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BASELINE WIDAL TITRE DETERMINATION AMONG THE HEALTHY POPULATION IN JODHPUR REGION, RAJASTHAN.

Microbiology

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ABSTRACT

Background: Enteric fever still continues to be endemic in several parts of developing countries. For diagnosis, Widal test is the second most commonly used test after blood culture. Four fold rise of anti-O agglutinin (TO) and anti-H agglutinin (TH) titre in paired serum samples is more relevant but at many occasion, interpretations are to be made on a single serum test results. For effective interpretations, we need to know the baseline antibody titre in healthy population of a particular geographical area based on which the cut-off titre or significant titre can be calculated.

Aim: The present study was undertaken to determine the baseline Widal titre in apparently healthy population in Jodhpur region, Rajasthan.

Material and Methods: The study was done between September 2016 and February 2017, which included seventy five (75) serum samples. These samples were from the pre-operative cases and were sent to the microbiology lab for viral marker tests like HIV, HCV and HBV. Widal tube agglutination test was performed to know the titre of Anti-TO and Anti-TH antibodies.

Results: Forty two (56%) sera showed positive agglutinations (titre of ≥1:20) and the remaining 33 were negative. 62.1% sera were positive for agglutinins of 'O' antigen of Salmonella typhi (TO) at a titre of 1:20 and 46.15% sera were positive for agglutinins of 'H' antigen of Salmonella typhi (TH) at a titre of 1:40.

Conclusion: It was recommended that the significant titre of TO be considered as 1:40 and that of TH as ≥ 1: 80 in this region.

KEYWORDS

Typhoid fever, Widal test, Baseline titre, Cut-off titre, Significant titre.

INTRODUCTION

Enteric fever includes typhoid fever caused by *Salmonella* Typhi and paratyphoid fever caused by *Salmonella* Paratyphi A, B and C.[1] Approximately 21 million cases and 222000 typhoid related deaths occur annually worldwide.[2] Incidence of Typhoid fever has decreased in developed countries due to improved water supply and sanitation facilities but is still endemic in developing countries.[1]

To diagnose Typhoid fever, Widal test is the second most commonly used test after blood culture. This test serves to make a serological diagnosis in such cases where the causal organism could not be demonstrated in patient's body fluid or excretions.[3] Antigen suspensions related to the prevalent enteric fever agents are used to compare either paired sera or a single serum to see the significant antibody levels against the somatic "O" antigen (TO) and the flagellar "H" antigen (TH) of *Salmonella* Typhi.[4,5] Demonstration of four fold rise in antibody titre in paired serum samples is more relevant. But often the diagnosis is to be made immediately based on a single serum sample, when the patients present late. For effective interpretation in such single samples, we need to know the Anti-TO and Anti-TH antibody levels in normal population (baseline titre), which may vary in different geographical areas due to the varying endemicity of Typhoid fever.[6] Based on the baseline antibody titre, the cut-off titre can be deduced and the diagnosis of Typhoid fever can be made.[1]

The purpose of this study was to find out the baseline antibody titre against the "O" and "H" antigens of *Salmonella* Typhi in the population and to develop a local recommendation for interpretation of the Widal test results in single serum samples. To the best of our knowledge, this is the first study being done in this part of Rajasthan.

MATERIAL AND METHODS

This pilot study was conducted in the Department of Microbiology, All India Institute of Medical Sciences Jodhpur, from September 2016 to February 2017. Approval from the Institute ethics committee was sought.

Serum samples from pre-operative cases which came to the microbiology laboratory for routine viral marker tests like HBV, HCV and HIV formed the study samples. Separate samples were not collected for this study, so individual consent was not obtained. The online hospital records were checked for the clinical and epidemiological details of these cases. Only those who belonged to Jodhpur, Rajasthan, were included as study subjects. Those with any history of fever in last 6 months or history of vaccination against Typhoid fever were excluded from the study.

About 2ml of blood was taken and serum was separated immediately, labeled and stored at 4°C for further processing. The serum samples were processed according to standard tube dilution method of the Widal test.[3] Commercially available antigens (Span Diagnostics Ltd) which contained the "O" and "H" antigens of *S.* Typhi were used. The test used two fold serially diluted sera of patients, dilution being from 1:20 to 1:320. A known positive control, negative control and antigen control were set up with each batch of the test. All the tubes were mixed well and they were incubated at 37°C overnight (16-20 hours.). After completion of incubation period, the tubes were examined for agglutination. Anti-*Salmonella* antibodies if present in the serum, reacted with the corresponding antigens to give agglutination. The 'O' antigen being a somatic antigen, brought about a coarse, compact, granular agglutination, whereas 'H' antigen being a flagellar antigen, brought about a large, loose, fluffy, agglutination. The antibody titre was reported as the highest dilution of serum which showed distinct agglutination. The titre showing the maximum number of agglutination was considered as the Baseline titre of the study population.

RESULTS

A total of 75 sera from 37 male and 38 female individuals of all age groups were tested. Among these, 42 (56%) showed agglutinations at a titre of ≥1:20 for either "O" or "H" antibodies against *Salmonella* Typhi and 33 sera did not show any agglutination [Table – 1]. 29 sera (38.66%) showed positive Anti-TO agglutinin (titre ≥ 1:20), whereas 39 sera (52%) showed positive Anti-TH agglutinin. The number and percentage of sera with corresponding end titres for agglutinins against *Salmonella* Typhi, has been presented in Table-2. Table-3 shows the comparative analysis between the present study and other studies as

regards to the total positive samples and Baseline titres of "O" and "H" agglutinins.

Table 1. Widal status of samples

Widal status	Frequency	Percentage
Positive for agglutinins (≥ 1:20)	42	56
Negative for agglutinins(< 1:20)	33	44
Total samples	75	100

Table 2. Percentage of sera with corresponding end titres for agglutinins against S. Typhi.

Antigen	Antibody	Dilutions					
		1:20	1:40	1:80	1:160	1:320	1:640
S typhi-O	Anti-TO	18 (62.1%)	9 (31%)	2 (6.9%)	0	0	0
S typhi-H	Anti-TH	14 (35.89%)	18 (46.15%)	4 (10.25%)	3 (7.69%)	0	0

Table 3. Comparative analysis of total positive samples and Baseline titres of "O" and "H" agglutinins.

Authors	Place	Year	Percentage of positive samples	Baseline titre	
				TO	TH
Gunjal S et al	Ahmednagar (Maharashtra)	2012	81.55	1:40	1:40
Saxena N et al	Hadoti region (Rajasthan)	2012	54	1:40	1:40
Sharma S et al	Jaipur (Rajasthan)	2013	42.6	1:40	1:80
Mittal G et al	Uttarakhand	2014	51	1:40	1:80
Vazhavandal G et al	Trichy	2014	53.4	1:80	1:80
Chauhan S et al	Muzaffarnagar city (UP)	2015	56.8	1:80	1:80
Present study	Jodhpur (Rajasthan)	2017	56	1:20	1:40

DISCUSSION

The mainstay of diagnosing Typhoid fever in clinical practice is a positive blood culture.[1,7] The sensitivity, specificity, and predictive values of Widal test varies in different geographical areas [7] but as this is a very simple and inexpensive test, it is still widely used for diagnosis.[8] At times, the diagnosis is to be made quickly on a single available serum sample. Widal test can be used in such cases but the test results need to be interpreted carefully taking into account the previous history of enteric fever and vaccination, in which widal test can prove to be less specific.[9] Interpretation of the results of Widal test is greatly helped by knowledge of the background levels of antibodies in the local healthy population as there is considerable variation in agglutinin levels among non-infected populations.[6]

In the present study, 56% of samples were found to be positive for the agglutinins (titre ≥ 1:20), which is in accordance with the findings of Chauhan S et al, Saxena N et al, Vazhavandal G et al[10,11,12] whereas 44% samples were negative (titre ≤ 1:20) **[Table 1]**.

Maximum serum samples, i.e. 62.1% showed titres of 1:20 for Anti-TO and 46.15% serum samples showed titres of 1:40 for Anti-TH. Therefore, as is evident from the above findings, the baseline titre for "O" and "H" antibodies of Salmonella Typhi was found to be 1:20 and 1:40 respectively [**Table 2**]..

In other studies from Rajasthan [**Table 3]**, baseline Anti TO titre was found to be 1:40 [11,13], but in our study, only 31% of the serum samples showed a titre of 1:40. As regards to baseline Anti TH titre, one of them found 1:80 to be the baseline titre[13], whereas in our study we found 1:40 to be the baseline titre, which is in accordance with the other study.[11]

Studies from other parts of the country [**Table 3]** showed the baseline Anti TO titre to be 1:40 at some places [14,15] and 1:80 at others.[10,12] A study done by Punia JN et al showed anti TO titre of 1:20 only in 34.1% participants.[5] The baseline Anti TH titre of 1:40 was reported in a study from Maharashtra [15] which is similar to our findings. Other studies found 1:80 to be the baseline Anti TH titre.[10,12,14] Such variations in the baseline titres may be due to the difference in endemicity of Enteric fever in different regions along with the variation in agglutinin levels among non-infected population.[6]

CONCLUSION

Widal test results in single serum sample should be interpreted depending on the prevalent baseline titre in that particular geographic area. In this study, the baseline titre for Anti-TO was 1:20 and that for Anti-TH was 1:40. So, it is recommended to consider the cut-off titre or significant titre of ≥1:40 and ≥1:80 for O agglutinin and the H agglutinin of *Salmonella* Typhi, respectively, whenever interpreting the Widal test results on a single serum sample in this region. As the baseline titre of Antibodies can vary at different places due to varying endemicity, regular studies need to be carried out at community levels at frequent intervals to effectively interpret the results of Widal test.

Though the sample size in this study was small, the results will definitely add to the existing knowledge owing to the fact that this is an establishing institute and this study was the first attempt to know the baseline titre in this region. Anti-AH and Anti-BH baseline antibody titres could not be measured, which could have added to the calculation of cut-off titre in cases of Enteric fevers due to *Salmonella* Paratyphi A and B.

REFERENCES:

1. Ananthanarayan R, Paniker C. Textbook of Microbiology. 10th ed. Kanungo R, editor. Hyderabad: Universities Press (India) Private Limited; 2017. 300-306 p.
2. WHO | Typhoid. WHO. 2015 [cited 2018 Jan 13]; Available from: http://www.who.int/immunization/diseases/typhoid/en/
3. Mackie, McCartney. Practical Medical Microbiology. 14th ed. Collee J, Fraser A, Marmion B, Simmons A, editors. New Delhi: Elsevier Inc.; 2007. 180-182 p.
4. Collier M, Balows A, Sussman M. Bacterial Infections Vol-III. In: Topley and Wilsons' Microbiology and Microbial Infections. London: Arnold Publishers; 1998. p. 459–78.
5. Punia JN, Joshi RM, Gupta V, Arora RK. Determination of baseline Widal titres from Chandigarh. Indian J Med Microbiol. 2003;21(2):144. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17643006
6. House D, Wain J, Ho VA, Diep TS, Chinh NT, Bay P V. Serology of typhoid fever in an area of endemicity and its relevance to diagnosis. J Clin Microbiol. 2001;39(3):1002–7.
7. Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ. Typhoid Fever. N Engl J Med. 2002;347(22):1770–82.
8. Wijedoru L, Mallett S, Parry CM. Rapid diagnostic tests for typhoid and paratyphoid (enteric) fever. Cochrane database Syst Rev. 2017 May 26 [cited 2018 Jan 9];5:CD008892. Available from: http://doi.wiley.com/10.1002/14651858.CD008892.pub2
9. Waddington CS, Darton TC, Pollard AJ. The challenge of enteric fever. J Infect. 2014;68(Supplement 1):S38–50.
10. Chauhan S SS. Study of baseline Widal titre amongst healthy individuals in and around Indore , India. Int J Pharm Bio Sci. 2016;7(1):152–5.
11. Saxena N, Maheshwari D DD. Baseline Widal Titres Among Apparently Healthy Individuals in Hadoti region, Rajasthan. J Evol Med Dent Sci. 2013;2(15):2425–9.
12. Vazhavandal G., Vallab Ganesh Bharadwaj B., Vasudevan K., Sarada V. UA. A Study on the Baseline Widal Titre Vazhavandal G ., Vallab Ganesh Bharadwaj B ., A Study on the Baseline Widal Titre. J Biol Chem Res. 2014;31(1):220–5.
13. Sharma S, Singh A, Parihar G VA. The-Baseline-Widal-Titre-Among-The-Healthy-Volunteers-In-Jaipur-Region-Rajasthan-India. Int J Res Rev Pharm Appl Sci. 2013;3(5):667–72.
14. Mittal G, Gupta P, Agarwal RK, Talekar M, Negi G, Gupta S. Evaluation of the baseline widal titres in healthy blood donors of Uttarakhand. Indian J Community Heal. 2014;26(3):264–7.
15. Gunjal S, Gunjal P, Patil N, Vanaparthi N, Nalawade A, Banerjee S KK. Determination of Baseline Widal Titres Amongst Apparently Healthy Blood Donors in Ahmednagar , Maharashtra, India. J Clin Diagnostic Res. 2013;7(12):2709–11.