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Microbiology Section

An Epidemiological Study to Estimate the Baseline Titres of Widal Test in Apparently Healthy Individuals of Bhubaneswar, Odisha

SUBHAJIT GIRI[1], SURYA NARAYAN MISHRA[2], BASANTA KUMAR BEHERA[3], DIPTI PATTNAIK[4]

ABSTRACT

Introduction: Enteric fever is one of the most prevalent community acquired infection especially in the developing countries; caused by Salmonella enterica serovar Typhi, Paratyphi A and Paratyphi B. The Widal test is a well-known test to detect the serological evidences of presence of Salmonella groups. As the baseline Widal titre varies substantially region wise; updating the baseline Widal titre among the healthy individuals is a must for the proper interpretation of the Widal test.

Aim: To determine the baseline Widal titre (titre of the antibodies to the O and H antigens of Salmonella enterica serovar Typhi and the H-antigens of S. Paratyphi A and Paratyphi B) amongst apparently healthy individuals of Bhubaneswar, Odisha.

Materials and Methods: To determine the baseline Widal titre among apparently healthy individuals of Bhubaneswar; a prospective study covering 500 blood samples was conducted using tube Widal test. Healthy young individuals who were neither vaccinated nor have a recent hospitalization history were included in the study.

Results: Out of the total 500 serum samples, 340 were positive for agglutinins (1 ≥ in 20) and 160 serum samples were negative for agglutinin (1 ≤ in 20). The distribution of the samples with an antibody titre ≥ 1:20 against different serotypes of Salmonella enterica subspecies enterica showed an antibody to anti O antigen in 340 samples (68%), an antibody to anti H antigen in 292 samples (58.4%), an antibody to anti AH antigen in 268 (53.6%), an antibody to anti BH antigen in 224 samples (44.8%) out of the total 500 samples. The baseline titre for Widal test in apparently healthy individuals of Bhubaneswar was calculated to be: 1:40 for the anti TO antibodies and of 1:20 each for the anti- TH, anti AH and anti BH antibodies.

Conclusion: It is clear that Salmonella agglutinins are common among apparently healthy people and as endemicity of typhoid in an area may change over time, information of baseline titre will help a better clinical diagnosis and treatment to the deserving patients.

Keywords: Agglutination, Salmonellosis, Typhoid

INTRODUCTION

Enteric fever is prevalent world over and continues to be a major public health problem in developing countries. Salmonella enterica serovar Typhi, Paratyphi A and Paratyphi B is the aetiological agent of enteric fever or typhoid fever. In India, though Salmonella enterica serotype typhi remains the predominant Salmonella species causing enteric fever [1]. The gold standard for diagnosis of enteric fever 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 [2]. The serological test, Widal test, is a well known test, used as an indirect test to detect the serological evidences of presence of Salmonella groups. The Widal test discovered by Georges-Fernand-Isidor 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. 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 [3].

A number of social and economic factors like poverty, lack of access to healthcare, antibiotic resistance, human migration pattern and changing environmental and developmental activities all contribute to the expanding impact of infectious diseases in a developing country like India [4,5]. The major part of the population of Odisha which is one of the most poor and backward states of India, stays in the costal districts which are also vulnerable to natural calamities like flood, draught and cyclones. Bhubaneswar being the capital city, people from all around the state as well as the neighbouring states come and settles in the city for a better living. This increases the slum areas as well as the resultant pollution making the people vulnerable to water and food borne infections and salmonellosis is one of them [6]. Simultaneously the change in food habits also has a direct impact on the growing population. Thus, the Baseline titer calculation for widal test among apparently healthy individuals in a locality will give good information to categorize and treat the infected individuals in that particular locality and also this may be considered as the first test to screen out the patient and thus preventing unnecessary exposure to antibiotics. This baseline titer should also be monitored on regular intervals.

MATERIALS AND METHODS

A prospective study was conducted at the Department of Microbiology, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha to determine the baseline Widal titre (titre of the antibodies to the O and H antigens of S. typhi and the H antigens of S. paratyphi A and B) amongst apparently healthy individuals of Bhubaneswar, Odisha. Being a study under the STS grant of ICMR; the study duration was for two months i.e., from June 2016 to August 2016. Necessary clearance was taken from both the Institutional research and ethical committee.

Inclusion criteria: Healthy young adults of age group 18-45 years who have no past history suggestive of vaccination against

salmonellosis and are not suffering from any co-morbid conditions like Diabetes mellitus, hypertension etc.

Exclusion criteria: The persons with a history of hospitalization in the recent six months or who are currently admitted in the hospital for any disease.

The health screening was done using a semi structured survey questionnaire which was previously validated [7,8]; as per Drugs and Cosmetics act. The form is a defined full-Length Blood Donor History Questionnaire, Version 2.0 May 2016 as per AABB (formerly known as the American Association of Blood Banks) guidelines [7,8]. The sample size was estimated by considering the prevalence of Widal agglutination titre as 42% from the study done by Pal S et al., [9]. The sample size thus estimated was 389.76. By considering 20% non-respondents the final size was estimated to be 467.7; which was then rounded to 500. After obtaining the informed consent, non- repetitive blood samples were collected in plain vacutainer from healthy blood donors (n=500) of the age group of 18-45 years, of both the sexes from various hospitals, slums as well as colonies of Bhubaneswar. The samples thus collected were sent without delay in appropriate sample transport containers to Department of Microbiology, KIMS, Bhubaneswar, Odisha. Commercially available antigens (VITAL WIDAL) which contain the Salmonella enterica serovar Typhi O and H antigens, the Salmonella enterica serovar Paratyphi AH antigen and the BH antigen was used. The tube agglutination test was carried out using 0.5 mL of two fold serially diluted donor's sera (dilutions from 1:20 to 1:320) in 0.9% normal saline and was tested by adding an equal amount of antigen. A negative control (without any sera) was included in each batch of the tests. The results were interpreted after overnight incubation of the samples at 37°C water bath [9]. The baseline titre for the O, H, AH and the BH agglutinins was the highest titre showing agglutination by any of the study samples [2,9]. The final data were calculated after compiling all the titre values which is considered as the Baseline titre for Widal test in Bhubaneswar.

RESULTS

A total of 500 serum samples from apparently healthy individuals were screened by widal tube agglutination test. Out of these 500 serum samples, 340 were positive for agglutinins (1 ≥ in 20) and 160 serum samples were negative for agglutinin (1 ≤ in 20) [Table/Fig-1].

Widal status	Frequency	Percentage
Positive for agglutinins (≥1:20)	340	68%
Negative for agglutinins (≤1:20)	160	32%
Total participants	500	100%

[Table/Fig-1]: Results of widal test.

The distribution of the samples with an antibody titre ≥ 1:20 against different serotypes of Salmonella enterica subspecies enterica showed an antibody to anti O antigen in 340 samples (68%), an antibody to anti H antigen in 292 samples (58.4%), an antibody to anti AH antigen in 268 (53.6%), an antibody to anti BH antigen in 224 samples (44.8%) [Table/Fig-2].

The distribution of the 340 samples (68%) with an anti O titre of ≥ 1:20 against Salmonella enterica subspecies enterica serotype typhi was as follows: an agglutination titre for TO – 1 in 20 of 74 samples (21.76%), for TO – 1 in 40 of 164 samples (48.24%), for TO – 1 in 80 of 92 samples (27.06%), for TO – 1 in 160 of 8 samples (2.36%) and for TO – 1 in 320 of 2 samples (0.58%) [Table/Fig-2].

The distributions of the 292 samples (58.4%) with an anti H titre of of ≥ 1:20 against Salmonella enterica subspecies enterica serotype typhi was as follows: an agglutination titre for TH – 1 in 20 of 122 samples (41.78%), for TH – 1 in 40 of 114 samples (39.04%), for TH –1 in 80 of 40 samples (13.70%), for TH – 1 in 160 of 14 samples (4.79%) and for TH – 1 in 320 of 2 samples (0.69%) [Table/Fig-2].

Serotype	Ag-gluti-nins	End Titres: Frequency (Percentage)					
		< 1:20	1:20	1:40	1:80	1:160	1:320
S. Typhi	Anti TO	160	74 (21.76%)	164 (48.24%)	92 (27.66%)	08 (2.36%)	02 (0.58%)
S. Typhi	Anti TH	208	122 (41.78%)	114 (39.04%)	40 (13.7%)	14 (4.79%)	02 (0.69%)
S. Paratyphi A	Anti AH	232	130 (48.51%)	98 (36.57%)	36 (13.43%)	04 (1.49%)	0
S. Paratyphi B	Anti BH	276	122 (54.47%)	86 (38.39%)	16 (7.14%)	0	0

[Table/Fig-2]: Number and percentage of sera with corresponding end titres for agglutinatins against S. Typhi, S. Paratyphi A, S. Paratyphi B.

The distributions of samples with anti AH titre of ≥ 1:20 was seen in 268 cases (53.6%). Out of which TAH ≥ 1:20 in 130 cases (48.51%), TAH ≥ 1:40 in 98 cases (36.57%), TAH ≥ 1:80 in 36 cases (13.43%) &TAH ≥ 1:160 in 4 cases (1.49%) [Table/Fig-2].

The distributions of samples with anti BH titre of ≥ 1:20 was seen in 224 cases (44.8%). Out of which TBH ≥ 1:20 in 122 cases (54.47%), TBH ≥ 1:40 in 98 cases (38.39%) and TBH ≥ 1:80 in 36 cases (7.14%) [Table/Fig-2].

Calculation of baseline titre: From the above findings, we can reach in the calculation of baseline titre for widal test in apparently healthy individuals of Bhubaneswar will be as follows:

For S. typhi: anti TO – 1:40

For S. typhi: anti TH – 1:20

For S. paratyphi A: anti AH – 1:20

For S. paratyphi B: anti BH – 1:20

DISCUSSION

More than 100 years after the introduction of the Widal test for diagnosis of typhoid fever, the controversy that surrounded the test has not been abated [8,9]. As bacterial culture is time consuming and not readily accessible especially in developing countries like ours, Widal test can be used as an alternative laboratory procedure for the diagnosis of enteric fever, provided a baseline antibody titre of healthy individual in the population is known.

In the current study, 340 (68%) out of total 500 serum samples were found to be positive for agglutinins for the Salmonella serotypes. Our findings were quite higher than those which were reported by other investigators except that by Gunjal SP et al., [8]. The lowest positivity was reported by Pal S et al., which was 42.60% [9]. The study done by Pang T et al., clearly showed that in an endemic area such as Malaysia, S typhi agglutinins against both H and 0 antigens may be present in the normal population at titres of up to 1/160 [10]. The positivity in that study was approximately 61%. According to the study done by Acharya T et al., and Peshattiwar P et al., the percentage of positivity was quite low i.e., 50.6% and 55.12% respectively, in comparison to our findings [11,12]. A comparative analysis of samples which were positive for agglutinins for Salmonella serotypes has been presented in [Table/Fig-3].

Author	Year	Percentage of positive samples
Pang T et al [10]	1983	61%
Gunjal SP et al. [8]	2012	81.55%
Peshattiwar P et al. [12]	2012	55.12%
Pal S et al. [9]	2013	42.60%
Acharya T et al. [11]	2013	50.6%
Present study	2016	72.8%

[Table/Fig-3]: Comparative analysis of samples positive for agglutinins for Salmonella serotypes.

Rising titre observed in paired serum samples collected 10 to 14 days apart is classically considered a standard serological evidence of typhoid fever. In typhoid fever, however, such a rise is not always demonstrable, even in the blood culture confirmed cases. This situation may occur because of the acute phase sample which is obtained late in the natural history of the disease, because of the high levels of the background antibody in a region of endemicity or because in some individuals, the antibody response is blunted by the early administration of an antibiotic [13]. Such type of demonstration of rising titre in paired serum samples is not patient friendly as this will compel the patient to wait for a prolonged period to receive the standard treatment. For practical purposes, the treatment decision must be made on the basis of the results which are obtained with a single acute phase sample. The cut off titre in a particular population depends on the background level of the typhoid antibodies and the level of the typhoid vaccination, which may vary with time [3,13].

In our study, sera of 164 (48.24%) out of 340 positive cases (for TO) showed titres of TO ≥ 1:40 and sera of 122 (41.78%) out of 292 positive cases (for TH) showed titres of TH ≥ 1:20. Therefore, as is evident from the above findings, the baseline titre for "O" antibody of Salmonella typhi was found to be 1:40 and that of "H" antibody of Salmonella typhi was found to be 1:20. Similar study done by Sharma N et al concludes that the current baseline antibody titre among the healthy individuals in and around Jabalpur region for S. typhi-O and S. typhi-H is 1:40(62.94%) and 1:40 (62.64%) respectively [13]. Other similar studies done by Pal S et al. and Madhusudhan NS [9,14] also reported a baseline titre of 1:40 for TO, but of 1:80 for TH. In another study done in Nepal by Pokhrel BM et al., which concluded that significant titres are those higher than1:80 for anti-O and higher than 1:160 for anti-H antigen of Salmonella enterica serotype Typhi [15].

The cut off level for both AH and BH in our study was ≥1: 20. Our values were similar to the study done by Patil AM which was conducted in a similar environment but covered the healthy children as study population [16]. In that study; 15 samples (92%) had a titre of ≤ 1:20 for the Salmonella enterica serovar Paratyphi B 'H' antigen. The baseline titre for the Salmonella enterica serovar Paratyphi B 'H' antigen was ≥ 1:20.

A comparative analysis of baseline titres of various agglutinins has been presented in [Table/Fig-4]. The variations in the titres could have been caused by differences in endemicity of typhoid in each area. The highest number of samples i.e., 130 out of 268 (for AH) i.e., 48.51% and 122 out of 224 (for BH) i.e., 54.47% respectively, showed titres of ≥ 1:20 for AH and BH. The highest level of titre for TO, TH was found to be 1:320 in our study. Two (0.58%) out of 340 positive (for TO) cases showed a titre of ≥ 1:320. Similarly two (0.69%) out of a total 292 positive (for TH) cases showed a titre of ≥ 1:320. Our findings were in accordance with those of Madhusudhan NS and Patil AM [14,16], who also observed that the highest level of titres for O, H and AH was 1:320. In another significant study carried out in Kerala, India, by Sreenath K et al., a higher titres of 1:160 against O and H antigens of S. typhi were seen [17]. The highest level of titre for AH in our study was 1:160 where as the highest titre for BH was 1:80. Four (1.49%) out of a total 268 positive (for AH) showed a titre of ≥ 1:320 whereas 16 (7.14%) out of a total 224 positive (for BH) showed a titre of ≥ 1:80. High titre of antibodies in our study may be caused by infections with cross reacting organisms other than Salmonella.

		Baseline Titre			
Author	Year	TO	TH	AH	BH
Pal S et al., [9]	2013	1:40	1:80	1:20	1:20
Peshattiwar P et al., [12]	2012	1:40	1:80	1:40	NR
Gunjal SP et al., [8]	2012	1:40	1:40	1:80	1:80
Present study	2016	1:40	1:20	1:20	1:20

[Table/Fig-4]: Comparative analysis of baseline titre of "O" & "H" agglutinins.
NR – Not Reported

Antibody titer may be high in healthy individuals in the presence of cross reacting antigens, such as malaria, brucellosis, dengue fever, healthy carrier state, chronic liver disease, endocarditis or other Enterobacteriaceae infections. There are more than 40 cross-reacting antigens between S. typhi and other members of family Enterobacteriaceae [13,14,17]. Persons who had past enteric infection or vaccinated with the old typhoid vaccine (TAB) may develop transient anamnestic reaction during an unrelated febrile illnesses, such as malaria [18,19]. In our study; persons with past history of vaccination were excluded (part of exclusion criteria).

Hence, the baseline titre of a particular area should be known. The probable reason for the low titre in our study could be the better health and hygiene conditions of the study group chosen.

More studies should be carried out to determine Salmonella agglutinin titre in apparently healthy populations, so that a better judgment which is based on the prevailing agglutinin titres can be made. Further scope of this study is to include a wider population with multi-centre involvement of various medical colleges of Odisha state to reach to a consensus regarding baseline Widal titres.

LIMITATION

The current study covers only a very selective and small group of healthy individuals. A broad and extensive study consisting of groups covering the entire city will give a more definitive conclusion. The duration of the study being limited to two months (part of the ICMR STS Project) can also be considered as one important limitation.

CONCLUSION

The final recommendation for the baseline titre for Widal test among apparently healthy adults in Bhubaneswar, Odisha can be: 1:40 for anti TO antibodies and 1:20 each for the anti TH, anti AH and anti BH antibodies. As the endemicity of typhoid in an area may change over time, more frequent and elaborate studies should be made to keep the recent baseline titer up-to-date.

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PARTICULARS OF CONTRIBUTORS:
1. Student, Department of Microbiology, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, India.
2. Assistant Professor, Department of Microbiology, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, India.
3. Professor, Department of Community Medicine, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, India.
4. Professor and HOD, Department of Microbiology, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Surya Narayan Mishra,
Assistant Professor, Department of Microbiology, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, India.
E-mail : surya.mishra@kims.ac.in

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