

Estimation of Baseline Widal Antibody Titers among Apparently Healthy Urban Population of District Jammu

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Abstract

Introduction: Definitive diagnosis of Enteric fever is by blood culture for *Salmonella enterica* serotype Typhi, Paratyphi A, and Paratyphi B which takes long turnaround time and is costly, whereas Widal test is simple, rapid, and cost-effective test whose interpretation depends on the baseline Widal titers among healthy individuals in a defined population. **Objectives:** To determine the baseline Widal titers among apparently healthy urban population of district Jammu (J&K). **Materials and Methods:** 302 individuals in the age group of 18–50 years were recruited. A pretested questionnaire was used to collect demographic and clinical details. The Widal testing was done using commercial *Salmonella* antigen kit. **Results:** A total of 302 samples were screened by Widal test. 138 samples (45.69%) were reactive for TO antigen and 64 (21.19%) tested reactive for TH antigen, 3 (0.01%) samples showed agglutination for AH antigen and 3 (0.01%) were positive for BH antigen. Majority of seropositive samples were in dilutions of 1:40 for both TO and TH antigens. **Conclusions:** Hence, next higher dilutions showing positivity for both TO and TH antigens, i.e., $\geq 1:80$ may be considered diagnostic for enteric fever in the urban population of Jammu district.

Keywords: Enteric fever, typhoid, Widal test

INTRODUCTION

Enteric fever is a serious global public health problem in developing countries including India with global estimate of >21.6 million cases annually.^[1] The causative organism is *Salmonella typhi* transmitted to human beings through feco-oral route resulting in considerable morbidity. It afflicts the local community and travelers to the endemic areas, the incidence rising during the rainy season due to water logging and contamination of water with fecal material. Social factors favoring it, are the pollution of drinking water supplies due to open defecation, urination, personal hygiene habits, and health ignorance.^[2]

Clinical diagnosis of typhoid may be difficult because of altered or atypical presentation of patients. In developing countries, often patients visit hospital late in course of illness or they take drugs as self or unauthorized prescription before reporting to doctors. The gold standard and definitive diagnosis is by isolation of *Salmonella enterica* serotype Typhi, ParatyphiA, and Paratyphi B from blood, bone marrow, stool, or urine which is about 90% in the 1st week of illness and decreases

to about 50% by the 3rd week.^[3] Blood culture has demerits such as unavailability, cost, and relatively long turnaround time (TAT), hence not much utilized test in developing countries. Hence, an alternative is Widal test which is simpler, rapid, and cost-effective.

Widal test is based on a serologic reaction involving clumping of a suspension of killed *Salmonella* cells as antigen by a specific antibody expected to be present in serum of a patient infected with organism. It detects agglutinins against O and H antigens of *S. typhi* and H antigens of *Salmonella paratyphi* A and B. Interpretation depends on the baseline titer of these agglutinins to O and H antigens of *S. typhi* and H antigen of *S. paratyphi* prevalent among healthy individuals in particular geographic area.^[4] Baseline titer of agglutinins varies from place to place (e.g., 1 part in 40–1 part in 160 for TH antigen

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and 1 part in 40-1 part in 80 for TO antigen) depending on the endemicity of enteric fever in that region as it varies from region to region. Updating the baseline Widal titer in a population is mandatory for proper interpretation of the test.^[2] Since no such data were available for urban population of Jammu, a study was conducted to estimate the baseline Widal titers among apparently healthy individuals of urban population of Jammu district.

MATERIALS AND METHODS

A cross-sectional study was designed to estimate the baseline Widal titer against *S. typhi* and *S. paratyphi* among apparently healthy urban population of district Jammu. For deriving sample size, we presumed seroprevalence of *S. typhi* infection in the general population to be 25%.^[5] Hence, the sample size derived was 300 participants using the formula $4pq/L^2$ (Where P stands for Prevalence of *S. typhi* infection, q stands for 100-p and L stands for allowable error which was kept as 20% of prevalence). The population chosen for the study was in the age group of 18–50 years of both sexes residing in Jammu district for last at least 5 years. The study participants were picked up from the educational institutions (both government and private) ranging from schools to colleges and Universities following the principle of consequent sampling, i.e., every participant qualifying the inclusion criteria were included in the study one by one. Clearance from the Institutional Ethics Committee was obtained. A list of educational institutions located in Jammu district was prepared. While preparing the list different departments of University of Jammu were taken as individual institutions. By simple random sampling technique involving draw of lots, the names of the educational institutions were picked up. Then, the head of institution was contacted by the investigator to explain purpose and procedure of the study who if agreed to allow the study in the institution, in turn invited the staff and students in the age group of 18–50 years and the investigator again explained the purpose and procedure of study to them. Only those volunteers who satisfied the inclusion criteria were included in the study. A pretested questionnaire explained to them in the language well understood by them, was administered after obtaining a written informed consent of the subjects. Under all aseptic precautions, 2–3 ml of whole-blood sample was drawn by venepuncture and transferred into a red top vacutainer. The puncture site was covered with antiseptic dressing. Whole-blood samples were transported in cold chain (vaccine carrier) to the institutional research laboratory for storage in refrigerator at 2°C–8°C after harvesting the serum. Widal testing was done within 6 days of collection by slide agglutination test, using stained Salmonella antigen kit (SPAN Diagnostics). It is a semi-quantitative test, involving antigen-antibody reaction between somatic O and flagellar H antigens of *S. typhi* and flagellar antigens [A(H), B(H)] of *S. paratyphi* with their respective antibodies if present in serum. In case the samples were positive for antibodies, serial dilutions of antibody titers were assessed ranging from 1:20, 1:40, 1:80, 1:160, and 1:320. An internal quality control

was used along with every test run. All the participants with very high antibody titers were advised to report to nearest hospital/clinics for further evaluation by clinicians.

RESULTS

A total of 302 samples were screened for the presence of anti-TO, anti-TH, anti-AH, anti-BH agglutinins by Widal slide agglutination test. Out of them, 159 (52.64%) were male and 143 (47.36%) were female. Age distribution of the subjects ranged between 18 and 50 years with mean age being 32.28 years with standard deviation = 10.045. Age and sex distribution of samples with dilutions showing maximum reactivity is mentioned in Table 1.

Out of all samples reactive for TO and TH antigens, 86 (62.31%) were reactive for TO antigen, 12 for TH antigen and 52 for both TO and TH antigens. Among the 138 samples reactive for TO antigen and 64 samples reactive for H antigen the reactivity in different dilutions is mentioned in Table 2. On studying distribution of reactivity of both TO and TH antigens in dilutions less than and more than 1:40, 66 out of 138 samples reacted for TO and 23 out of 64 reacted for TH antigen. However, the findings were not statistically significant ($\chi^2 = 2.507 P = 0.1132$).

DISCUSSION

Blood culture remains the gold standard for definitive diagnosis of enteric fever but lack of the facility and TAT limits its use in the developing countries. The Widal test which detects agglutinating antibodies to Salmonella enteric subspecies enteric serotype Typhi “O” and “H” antigens and “H” antigens of Salmonella enteric subspecies enteric serotype Paratyphi A and B is widely used in the developing countries due to the ease of performing the test, low cost, and relatively rapid results. Widal test is also useful for the diagnosis of patients already on antibiotics which may inhibit the growth in culture.^[3] In the present study, a total of 302 apparently asymptomatic subjects were included and tested for antibody titers against *S. typhi* infection. Out of them 159 (52.64%) were male and 143 (47.36%) were female. In similar study conducted to assess baseline Widal titer among healthy individuals in and around Indore, Madhya Pradesh, 86.63% individuals were males, whereas 13.37% were female.^[4] In our study, the gap in male female ratio was very less as compared to that study. It could be due to the fact that majority of our subjects were taken from teaching community where proportion of females among teachers is comparable to the male population, whereas in their study, the individuals were drawn from blood donors among whom majority were males only.

Recent study done by Sreenath *et al.* showed the significant titers should be >1:80 for anti-TO and >1:160 for anti-TH for a presumptive diagnosis of typhoid fever.^[6] In the present study, 45.69% ($n = 138$) samples tested positive for TO antigen in dilutions >1:20 and for TH antigen 21.19% ($n = 64$) in dilution of >1:20. In study conducted by Shrikant Kogekar *et al.* at Indore, the positivity for TO antigen was comparable with the present

Table 1: Age group and sex-wise distribution of subjects with reactive samples

Age group	Sex	Total samples	Positive for TO antigen, n (%)	Majority titer seen	Titer, n (%)	Positive for TH antigen, n (%)	Majority titer seen	Titer, n (%)
18-26	Male	66	30 (45.45)	1:40	19 (63.33)	7 (10.60)	1:40	5 (71.42)
	Female	36	25 (69.44)	1:80	9 (36.00)	11 (30.55)	1:40	5 (45.45)
26-34	Male	30	11 (36.66)	1:40	5 (45.45)	6 (20.00)	1:40	4 (66.66)
	Female	35	19 (54.28)	1:40	8 (42.01)	6 (17.14)	1:80	4 (66.66)
34-42	Male	24	10 (41.66)	1:40	7 (70.00)	7 (29.16)	1:40	6 (85.71)
	Female	45	24 (53.33)	1:40	13 (54.16)	13 (28.88)	1:40	7 (53.84)
42-50	Male	39	11 (28.20)	1:40	10 (90.90)	5 (12.82)	1:40	3 (60.00)
	Female	27	7 (29.62)	1:80	5 (71.42)	9 (33.33)	1:40	6 (66.66)
Total	Males	159	62		41	25		18
	Females	143	75		35	39		22

Table 2: Distribution of reactivity among different dilutions of TO and TH antigens

Antigens	≤1:20 (%)	≤1:40 (%)	≤1:80 (%)	≤1:160 (%)	≤1:320 (%)	Total	P (χ^2 , df, P)
TO antigen	00 (00.00)	72 (52.17)	47 (34.05)	12 (8.69)	7 (5.07)	138	109.2, 3, <0.0000001
TH antigen	03 (4.68)	38 (59.37)	15 (23.43)	7 (10.93)	1 (1.56)	64	88.75, 4, <0.0000001

TO: Autigen, TH: Autigen

study (49.72%), while for TH antigen, it was relatively very high to the tune of 52.26% of samples.^[4] In another study conducted by Bijapur *et al.* in North Kerala, 25.2% were found positive for TO antigen and for TH antigen, 15.2% of total samples were positive.^[7] In the present study, majority of seropositive samples were in dilution of 1:40 for both TO and TH antigens. In the study conducted by Sreenath *et al.* at Kollam Kerala, the majority of samples showing seropositivity were positive in dilutions 1:40 for TO as well as TH antigen. Hence, higher dilutions showing positivity for TO antigens i.e., ≥1:80 were considered diagnostic for enteric fever in our study population.

From public health point of view, an easy, economical, and less time-consuming diagnostic test is always handy while dealing with the spurt of cases of pyrexia with atypical presentations, especially during summers and rainy season when other communicable diseases such as malaria and dengue chikungunia fever have high incidence and making a definitive diagnosis is an urgent need. At the same time, the interpretation of this test depends on the prior knowledge of the baseline levels of Widal antibodies among the particular population is main need, which the present study satisfied.

CONCLUSIONS

In the present study, majority of seropositive samples were in dilution of 1:40 for both TO and TH antigens. Hence, next higher dilutions showing positivity for both TO and TH antigens, i.e., ≥1:80 may be considered diagnostic for enteric fever in the urban population of Jammu district.

Limitations

It was an ICMR funded, students research project (STS) to develop the research acumen among undergraduate medical students. The test tube method was intended to be employed but because of the

cost issues and easy in performance, the slide agglutination test was employed after seeking approval from ICMR.

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Conflicts of interest

There are no conflicts of interest.

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