ORIGINAL ARTICLE

Evaluation of the baseline Widal titres in healthy blood donors of Uttarakhand

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Abstract

Introduction: The interpretation of Widal test depends upon the baseline titre which is prevalent amongst healthy individuals in a particular geographical area. **Aim and Objective:** The aim of this study was to determine the baseline Widal titre of the apparently healthy population of a tertiary care centre of Uttarakhand. **Material and Methods:** Blood samples were collected from healthy blood donors (n=500) of the age group of 18-60 years, of both the sexes, who attended our blood bank from September 2013 to November 2013 and were analyzed for the presence of Salmonella antibodies by carrying out the Widal tube agglutination test. **Results:** Of the 500 serum samples which were tested, 255 (51%) serum samples were positive for agglutinins (≥ 1:20) and 245 were negative. The most frequently recorded titre of the reactive sera was 1:40 for the anti-O antibodies and 1:80 for the anti-H antibodies for Salmonella enterica serotype typhi and this was the baseline titre. While the baseline titre of the 'H' agglutinins of Salmonella enterica serotype paratyphi A was 1:20 and of paratyphi B was also1:20. **Conclusions:** Based on the results of our study, it has been recommended that the significant titre of 1:80 for the anti-O antibodies and of 1:160 for the anti-H antibodies may be considered as diagnostic for enteric fever in the region of Uttarakhand, India.

Key Words

Baseline Widal titre; Typhoid Fever; Salmonella; Widal

Introduction

Widal test has been used very extensively in the sero-diagnosis of typhoid fever and in developing countries, remains the only practical test available. Enteric fever is endemic in India and it continues to be one of the major health problems here. [1] The Widal agglutination test, developed by F Widal in 1896 has been in use for more than a century as an aid in the diagnosis of typhoid fever. [2] It utilizes a suspension of killed Salmonella typhi as antigen, to detect typhoid fever in serum from suspected S typhi-infected patients who present with febrile illness.

It is a tube dilution test which measures agglutinating antibodies against the lipopolysaccharide O and protein flagellar H antigens of S. typhi, S. paratyphi A and B. 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. Updating the baseline Widal titre is a must for the proper interpretation of the Widal test. [3,4]

Aims & Objectives

To determine the baseline Widal titre (titre of the antibodies to the O and H antigens of S. typhi and to the H antigens of S. paratyphi A and B) amongst healthy blood donors of Uttarakhand. It was also aimed to define the significant titre for the Widal

agglutination test for the diagnosis of enteric fever in an endemic area in a single serum test.

Material and Methods

This cross sectional study was conducted in the Department of Microbiology and Blood bank (Pathology), Himalayan Institute of Medical Sciences, Uttarakhand India. After obtaining the informed consent, blood samples were collected in plain vacutainer from healthy blood donors (n=500) of the age group of 18-60 years, of both the sexes, who attended our blood bank from September 2013 to November 2013. The health screening was done using a semi structured survey questionnaire (blood donor questionnaire as per Drugs and Cosmetics act). Those who were vaccinated for enteric fever in the preceding three years and those with a recent history of fever of unknown origin were excluded from the study.

Commercially available antigens which contained the Salmonella enterica serovar typhi O and H antigens, the Salmonella enterica serovar paratyphi AH antigen and the paratyphi BH antigen were used (Span diagnostics Ltd). 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 was included in each batch of the tests. The results were interpreted after overnight incubation of the samples at 37^{o}C in water bath. The results were analyzed. The baseline titre for the O, H, AH and the BH agglutinins was the highest titre showing agglutination by any of the study samples.

Results

A total of 500 healthy blood donors were screened for the agglutinins against the Salmonella enterica subspecies enterica serotypes, Typhi, Paratyphi A and Paratyphi B by the Widal tube agglutination test. Out of 500, 468 (93.6%) were males and 32 (6.4%) were females. Maximum patients were in age group of 18-27 years (53.4%).

255 (51%) samples were positive for the agglutinins (≥1in 20) whereas 245 (49%) samples did not show agglutinins (< 1in 20) [Table 1].

The distribution of the samples with an antibody titre of ≥ 1: 20 against different serotypes of Salmonella enterica subsp. enterica. Antibodies to the anti-O antigen was seen in 147 samples (29.4%), antibodies to the anti-H antigen in 255 samples (51%), to the anti-AH antigen in 21 samples (4.2%) and to the anti-BH antigen in 12 samples (2.4%) [Table 2].

The distribution of 147 samples with the anti-O titre of ≥1:20 to the Salmonella enterica serotype typhi showed that 34 samples (6.8%) had a titre of 1:20, 96 samples (19.2%) had a titre of 1:40 and 14 samples (2.8%) had a titre of 1:80, while only 3 samples (0.6%) had the highest titre of 1:160. Similarly, among the 255 samples which showed the anti-H titre of ≥1:20 to the Salmonella enterica serotype typhi, 54 samples (10.8%) had a titre of 1:20, 50 samples (10%) had a titre of 1:40, 131 samples (26.2%) had a titre of 1:80 and 19 samples (3.8%) had a titre of 1:160 .The highest titre of 1:320 was found in 1 sample (0.2%) [Table 3].

21 samples (4.2%) showed an agglutination titre of ≥ 1: 20 against the H antigen of the Salmonella enterica serotype paratyphi A, among which 14 samples (2.8%) had a titre of 1:20 and 7 samples (1.4%) had a titre of 1:40. Only 12 samples (2.4%) had an anti-H titre of ≥1:20 for the Salmonella enterica serotype paratyphi B.

Discussion

The specific purpose of this study was to develop a local recommendation for the interpretation of the Widal test results. Although the Widal test is widely used in our region for the diagnosis of typhoid fever, no previous attempts were made to know the baseline Widal titre amongst the local population, as per our knowledge. Among the 500 blood samples of healthy blood donors who were tested, 255 (51%) were positive for agglutinins for the Salmonella serotypes. The agglutinins to S. typhi were the most prevalent among the sera which were tested at various dilutions. The most frequently recorded titre for O agglutinin (19.2%) was found to be 1:40 and this was considered as the cut off titre. For the H agglutinins, we observed that a majority of the study population (26.2%) had a titre of 1:80 and this was taken as the cut off titre. This study concludes that the current baseline titre for the diagnosis of typhoid fever in this region is 1:40 for the anti-O agglutinins (TO) and that it is 1:80 for the anti-H agglutinins (TH). The baseline anti-H agglutinin titre of the paratyphoid A(AH) and B(BH) groups was found to be 1:20, which suggested that the paratyphoid groups were less prevalent in this area as compared to S. typhi.

It has been evident from the various studies which have been conducted across our country [Table 4] that the baseline titre is subject to variations,

depending on the geographical area and the sanitary conditions of the region. Our results were in concordance with the study which was reported by Garhwal region of Uttarakhand, India. [9] However another study from Dehradun city showed slightly variable results. Hence, the baseline titre of a particular area should be known.

Conclusion

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. 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.

It is concluded, that even today, the Widal test remains one of the best, easily accessible, cheap and simple method for the diagnosis of typhoid fever. Based on the above results of our study, it is recommended that the cut-off titre of 1:80 for the anti-O antibodies and of 1:160 for the anti-H antibodies may be considered as diagnostic for enteric fever in this region of Uttarakhand, India. Further scope of this study is to include a wider population with multi-centre involvement of various medical colleges of Uttarakhand to reach to a consensus regarding baseline Widal titres.

Public Health Importance: Proper interpretation of a single Widal test depends on the pre-existing levels of antibodies detectable in the normal individuals of different areas since it varies considerably by time and place depending on the endemicity of the disease.

Besides sensitivity and specificity, positive predictive value (PPV) is the most important measure of a clinical diagnostic method since it represents the proportion of patients with positive test results that are correctly diagnosed and PPV is not intrinsic to a test; it is affected by prevalence of the disease. Hence establishing base line titer of antibodies is of value in correctly diagnosing the disease early and thus help in reducing morbidity and mortality.

Furthermore studying trends in the antibody titers over a period of time indicates the changing trends of the endemicity/prevalence of the disease in the community which indirectly reflects the changing conditions of public sanitation & personal hygiene along with impact of public health management systems in the community.

Authors Contribution

GM, PG & RK: Conception and design, Analysis and interpretation of data, Drafting the article or revising it critically for intellectual content, final approval of the version. MT, GN & SG: Drafting the article or revising it critically for intellectual content, final approval of the version. SG also helped in Analysis and interpretation of data.

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TABLES

TABLE 1 RESULTS OF WIDAL TEST

Widal status	Frequency	Percentage
Total participants	500	100
Positive for agglutinins (≥ 1:20)	255	51
Negative for agglutinins(< 1:20)	245	49

TABLE 2 DISTRIBUTION OF SAMPLES WITH ANTIBODY TITRE ≥ 1:20 AGAINST DIFFERENT SEROTYPES OF SALMONELLA ENTERIC SUBSPECIES ENTERIC. (TOTAL NO. OF SAMPLES N=500)

Serotype	Antibody type	Frequency	Percentage
S. typhi	Anti O antigen	147	29.4
S. typhi	Anti H antigen	255	51
S. paratyphi A	Anti H antigen	21	4.2
S. paratyphi B	Anti H antigen	12	2.4

TABLE 3 NUMBER AND PERCENTAGE OF SERA WITH END TITRES IN HEALTHY VOLUNTEERS.

Antigen	No. of positive samples (%)	Dilution (1:20) No. (%)	Dilution (1:40) No. (%)	Dilution (1:80) No. (%)	Dilution (1:160) No. (%)	Dilution (1:320) No. (%)
S. typhi O	147(29.4)	34(6.8)	96(19.2)	14(2.8)	3(0.6)	0
S. typhi H	255(51)	54(10.8)	50(10)	131(26.2)	19(3.8)	1(0.2)
S. paratyphi AH	21(4.2)	14(2.8)	7(1.4)	0	0	0
S. paratyphi BH	12(2.4)	10(2.0)	2(0.4)	0	0	0

TABLE 4 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 S et al [5]	Central India	1997	1:80	1:80	0	0
Punia JM et al [1]	Chandigarh	2003	1:80	1:160	1:20	1:20
Patil Anand M et al [6]	Karnataka	2007	1:80	1:80	1:40	1:40
A J Sneha [7]	Pondicherry	2011	1:80	1:80	1:40	1:40
Peshattiwar P [8]	Andhra Pradesh	2011	1:80	1:80	1:40	1:20
Pal S et al [9]	Srinagar Garhwal	2011	1:40	1:80	1:20	1:20
Kataria VK et al [10]	Dehradun city	2012	1:80	1:80	1:40	1:40
Present study	Uttarakhand	2013	1:40	1:80	1:20	1:20