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STUDY OF BASELINE WIDAL TITRE AGAINST SALMONELLA SPECIES AMONGST HEALTHY INDIVIDUALS IN THIRUVANANTHAPURAM DISTRICT OF KERALA, SOUTH INDIA

ABSTRACT

Objective: To determine the distribution pattern of agglutinating antibodies to Salmonella enterica serotype typhi, paratyphi A and paratyphi B antigens in normal population of Thiruvananthapuram District, Kerala, South India.
Methods: One hundred apparently healthy different age group volunteers both male and female free from infectious and other cardiac, lung and kidney diseases were enrolled for blood collection. Sera were separated from collected blood and serially diluted in such a way that 1:20, 1:40, 1:80, 1:160 and 1:320 by using isotonic normal saline. Widal test was performed and after incubation the test tube was examined visually for agglutination.
Results: Among the 100 blood samples collected from healthy individuals from different areas of Trivandrum District, 64 individuals had significant antibody titers (≥ 1:20) against one of the four antigens against S. enterica. In this 51 samples with an anti-O titer against serotype typhi, 16 and 33 samples had titers of ≥ 1:60 and ≥ 1:40, respectively. A proportion (2% of all) had anti-O titer of ≥ 1:80. Similarly, among the 59 samples demonstrating anti-H titers of ≥ 1:20 to S. enterica serotype typhi, 24 had a titer of ≥ 1:80 and 14 had 1:160. For S. enterica serotypes paratyphi A and B, anti-H titers of ≥ 1:20 were found only in 12% and 3%, respectively of all samples tested.
Conclusion: It is found out that the cutoff value of antibody titers against Salmonella enterica typhi, paratyphi A and paratyphi B is 1:80 in case of O antigen and 1:160 in case of H antigen. From this study it is clear that the baseline titre of Salmonella antibodies in healthy individuals in a locality should be observed at regular intervals.

Key words: Enteric fever, Widal test, Baseline Widal titre

1. INTRODUCTION

Typhoid fever continues to be a global health problem, especially in tropics and subtropics[1]. There was a global estimation of more than 21.6 million of cases of typhoid fever in 2000 and 5412,744 illness were due to paratyphoid fever. These fevers are considered as a major cause of morbidity and mortality in developing countries with more than 90% of cases found in Asia only[2,3]. The diagnosis of typhoid fever in developing countries is based mainly on clinical ground and is difficult, as the presenting symptoms are diverse and similar to those observed in other common febrile illness[1,4,5]. The traditional salmonella laboratory confirmation of clinical suspicion of typhoid fever is based on culture on selective media and identification of suspected colonies by biochemical and serological tests[1,6]. These methods are generally time consuming, laborious and may give false negative results. An alternative is typhoid serology using the Widal test that is widely used in many developing countries because of its feasible, fast and easy[1]. Several reports showed that Widal test is valuable one in the diagnosis of typhoid fever when performed reliably and interpreted with care even though various factors such as previous immunization, sharing of antigenic determinants with other bacteria, stage of the disease etc., interfere with the Widal test results. Updating the baseline Widal titre is a must for the proper interpretation of the Widal test[7,8,9].

Enteric fever is endemic in South India including the Thiruvananthapuram district of Kerala. The baseline titre of agglutinating antibodies against Salmonella enterica serotype typhi, paratyphi A and paratyphi B antigens in healthy individuals in Thiruvananthapuram District have not been established. Therefore, the present study was designed to determine the same and also to assess some of the factors which may be of importance in the interpretation of Widal test in the study area. The outcome of this study would provide a good platform for the diagnosis of enteric fever in the study area.

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2. Methods

The present study was conducted at Noorul Islam Institute of Medical and Dental Science which is a tertiary care hospital and academic centre of 450 beds, located in Neyyatinkara, Thiruvananthapuram District, Kerala, South India.

One hundred apparently healthy different age group blood donors both male and female from various parts of Thiruvananthapuram district were enrolled. None of the volunteers had a history of recent infections including malaria, viral hepatitis, tuberculosis, HIV infection, sexually transmitted diseases and other infectious diseases. They were also free from cardiac, lung and kidney diseases. Widal antigen kits (Antigen suspension of Salmonella enterica serotype typhi, paratyphi A and B) were obtained from Tulip Diagnostics Private Ltd., Ernakulam, Kerala, India.

Venous blood sample was collected from each participant, left to clot for 15 minutes in the room temperature. Sera were separated by using micropipette. The separated sera were properly labeled and stored in -20^{o}C for further study.

The Widal test was started with serial serum dilutions: 1:20, 1:40, 1:80, 1:160 and 1:320 by using isotonic normal saline. 0.5ml of each of the antigen suspension was added to corresponding tubes, mixed well and incubated at 37^{o}C for 24 hours. The tubes were then examined visually for agglutination. 50% agglutination was considered and recorded as the titre of antibodies present in the individuals against Salmonella.

3. Results

This study was conducted to find out the distribution pattern of agglutinating antibodies against Salmonella enterica serotype typhi, paratyphi A and B antigens in normal population of Thiruvananthapuram District, Kerala. The study was conducted from June – 2013 to September -2013. A total of 100 blood samples were collected from apparently healthy persons from different places of Thiruvananthapuram District especially the coastal regions such as Poovar, Vizhinjam and Sankhumukham. 42 of 100 donors belong to Poovar region and 28 persons were from Vizhinjam and the remaining 30 persons belong to Sankhumukham region. Majority of blood donors, 79 of 100 were male and the remaining 21 donors were female. 22 donors were aged between 21 – 30 years. 20 donors were aged between 11 – 20 years. 19 donors were aged between 31 - 40 years. 16 donors were aged between 41 – 50 years. 12 donors were aged between 51 - 60. 7donors were above 60 years age and 4 donors were below 10 years age.

Sera were separated from collected blood samples and the distribution of antibody titres against various Salmonella enterica serotypes were determined by standard Widal agglutination tube test. Among the total 100 samples, 64 samples showed agglutination at the titre ≥ 1:20 for the O or H antibodies against Salmonella enterica serotypes typhi, para typhi A and B. Rest of the 36 samples did not show agglutination. The distribution of 51 samples showed anti – O titre of ≥ 1:20 to Salmonella enterica serotype typhi, 15% of all samples tested had a titre ≥ 1:60 while more than 36% of all the samples had a titre of 1:40. Two individuals had an anti – O of 1:80. The median and mean anti body titres against the “O” antigen were 1:20 and 1:30 (Standard deviation ≥ 1:30).

Among the 59 samples showed anti – H titre of ≥ 1:20 to Salmonella enterica serotype typhi, 29 samples were positive at a titre of ≥ 1:80 and 12 had a titre of 1:160. One sample showed a titre of 1:320. The median and mean anti body titres against the “H” antigen were 1:20 and 1:64 (Standard deviation ≥ 1:123). Altogether 12 samples showed agglutination titre of ≥ 1:20 against H – antigen of Salmonella enterica serotype para typhi A among which 8 samples had ≥ 1:40 and one had ≥ 1:80 titres. The median and mean anti body titres against the “H” antigen of serotype paratyphi A were 1:10 and 1:13 (Standard deviation ≥ 1:10). Only two samples had anti –H titre of ≥ 1:20 to H – antigen of Salmonella enterica serotype paratyphi B. (Table 1).

4. Discussion

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Results of the present study conducted among the healthy population of coastal regions of Thiruvananthapuram District of Kerala, confirmed the presence of high agglutinin titres. 14% of the sample had anti –O antibody titres of ≥ 1:80 and 17% had anti – H antibody titres of ≥ 1:160 against Salmonella enterica serotype typhi. In many parts of Kerala, the present baseline titre of Widal test for the diagnosis of typhoid fever is 1:100 for both O and H agglutinins. Therefore the results of present study showed that the level of agglutinins for Salmonella enterica typhi in these healthy individuals were greater than those used to diagnose the typhoid fever currently in Kerala. Based on these results, the significant titres should be greater than 1:80 for anti – O and greater than 1:160 for anti – H for a presumptive diagnosis of typhoid fever. The present study also showed that anti – H agglutinin titre to Salmonella enterica serotype paratyphiA in Thiruvananthapuram district was less than that against Salmonella enterica serotype typhi.

5. Conclusion

In many developing countries including India, the laboratories mainly rely upon Widal test for typhoid serology. So that the present study was done to determine the baseline titre of agglutinating antibodies against various serotypes of S.enterica in normal population of Thiruvananthapuram District, Kerala, South India. The typhoid fever is endemic in many parts of South India including the coastal regions of Thiruvananthapuram District, Kerala. In the present study, the blood samples were collected from hundred healthy persons from the coastal regions of Thiruvananthauram District such as Poovar, Vizhinjam and Sankhumukham. The results of present study showed the presence of high agglutinin titre in a significant proportion of healthy individuals. Lack of proper hygiene and poor sanitation measures are the main reasons for the high antibody titre. In comparison with developing countries the baseline titre for Widal test islesser in developed countries. According to the previous literatures, the Widal test results may depend on the levels of antibodies to cross-reacting antigens to various Salmonella species. In the Kauffmann – White classification, the genus Salmonella is subdivided in to more than 2300 serotypes containing different combinations of antigens. Salmonellae are divided in to serological groups on the basis of O or somatic antigens. Cross-reactions producing a false positive anti – O titre in the Widal test can therefore occur with any of these serotypes. Widal test shows a false positive reaction in some other clinical conditions like malaria, syphilis, dengue fever etc., and these clinical illnesses should be differentiate correctly from enteric fever by clinical diagnosis and other laboratory tests. Finally, from this study it is clear that the baseline titre of Salmonella antibodies in healthy individuals in a locality should be observed at regular intervals.

Serotype	Antibody type	Frequency	Percentage
Typhi	Anti O antigen	51	51%
Typhi	AntiH antigen	59	59%
Paratyphi A	Anti H antigen	12	12%
Paratyphi B	Anti H antigen	2	2%

Total number of sample, N=100

Table 1: Distribution of the sample with antibody titer ≥1:20 against different serotypes of Salmonella enterica

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