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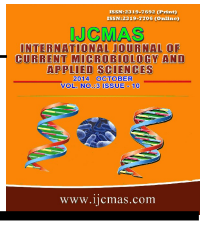

Original Research Article

Baseline titer for Widal test in Haryana, India

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A B S T R A C T

The widal test is one of the extensively used and only available serodiagnostic test of typhoid fever in developing economies. Its diagnostic titer value depends upon the baseline titer in a particular geographical region. Aims: To determine the baseline titer in widal test Settings and Design: This prospective study was conducted in time period of three months, from Nov. 2013- Jan 2014 in Pt. B.D.Sharma PGIMS , Rohtak. Methods and Material: Blood samples from healthy blood donors were collected and subjected to widal tube agglutination test, 0.4 ml of two fold serially diluted patients' sera (dilutions from 1:20 to 1:320) in 0.9% normal saline and was tested by adding an equal amount of antigen. Results: Out of total 250 samples from blood donors 149 samples showed titer of ≥ 1:20 and 101 samples ≤ 1:20 against TO,TH ,TAH. Out of these 70(28%) and 107(42%) samples showed a titer >1:20 to the O and H antigen of Salmonella enterica serovar typhi respectively. 63(25%) samples were reactive for anti paratyphi A agglutinins upto 1:20 titer. Conclusions: Based on present study it was recommended that the significant titre of the 'H' agglutinins and the 'O' agglutinins of Salmonella enterica serovar typhi was ≥ 1:160 and ≥ 1: 80 respectively. Similarly, the significant titer of the 'H' agglutinins of Salmonella enterica serovar paratyphi A was ≥ 1: 40.

Introduction

Enteric fever is a common cause of pyrexia of unknown origin in India and diagnosis of uncomplicated cases based on the signs and symptoms is not specific. Definitive diagnosis of typhoid can be made by the isolation of *Salmonella typhi (S.typhi)* from blood, faeces or bone marrow. Such culture techniques are often unavailable in developing nation and widal test serves as most efficient serological diagnostic tool in the diagnosis of the enteric fever.

It is a tube dilution test which measures agglutinating antibodies against the lipopolysaccharide O and protein flagellar H antigens of *S. typhi*. There is no consensus regarding diagnostic criteria for interpretation of the test. Classically, a four fold rise of antibody in paired sera 10-14 days apart is considered diagnostic of typhoid fever (Parker, 1984).

The interpretation of the widal test depends upon the baseline titer in healthy population and which in turns depend upon the endemicity of enteric fever in a specific area. It 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. Therefore the present study was done to determine the baseline titer of agglutinins in healthy population and to define the significant titer of the widal agglutination test for the diagnosis of enteric fever in an endemic area in a single serum test.

Materials and Methods

This study was conducted on sera of 250 healthy blood donors in a time period of three months from Nov 2013 to Jan 2014. All the blood doors were male from the age group of 18-50 years. These samples were screened for Malaria, HBsAg and antibodies to HIV, HCV and *Treponema pallidum.* The samples which found to be positive were not included in the study.

The widal tube agglutination test was done on all sera by the conventional tube agglutination method using commercially available antigens (SPAN Diagnostic Private Limited) (Cruickshank *et al.,* 1975). 0.4 ml of two fold serially diluted patients sera (dilution from 1:20 to 1:320) in 0.9% normal saline were tested by adding an equal volume of antigen. A negative saline control was included in each batch of the test. The tubes were incubated at 37°C for 2 hours and kept at room temperature overnight and then examined for agglutination.

Results and Discussion

Typhoid fever continues to be a major health problem in developing countries, because of more common unhygienic practices. Definitive diagnosis of enteric fever depends on isolation of salmonellae from blood, stool, urine, bone marrow, bile or other body fluids (Manson-Bahr, 1987; Gilman, 1975; Geddes, 1974). However, all these facilities are expensive and not available easily. So, widal test remains as an alternative for the diagnostic purposes in such situations. This test is based on demonstration of the presence of agglutinin (antibody) in the serum of an infected patient, against the H (flagellar) and O (somatic) antigens of *Salmonella enterica* serotype *typhi*, *paratyphi A* and *paratyphi B*, during the acute and convalescent period of infection (Washington and Henry, 1984).

The earliest serological response in acute typhoid fever is a rise in the titer of the O antibody, with a gradual elevation of the H-antibody titer, but persisting longer than that of the O-antibody cut off titer. Usually up to 70% of adults show an early rise of antibody titer in the first week of infection (Chessbrough. 1987).

The frequency of H agglutinins in a give population reflects the latent infection. On the other hand the concentration of O agglutinin does not vary frequently in different regions and it reflects the recent infection with *Salmonella* species (spp.).

But there are many difficulties associated with evaluation of widal test. As there is variation in the level of antibodies detectable in apparently healthy population of different areas and this variation can effected by cross infection with other *Salmonella* spp. False positive results can be obtained in healthy individuals by the presence of cross reacting antigens for example malaria, brucellosis, dengue fever and other enterobacteriaceae infections, vaccinated individual (Colle *et al.,* 1996).

For the diagnostic purposes a four fold rise in antibody titers between acute and convalescent phases is considered significant, but this type of comparison is not feasible in practical use. Therefore, a single cut off value on average baseline titer among healthy individuals needs to be determined. So, the purpose of the present study was to develop recommendations for the interpretation of widal test results in the local region.

Many other studies have reported lower and higher (Chew *et al.,* 1992; Hamze and Vincent, 2004; Abraham *et al.,* 1981). The lower level of cut off values in widal test was because of proper hygienic practices in developed countries and vice versa.

In the present study, the baseline titers upto ≥1:20, ≥1:40, 1:80 was present in 138 (55%) individuals, 70(19.6%), 21(8.4%) individuals respectively, against TO antigen of *Salmonella enterica serovar typhi* . The highest titer against TH upto ,1:80, 1:40 and 1:20 was present in 48(19%) 58(23%) and 88(35%) cases, respectively. So, the base line titers for the O and H antibodies of *S. typhi* was found to be 1:40 and 1:80. Similar results were reported in other studies as well (Kulkarni and Rego, 1994; Peshattiwar, 2012; Pang and Puthucheary, 1983; Bharat *et al.,* 2009).

According to this study the prevalence of *Salmonella paratyphi A* was found in 63(25%) individuals with a base line titer of 1:20. Therefore, it was concluded that the cut off value for TO, TH and TAH was 1:80, 1:160, 1:40 for the diagnosis purposes of enteric fever inthis region.

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