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Short Communication

Endemic titer of Widal test in Kolhapur, India

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K e y w o r d s

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A B S T R A C T

The widal test is one of the commonly used sero diagnostic test for typhoid fever in developing countries. Its diagnostic titer value depends upon its endemic titre in that particular geographical area. Aims: To determine the endemic titer of widal test. This prospective study was conducted in time period of six months, from January 2014 to June 2014 in Apple Sarswati Multispeciality Hospital, Kolhapur. Blood samples from healthy blood donors were collected and subjected to tube agglutination test; 0.4 ml of two fold serially diluted patients' sera (dilutions from 1:20 to 1:640) in normal saline and was tested by adding an equal amount of antigen. Out of total 300 samples from healthy blood donors 169 samples showed titer of ≥ 1:20 and 131 samples ≤ 1:20 against TO,TH and TAH. Out of these 90 (30%) and 135 (45%) samples showed a titer >1:20 to the O and H antigen of *Salmonella enterica serovar typhi*, respectively. Seventy (23.33%) samples were reactive for anti paratyphi A agglutinins up to 1:20 titer. 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 ≥ 1:80 and ≥ 1: 80, respectively. Similarly, the significant titer of the H agglutinins *of Salmonella enterica serovar paratyphi A* was ≥ 1: 40.

Introduction

Enteric fever also called as typhoid fever caused by *S. typhi*. Typhoid fever was once prevalent all over the world and was not well demarcated from other prolonged fevers. A detailed study of the disease was presented by Bretonneau (1826) who identified the intestinal lesion. The name typhoid was given by Louise (1829) to distinguish it from typhus fever. The infection as acquired by ingestion ID50 was found to be 10^3 to 10^6 bacilli. Incubation period is usually 7–14 days. Fever typically relative bradycardia and toxaemia. Bacteriological diagnosis of typhoid consists of demonstration of bacilli and antibodies from patients' serum. Demonstration of a rise in titre of antibodies, by testing two or more serum samples is more significant than single test. The results of a single test should be interpreted with caution, for that it is necessary to obtain information on the distribution of agglutinin levels in normal sera in different areas. Therefore the present study was done to determine the endemic

titer of widal 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 like our in a single serum test.

Materials and Methods

This study was conducted on sera of 300 healthy blood donors in a time period of six months from January 2014 to June 2014. All the blood donors were male from the age group of 20–45 years. These samples were screened for Malaria, HBsAg, Brucella, Lepto and antibodies to HIV, HCV and *Treponema pallidum.* The samples which found to be positive were not included in this study. The widal agglutination test was done on all sera by the conventional tube agglutination method using commercially available antigens (SPAN Diagnostic Private Limited) 0.4 ml of two fold serially diluted patients sera (dilution from 1:20 to 1:640) in 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 2-3 hours and kept at room temperature overnight and then examined for agglutination.

Results and Discussion

Typhoid fever is one of the major health problems in developing countries. Definitive diagnosis of enteric fever depends on isolation of *salmonellae* from blood, stool, urine, bone marrow, vomitus, pus, bile or other body fluids (Manson-Bahr and Bell, 1987; Gilman, 1975; Geddes, 1974). However, all these test facilities are tedious, skill requiring and expensive 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 and O antigens of *Salmonella enterica* serotype, *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 regarding antibody 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 hurdels associated with evaluation of widal test. As there is variation in the level of antibodies detectable in apparently healthy population of different areas of different region may be effected by cross infection with other *Salmonella* spp. Also false positive results can be obtained in healthy individuals by the presence of cross reacting antigens for example malaria, brucellosis, dengue, Enterobacteriacae infections and vaccinated individual also. (Colle *et al.,* 1996). For the diagnostic purposes a four fold rise in antibody titer between acute and convalescent phases is considered significant. Therefore, a single cut off value on average 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; Mittal and Bela, 2014, peshattiwar, 2012). 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 titers up to 1:20, 1:40, 1:80 was present in 168 (56%) individuals, 85(28.33%), 25 (8.33%) individuals, respectively, against TO antigen of *Salmonella enterica serovar typhi*. The highest titer against TH up to 1:80, 1:40 and 1:20 was present in 49(16.33%) 80(26.66%) and 111(37%) cases, respectively. So, the endemic titer 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 80(26.67%) 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, 1:80, 1:40 for the diagnosis purposes of enteric fever in this region.

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