



Original Research Article

Detection of baseline Widal titres among the blood donors: A population based study

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A B S T R A C T

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Enteric fever is one of the most infectious disease on a global scale and endemic in all parts of India. Widal test is widely used for its diagnosis. The testing method has some limitations, due to low titre agglutinins present in some individuals that react with *Salmonella* antigens. We studied about the average baseline titre of blood donors in our region Kollam, Kerala. The present study indicates that the cut off titres of 1 in 80 for anti O antibodies and 1 in 160 for anti H antibodies may be considered as the baseline titre for enteric fever in our region. Our study high lightens the importance of need for paired sampling to demonstrate the raising level of agglutinins. Classically, a fourfold rise of antibody in paired sera is considered diagnostic of typhoid fever.

Introduction

Enteric fever has a high profile as a major human bacterial infection (Crump *et al.*, 2004). Hospital based studies and outbreak reports from India indicate that occurrence rates of enteric fever has risen sharply in recent years. The most common etiological agent being *Salmonella enteric serovars typhi (S.typhi)*, apparently increasing number of cases are reported due to *S.paratyphi A* (Pang, *et al.*, 1995). An optimal diagnosis of enteric fever in clinical grounds alone is very difficult because of the nonspecific signs and symptoms of the disease. The gold standard in diagnosis of enteric fever remains when the pathogen can be

recovered from blood during the first week of the disease (Pearson, *et al.*, 2000). In a developing country like India, the bacterial culture facilities are often unavailable or limited to teaching hospitals and accredited laboratories. The Widal test continues to be the most simple and popular test in presumptive diagnosis of Typhoid fever (Jog *et al.*, 2008). Widal test, first introduced by Ferdinand Widal in 1896 is a tube agglutination test which measures agglutinating antibodies against the lipopolysaccharide O and flagellar protein H antigens of *S.typhi*. Even though the method is easy to perform, technically not demanding, concerns remain about the

reliability, sensitivity and specificity of the test (Peshattiwar 2012). The testing method has some limitations, due to low titre agglutinins present in some individuals that react with Salmonella antigens. Cross reactivity with other Salmonella species may occur and the test cannot distinguish between a current and a previous infection or vaccination against enteric fever. Hence a rising titre gives more significance than a single test (Ibweke *et al.*.,2008).

Further the interpretation of Widal test depends up on the baseline titre which is prevalent amongst the healthy individuals in a particular geographical area (Olopoenia *et al.*.,2000). In an endemic country like India, for an infection like enteric fever, sera of a proportion of healthy individuals contain antibodies capable of reacting to a variable titre in Widal test due to previous stimuli or as under the criteria of anamnestic reactions. These titres may differ substantially and it depends upon the endemicity of typhoid in each area which has been changing over time. Updating of the baseline titres at regular intervals is highly essential for proper interpretation of Widal test (Shukla *et al.*.,1997, Pang, *et al.*.,1983, Parry, *et al.*.,1993). Here we have formulated the baseline titres by a population based study among blood donors in Kollam for better correlation, evaluation and reporting.

Materials and Methods

The study was conducted over a period of one year from July 2012 to 2013 in the Department of Microbiology, Travancore medical college hospital in Kollam, Kerala, India.

Study group

The test was done on 550 voluntary blood donors of both sexes. All individuals were

> 18 years of age and those with a recent history of fever were excluded from the study.

Widal test

The test was done by classical tube agglutination method, in brief- Serum was serially diluted in 0.9% normal saline starting at a dilution of 1 in 20. For H and O agglutinins, 0.5 ml of serum was added to glass tubes followed by equal volume of antigenic suspension (total volume of 1 ml); all tubes were incubated overnight at 37°C before reading. Antigenic suspension comprised of stained suspensions of O and H antigens from *S. typhi*, *S. paratyphi A* and *S. paratyphi B* (Span diagnostics Ltd, India). The results were interpreted and evaluated as per standard guidelines. A negative control was included in each batch of the tests. The Widal anti O (TO) and anti H (TH, AH, and BH) titres were taken as the highest dilution of the serum with a visible agglutination.

Results and Discussion

Of the 550 blood donors positive agglutinins (>1 in 20) for *S. typhi*, *paratyphi A* and *paratyphi B* were present in 274 samples (49.88%), whereas 276 samples (50.18%) were negative (did not show agglutinins). Among the samples tested, agglutinins to *S.typhi* were predominant (41.8% for H and 32.25% for O antigen) and agglutinins for *S. paratyphi AH* and *BH* were very low (2.5% for *AH* and 0.9 % for *BH*). The table-1 showed among 193 positive samples with the anti O titre of > 1 in 20, to *S. typhi*, 73 samples (13.3%) had a titre of 1 in 20, 84 samples (15.3%) had a titre of 1 in 40, 29 samples (5.3%) had a titre of 1 in 80 and only 7 samples (1.3%) showed a high titre value of 1 in 160.

Table.1 Numbers and percentage of sera with end titres in Blood Bank donors

Antigen	No. of Positive samples	Dilution (1 in 20)	Dilution (1 in 40)	Dilution (1 in 80)	Dilution (1 in 160)	Dilution (1 in 320)
<i>S.typhi O</i>	193 (35)	73 (13.3)	84 (15.3)	29(5.3)	7(1.3)	Nil
<i>S.typhi H</i>	230 (41.8)	74 (13.5)	98 (17.8)	50(9.1)	8 (1.5)	Nil
<i>S.paratyphi AH</i>	14 (2.5)	12 (2)	2 (0.4)	Nil	Nil	Nil
<i>S.paratyphi BH</i>	5(0.9)	5(0.9)	Nil	Nil	Nil	Nil

Among 230 samples which showed anti H titre of >1 in 20 to *Salmonella typhi*, 74 samples (13.5%) had a titre of 1 in 20, 98 samples (17.8%) had a titre of 1 in 40 and 50 samples (9.1%) had a titre of 1 in 80. Only 8 (1.5%) samples expressed a high titre of 1 in 160.

Only 14 samples (2.5%) showed an agglutination titre of >1 in 20 against the H antigen of *Salmonella paratyphi A*, among them 12 samples (2%) had a titre of 1 in 20 and the rest 2 samples (0.4%) had a titre of 1 in 40. Among the 550 samples tested only 5 samples (0.9%) showed agglutinins at 1 in 20 dilutions for *S. paratyphi B*.

Definitive diagnosis of enteric fever depends on isolation of *Salmonellae* from blood, stool, urine, bone marrow, bile or other body fluids. However, it is a relatively specific method and is not always available in developing countries such as India. Widal agglutination test is an alternative laboratory test widely used for serological diagnosis of enteric fever.

The present study shows that in an endemic area such as India, *S. typhi* agglutinins against both O and H antigens. The lower titres were detected in Paratyphoid A as only 2 tested samples (0.4%) showed a titre of 1 in 40. This finding is in agreement with a study carried out in Sri Lanka, and other endemic areas in India (Senewiratne B et al 1977, Patil *et al.*, 2007, Sneha, 2011), where agglutinin titres of up to 1 in 80 (O Ag) and 1 in 160 (H Ag) were discovered in the normal population. It is thus clear that any interpretation as to the significance of a Widal test result must be made against this "baseline" information.

Therefore, a fourfold rise in antibody titres between acute and convalescent phases is considered as a significant change to establish the diagnosis. The result of a single Widal test should be evaluated based on the average baseline titres present in healthy individuals and antibody titres beyond a cut off value should be regarded as significantly elevated titres which may be used for diagnosis in an appropriate clinical setting.

As the Widal test is easy, inexpensive, non-invasive method, it can be of diagnostic value when blood culturing facilities are not available. However the results of Widal test should be evaluated on the basis of endemicity and cross reaction of antigens with other salmonella species. The present study results indicates that the cut off titres of 1 in 80 for anti O antibodies and 1 in 160 for anti H antibodies may be considered as significant baseline titres for enteric fever in our region. Our study highlightens the importance of need for paired sampling to demonstrate the raising level of agglutinins. Classically, a fourfold rise of antibody in paired sera is considered diagnostic of typhoid fever.

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