

## Study of Baseline Widal Titre against *Salmonella* Species amongst Healthy Individuals in Kollam District of Kerala, South India

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Received on: 01-05-2014; Revised and Accepted on: 19-05-2014

### ABSTRACT

This study was designed to determine the distribution pattern of agglutinating antibodies to *Salmonella enterica* serotype typhi, paratyphi A and paratyphi B antigens in normal population of Kollam District, Kerala, South India. One hundred and eighty three apparently healthy different age group volunteers both male and female free from infectious and other cardiac, lung and kidney diseases were enrolled for the study. Venous blood sample was collected from each participant. 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 of the present study showed that the mean anti-O titres of *S.typhi* in the overall population were  $17.0 \pm 16.8$  and the anti-H titre were  $14.2 \pm 9.8$ . The mean anti-H titres of *S.paratyphi A* were  $20.2 \pm 20.3$  and *S.paratyphi B* were  $28.4 \pm 42.2$ . Results showed that the baseline anti-O titre of *S.typhi* was 1:160 and anti-H titre of *S.typhi*, *S.paratyphi A* and *B* was 1:80 in the selected study area. 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.

### INTRODUCTION

Enteric fever which includes typhoid and paratyphoid fever is a systemic febrile illness caused by the bacterium *Salmonella enterica* serovar typhi and *Salmonella enterica* serovar A, B or C respectively and *S. enterica* ser. typhi is found to be associated with more than 90% of cases of enteric fever. Paratyphoid fever is clinically similar but milder than typhoid fever [1]. Typhoid fever continues to be a global health problem, especially in tropics and subtropics [2, 3]. 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 [1, 3, 4]. 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 [2, 3, 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 [2, 3, 6]. These methods are generally time consuming, laborious and may give false negative results. An alternative to these methods is Widal test [2]. This test was developed by F. Widal in 1896. The Widal agglutination test is the diagnostic test which is most commonly used for the diagnosis of enteric fever, ever since its introduction 100 years back [7, 8]. This test detects the antibodies against the O and H antigens of *Salmonella typhi* and against the H antigens of *Salmonella 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 [7, 9].

Enteric fever is endemic in India and it continues to be one of the major health problems here [7]. Updating the baseline Widal titre is a must for the proper interpretation of the Widal test. In our previous study, we examined the baseline Widal titre of

healthy individuals from the coastal regions of Thiruvananthapuram District, Kerala [3]. Now the present study was designed to determine the baseline titre of agglutinating antibodies against *Salmonella enterica* serotype typhi, paratyphi A and paratyphi B antigens in healthy individuals in Kollam District of Kerala. The outcome of this study would provide a good platform for the diagnosis of enteric fever in the study area.

### 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 and eighty three apparently healthy different age group blood donors both male and female from various parts of Kollam 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°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°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*.

### RESULTS AND DISCUSSION

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 Kollam District, Kerala. The specific purpose of this study was to develop a local recommendation for the interpretation of the Widal

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test results. The study was conducted from January - 2013 to May - 2013. A total of 183 blood samples were collected from apparently healthy persons from different places of Kollam District especially the coastal regions such as Sasthamkotta, Anchal, Thazhava, Thavalakkara and Kundara. Majority of blood donors, 108 of 183 were male and the remaining 75 donors were female. 43 of 183 donors belong to Sasthamkotta region, 40 persons were from Anchal, and 38 persons belong to Thavalakkara region. 32 persons were from Thazhava and the remaining 30 persons from Kundara regions. 63 donors were aged between 26 - 45 years, among them 29 candidates are males 34 candidates are females. 56 donors were aged between 6 - 25 years, 39 members are males and remaining 17 members are females in this category. 35 donors were aged between 46 - 65 years, among them 21 donors are males and remaining 14 donors are females. 29 donors were above 65 years, this category includes 19 males and 10 females.

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. The Table 1 & 2 showed the mean titre

value of antibodies against *Salmonella* species in the study population. The mean anti-O titre of *S.typhi* in the overall population were 17.0 ± 16.8 and the anti-H titre were 14.2 ± 9.8.

The mean anti-H titres of *S.paratyphi A* were 20.2 ± 20.3 and *S.paratyphi B* were 28.4 ± 42.2. The results showed that the blood donors above 65 years age had significantly lower anti-H titres of *S.paratyphi A* and *B*. But these titres were not significantly different from the titres of other age groups. Generally there was no significant difference in the titres between males and females (P > 0.05). But in the age group of 6 - 25 years, the mean anti-H titre of *S.typhi* was 0.9 ± 0.20 in case of males and in females, it was 1.14 ± 0.26 (P < 0.05, t = 2.168). In the age group of above 65 years, the anti-H titre of *S.paratyphi A* were 1.05 ± 0.23 in males and 1.24 ± 0.24 in females (t = 2.056, PC 0.05). Any titre above the baseline titre can be taken as a diagnostic titre for the diagnosis of enteric fever. Results of the present study showed that the baseline anti-O titre of *S.typhi* was 1:160 and anti-H titre of *S.typhi*, *S.paratyphi A* and *B* was 1:80 in the selected study area.

**Table No. 1: Mean anti - O titres of *Salmonella* serotype in the overall population of study area**

Serotype	Age group			
	6-25	26-45	46-65	Above 65
<i>S.typhi</i>	16.9 ± 13.2 (n = 56)	15.6 ± 23.0 (n = 62)	19.1 ± 9.8 (n = 35)	17.6 ± 10.5 (n = 29)

n - Number of samples

**Table No. 2: Mean anti - H titres of *Salmonella* serotypes in the overall population of study area**

Serotype	Age group			
	6-25	26-45	46-65	Above 65
<i>S.typhi</i>	13.0 ± 11.4 (n = 56)	13.3 ± 6.3 (n = 63)	15.3 ± 12.3 (n = 35)	17.1 ± 9.4 (n = 28)
<i>S.paratyphi A</i>	40.4 ± 31.4 (n = 54)	48.7 ± 59.4 (n = 63)	37.7 ± 39.9 (n = 35)	30.3 ± 25.4 (n = 29)
<i>S.paratyphi B</i>	27.4 ± 47.1 (n = 55)	22.1 ± 40.1 (n = 63)	37.1 ± 52.9 (n = 35)	33.7 ± 29.0 (n = 27)

n - Number of samples

## 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 Kollam District, Kerala, South India because the typhoid fever is endemic in many parts of South India including the coastal regions of Kollam District, Kerala. In the present study, the blood samples were collected from one hundred and eighty three healthy persons from the various parts of Kollam District especially the coastal regions such as Sasthamkotta, Anchal, Thazhava, Thavalakkara and Kundara. The present study revealed the baseline titer level of antibodies against *Salmonella* species in healthy population of study area. Lack of proper hygiene and poor sanitation measures are the main reasons for the spread of infection. In comparison with developing countries the baseline titre for Widal test is lesser 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.

## ACKNOWLEDGEMENT

We would like to thank Dr. Sadiq Hussain, MDS, Principal, Noorul Islam Institute of Dental Science, Neyyattinkara, Thiruvananthapuram District, Kerala for his valuable suggestions and support for the successful completion of this work and Mr. J. Kumaran. M.Pharm., Assistant Professor, P.S. College of Nursing, Thalakkulam, Kanyakumari District for his assistance in the preparation of this manuscript.

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## How to cite this article:

I.S. Aruni, P. Prabakaran: Study of Baseline Widal Titre against *Salmonella* Species amongst Healthy Individuals in Kollam District of Kerala, South India. *J. Pharm. Res.*, 2014; 3(5): 74-75.

**Conflict of interest:** The authors have declared that no conflict of interest exists.

**Source of support:** Nil