ORIGINAL ARTICLE

Determination of Baseline Antibody Titre for Widal Agglutination Test in a Specific Geographical area of Poonch, Rawalkot, Azad Kashmir

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ABSTRACT

Aim: To establish baseline antibody titre for Widal agglutination test in local population of Poonch, Rawalakot, Azad Kashmir.

Study settings: Department of Pathology, Microbiology section, Poonch Medical College, Rawalakot, Azad Kashmir from May 2019 to October 2019.

Method: 450 blood samples were collected form 18-60 years old healthy adult individuals. Widal agglutination test was performed on serum of these samples after making doubly dilutions with normal saline to determine baseline antibody titre.

Results: 80 out of 450 individuals showed positive results. Among positive 80 individuals 36 were positive for salmonella typhi O antigen, 43 were positive for Salmonella typhi H antigen, 1 sample was positive for *P.typhi* AH antigen while 14 were detected positive for Paratyphi BH antigen. Cut off value for Salmonella typhi O antigen is 1:40 and H antigen is 1:80. Baseline antibody titre for H antigen of Salmonella paratyphi A is 1:40 and for paratyphi B is 1:80, so Widal test should be interpreted according to these values in this area.

Keywords: Widal agglutination test, antibody,

INTRODUCTION

Typhoid fever, a potentially life-threatening infection spread through orofecal route. Clinical symptoms include high grade fever, vomiting and headache. It has case fatality rate of 15%. The main causes of its spread in poor and developing countries including Pakistan are poor hygiene, substandard or lack of sanitation and water contamination. WHO reports 21 million cases and 222,000 deaths due to the infection worldwide per year[1].

Typhoid fever also known as enteric fever is caused by *salmonella enterica* subspecies, *salmonella typhi* and *salmonella paratyphi A, B and C* [2].

The standard test for the diagnosis of typhoid fever is the isolation of the organism from culture.[3] Blood and bone marrow culture are cultured for the isolation of the organism and considered as gold standard. Culture of the organism can be done in special facilities where facilities are available. However, in developing countries culture facilities are not readily available, so Widal test remains the main diagnostic test that detects anti salmonella antibodies in patient's blood. In healthy population the antibody titre can be higher than baseline titre. This higher antibody titre can be due to chronic liver disease, vaccinated against typhoid or even it can be healthy carrier individual[4].

Widal test can be accurately performed by taking two samples first one in the acute phase and second one in convalescent phase. To achieve a standard four-fold rise in antibody titre, the samples should be taken with an 8-14 days interval[5].

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In a specific geographical area, it is necessary that base line titre of the population of healthy individuals should be known that can be used for the interpretation of titre from a single Widal Test[6,7].

To establish the baseline antibody titre of healthy population in Rawalakot AJK, this study was carried out, because base line titre was not established for this area. Typhoid fever in one of the major infectious disease in this area so it will help in the diagnosis.

MATERIAL AND METHODS

This study was conducted on 450 blood samples collected from participants in the Department of Pathology, Microbiology section, Poonch Medical College, Rawalakot, Azad Kashmir from May 2019 to October 2019. All samples were collected from healthy adult individuals and the samples were processed as per set protocols and the results were recorded accordingly. An informed consent was taken, and the individuals were screened through a questionnaire about any past six months history of hepatitis, malaria, dengue fever or AIDS infection.

Inclusion Criteria: Healthy adult individuals between the age of 18 years to 60 years were included in the study. All individuals were had no history of any enteric fever infection, malaria, hepatitis or aids in the past six months. Only local individuals were included in the study.

Exclusion Criteria: Individuals who were less than 18 years old or above 60 years were not included in the study. Similarly, individuals having past 6 months history of typhoid fever, malaria, hepatitis of aids were also excluded from the study. Nonlocal individuals were also excluded: Blood samples were collected from healthy donors through

a questionnaire, who had neither been vaccinated with TAB (Typhoid and Paratyphoid A and B) vaccine or oral typhoid vaccine/Vi vaccine nor had suffered from enteric fever in the past six months. A venous blood sample of 5ml was collected in a plain tube and allowed to clot at room temperature for about 30 minutes. The samples were then centrifuged at 3000rpm for 10 minutes in order to separate the serum from the blood. Sera were transferred to clean and dry sterile storage vials. The serum samples were refrigerated at −20◦C. Stained *Salmonella* antigen test kit for tube test. This test kit contained smooth suspensions of antigens "O" and "H" of *Salmonella* serotype Typhi, "H" antigen of *Salmonella* serotype Paratyphi A, and "H" antigen of *Salmonella* serotype Paratyphi B. The test was performed after bringing sera and reagents to room temperature. The test was performed in batches, each batch contained 50 samples. Widal titre was estimated by confirmatory quantitative tube agglutination test using standard agglutination test procedure[4]. As per the manufacturer's instructions, 0.5ml of the increasingly diluted sera, dilutions ranging from1 : 20 to 1 : 320 in 0.9% normal saline, was tested by adding an equal amount (0.5ml) of antigen, thereby giving final dilution ranging from1 : 40 to 1 : 640, and the tubes were then incubated overnight at 37◦C in a water bath. Positive and negative (saline) controls were added in every set of the test. The tubes were mixed well and incubated in a serological water bath maintained at 37◦C for 16–20 hours and were observed for agglutination. In a positive test, O antigen showed granular agglutination and H antigen showed fluffy agglutination. The last tube showing visible agglutination with the naked eye was taken as an endpoint of the test. The titre was reported out as the reciprocal of the endpoint. Absence of agglutination suggested a negative test. The method used for finding the baseline titre was Widal tube agglutination test. The amount of antigen suspension used was the same as the amount of diluted sera making the dilution 2-fold, and hence the readings in our study begin with titre 1: 40, which is in accordance with the manufacturer's instructions and the standard lab protocols.

Statistical Analysis: Statistical analysis was done using Microsoft excel spread sheet. The results were presented as percentages in the form of tables.

All 450 blood serum samples from healthy individuals were tested against antibodies to salmonella species and the results of their titres were recorded. These titres were recorded using Widal tube test agglutination method. 350 individuals out of 450 volunteers were male and rest were female. 78 samples out for 450 were positive for baseline antibody titre, while rest were unable to show any kind of agglutination against any type of antibody against salmonella species. It shows that roughly positive samples showing baseline antibody titre was roughly 17.78% of the total samples examined and rest 82.22 were negative for any salmonella antibody (Table A).

Among positive 80 individuals 36 were positive for salmonella typhi O antigen, 43 were positive for Salmonella typhi H antigen, 1 sample was positive for *P.typhi* AH antigen while 14 were detected positive for *Paratyphi* BH antigen (Table B).

Table C, shows that the individuals with positive results of antibody titre. 36(45%) individuals who were positive for O antigen of *S. typhi*, 22(61.11%) had a titre of 1:40, 12(33.4%) had titre of 1:80, 5(8.4%) individuals had a titre of 1:160 and 1(2.8%) had a titre of 1:320. 43(53.75%) samples were positive for H antigen against *salmonella typhi*, 10(23.25%) had antibody titre of 1:40, 28(65.11%) had a titre of 1:80, and 5 (13.88%) had a titre of 1:160. Only 1(1.25%) was positive for *Salmonella paratyphi* AH antigen its titre was 1:40. 14(17.5%) individuals were positive for *Salmonella paratyphi* BH antigen, 5(33.71%) had titre 1:40, 8(57.14%) had 1:80 and 1(7.14%) had titre of 1:160.

Table A: Positive and negative individuals and percentages.

Total no. of samples (n)	450
Ab titre ≤ 1:40 and percentage	370 (82.22%)
Ab titre ≥ 1:40 and percentage	80 (17.78%)

Table B: Positive percentages for different types of antibodies of different *salmonella* species.

Salmonella serotype	**Type of Antibody**	**No. of positive and %age**
Salmonella typhi	Anti-TO	36 (45%)
Salmonella typhi	Anti-TH	43 (53.75%)
S. Paratyphi A	Anti-AH	1 (1.25%)
S. Paratyphi B	Anti-BH	14 (17.5%)

RESULTS

Table C, showing different titres and their percentages against different antigens and antibodies.

Salmonella serotype	**Type of Antibody**	**No. of positive and %age**	**Titre 1:40**	**Titre 1:80**	**Titre 1:160**	**Titre 1:320**
Salmonella typhi	Anti-TO	36 (45%)	22(61.11%)	12(33.4%)	3(8.4%)	1(2.8%)
Salmonella typhi	Anti-TH	43 (53.75%)	10(23.25%)	28(65.11%)	5(13.88%)	--
S. Paratyphi A	Anti-AH	1 (1.25%)	1(100%)	--	--	--
S. Paratyphi B	Anti-BH	14 (17.5%)	5(35.71%)	8(57.14%)	1(7.14%)	--

DISCUSSION

The gold standard test for the diagnosis and isolation of salmonella species is bone marrow and blood culture. However, in the developing countries like Pakistan culture of the organism from blood or bone marrow is considered as expensive and its facilities are not readily available. Culture of the organism is time consuming process and expensive in this part of the world. Therefore, Widal agglutination is most commonly used in clinical and laboratory practice in Pakistan owing to its low cast and readily availability of results. In this part of the developing world the test remains relevant although it has been it is not usually performed in the developed countries. It is still significant if its titre in normal population is known and the test performed as per protocol[6].

The present was done to determine the baseline titre in normal healthy population. As far our best knowledge to

such published study is available at local level. This will help in establishing baseline titre at local level. Our study results are archaic in nature in local healthy people. However, it determines high antibody titre in healthy adult population. The results of our study depict that 80(17.78%) out of total 450 individuals showed agglutination to one or more antigens. Among positive 80 individuals 36(45%) were positive for salmonella typhi O antigen, 43(53.75%) were positive for Salmonella typhi H antigen, 1(1.25%) sample was positive for P.typhi AH antigen while 14(17.5%) were detected positive for Paratyphi BH antigen. So, baseline antibody titre based on our results for O and H antigens of Salmonella typhi are 1:40 and 1:80 respectively. This results are similar a study conducted in India that shows 1:40 titre for O antigen and 1:80 titre for H antigen[8,9]. However, a study conducted in Kathmandu, Nepal show high titres of 1:80 for O antigen and 1:160 for H antigen of Salmonella typhi. These results are much higher than our study[10]. Widal test against salmonella species can show higher results in individuals with immunization against the organism, infection with members of the enterobacteriacae, malaria, dengue or due to poor standardization of antigen production at commercial level[11].

According to a study conducted in Papua New Guinea by Cleg et al shows that there was sharp rise in the antibody titre over a short span of time at a community level. This indicates that baseline antibody titre varies in the population based on the endemicity of the infection. So, baseline cut off value is required for the diagnosis[12]. Enteric fever is a major public health concern in developing countries including Pakistan. Widal test will remain preferred method for diagnosis because its readily availability and cost effectiveness[13].

CONCLUSION

Test results of Widal tube agglutination should always be interpreted on the basis of baseline antibody titre in specific area especially in the developing countries. In our study cut off value for Salmonella typhi O antigen is 1:40 and H antigen is 1:80. Baseline antibody titre for H antigen of Salmonella paratyphi A is 1:40 and for paratyphi B is 1:80. The results of our study indicate that in this geographical area these baseline titres should be considered as cut off value and more higher than this value should be counted as positive results. However, Blood and bone marrow culture will remain the gold standard.

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