy donors who had neither been vaccinated with TAB vaccine with in a last year nor had suffered from any type of fever in last 6 months. These samples were screened for MP, Hbsag, *Brucella, Leptospira a*nd antibodies to HIV and HCV and *Treponema palladium.* The samples which found to be positive were excluded from the study.

Serum samples were subjected to widal tube agglutination test using commercially available antigens containing Typhi O, H and Paratyphi AH, BH antigen (Span Diagnostics Private Limited, India). In brief 0.4ml of two fold serially diluted patients

sera(dilution from 1:20 to 1:1280) in 0.9% normal saline were tested by adding an equal volume of antigen and tubes were then incubated overnight at 37^0C in a water bath. A negative saline control was included in each batch. When the colored, smooth attenuated antigen suspensions were mixed and incubated with individual's serum, anti- salmonella antibodies present in the serum react with the corresponding antigens to give agglutination. The 'O' antigen being a somatic antigen brings about a coarse, compact, granular agglutination whereas 'H' antigen being a flagellar antigen brings about larger, loose, fluffy agglutination[7,8]. The last tube showing visible agglutination with naked eye was taken as endpoint of the test. The titre was reported out as the reciprocal of the end point. All lab procedures were performed by trained lab technicians under the supervision of microbiologists. Ethical clearance was obtained by Institution's ethical clearance committee.

Results

A total of 493 healthy volunteers were screened for the agglutinins against the *Salmonella enterica subspiecies enterica serotype Typhi, Paratyphi A and Paratyphi B* by standard widal tube agglutination test. Out of 199 (40.13%) serum samples were positive for one or more type of agglutinins ≥1:20 and 294(59.63%)serum samples were negative for agglutinins(<1:20)(Table 1). The distribution of the samples with an antibody titre of ≥1:20 against different serotypes of Salmonella enterica subspecies enterica showed an antibody to the anti 'O' antigen in 340(68.96%) samples, an antibody to the anti 'H' antigen in 306(62.06%), an antibody to the anti AH antigen in 85(17.24%) samples and an antibody to the anti BH antigen in 119(24.13%) samples(Table 2).

Among the 340(62.06%) samples which showed the anti 'O' titre of ≥1:20 to the *Salmonella enterica subspecies enterica serotype Typhi*, 112(22.71%) samples had a titre of 1:20, 146(29.61%) samples had a titre of 1:40 and 68(13.79%) samples had a titre of 1:80.The highest titre of 1:160 was found in 14(2.83%)(Table 3). Similarly, among the 306 samples showing anti 'H' titres of ≥1:20 to *Salmonella enterica subspecies enterica serotype Typhi*, 154(31.23%) samples were positive at titre of 1:40,44(8.92%) had a titre of 1:80 and 23(4.66%) samples had a titre of 1:160(Table 4).

Altogether 85(17.24%) samples showed agglutination titre of ≥1:20 against anti 'H' antigen of *Salmonella enterica subspecies enterica serotype Paratyphi A*. Among which 26(8.27%) had a titre of 1:40 and 8(1.82%) had a titre of 1:80 and the rest of the 51(10.34%) samples had a titre of 1:20(Table 5). Among the 119(40.13%) samples with anti BH titre against *Salmonella ser.Paratyphi B* a titre of 1:40 was seen in 32(6.49%) samples(Table 6).

Table 1: Results of Widal test

Widal Status	Frequency	Percentage
Total Sample	493	100
Positive for (≥1:20) Agglutinin	199	40.13%
Negative for(1:20) Agglutinin	294	59.63%

Table 2: Distribution of the samples with antibody titer ≥1:20 against different serotypes of Salmonella enterica

Serotype	Antibody type	Frequency	Percentage
Typhi	Anti 'O' antigen	340	68.96
Typhi	Anti 'H' antigen	306	62.06
Paratyphi A	Anti 'H' antigen	85	17.24
Paratyphi B	Anti 'H' antigen	119	24.13

Table 3: Distribution of 340 samples with anti 'O' titre ≥1:20 against Salmonella serotype Typhi

Agglutinating titre	Frequency	Percentage
1:20	112	22.71
1:40	146	29.61
1:80	68	13.79
1:160	14	2.83

Table 4: Distribution of 306 samples with anti 'H' titre ≥1:20 against *Salmonella serotype Typhi*

Agglutinating titre	Frequency	Percentage
1:20	85	17.24
1:40	154	31.23
1:80	44	8.93
1:160	23	4.66

Table 5: Distribution of 85 samples with anti AH titre ≥1:20 against *Salmonella serotype Paratyphi A*

Agglutinating titre	Frequency	Percentage
1:20	51	10.34
1:40	26	8.27
1:80	08	1.62

Table 6: Distribution of 119 samples with anti BH titre ≥1:20 against *Salmonella serotypes Paratyphi B*

Agglutinating titre	Frequency	Percentage
1:20	87	17.24
1:40	32	6.49

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

Author	Place	Year	TO	TH	AH	BH
A. J. Sneha[14]	Puducherry	2011	1:80	1:80	1:40	1:40
Shekar Pal et al	Garhwal region(Uttarkhand)	2012	1:40	1:80	1:20	1:20
Naveen Saxena et al[15]	Hadoti region(Raj)	2012	1:40	1:40	1:20	1:20
Seema Mittal et al	Rohtak(Haryana)	2014	1:40	1:80	1:20	0
Hemangi Walke et al	Kolhapur(Maharastra)	2014	1:80	1;80	1;40	0
Present study	Tumkuru(Karnataka)	2016	1:80	1:80	1:40	1:40

Discussion

Enteric fever afflicts the local community and the travelers to the endemic areas, the 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 defaecation, urination, substandard food, personal hygiene habits and health ignorance[9,10].

Bacteriologically diagnosis of typhoid consists of demonstration of bacilli and antibodies from patient's serum[11]. The isolation of Salmonella species is a gold standard for the diagnosis but in developing countries, since patient often receive antibiotics prior to medical diagnosis, bacteria can be isolated from the blood cultures in only 40-60% of the cases. However the culture facilities are skill requiring, expensive, tedious and may not be available easily. This makes the Widal agglutination test as the most common alternative laboratory procedure for the diagnosis of enteric fever[11,12,13,14].

The serological diagnosis relies classically on the demonstration of the rising titre of the antibodies in paired sample at an interval of 10-14 days[15]. There are several difficulties associated with evaluation of the Widal test. These include high endemicity, non-availability of paired sera for the demonstration of rising titres, poorly standardized antigens, the sharing of effects of treatment with antibiotics and previous immunisation with TAB vaccine[16,12,17]. False-positive Widal results have been reported for patients with non-enteric fever salmonellae infections, malaria, typhus, Cryptococcus neoformans meningitis, chronic liver disease, collagenous and immunological diseases. There are more than 40 cross reacting antigens between S.typhi and other Enterobacteriaceace[13,18].

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[12]. In the present study, the baseline titre for the 'O' and 'H' antibodies of *Salmonella ser. Typhi* was found to be 1:80. Similarly the baseline titre for the H antigen of *Salmonella* ser.*Paratyphi A* and *Salmonella ser.Paratyphi B* was found to be 1:40. The results which were obtained in the present study were in accordance with the results of previous studies which were done by Sneha AJ in Puducherry(Table 7). Studies support that reevaluation of the Widal baseline titre for healthy individuals should be done at regular intervals.

Conclusion

Based on the results of our study, it has been recommended that the significant titre of the 'O' and the 'H' agglutinins of *Salmonella enterica subspecies enterica serotype Typhi* was ≥1:160.While the significant titre of the 'H' agglutinin of *Salmonella enterica subspecies enterica serotype Paratyphi A* and *Paratyphi B* was ≥1:80 for the diagnosis of enteric fever in Tumkuru, Karnataka.

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Conflict of interest: Nil

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