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EVALUATION OF THE BASELINE WIDAL TITRE AMONG APPARENTLY HEALTHY INDIVIDUALS IN MEERUT, UTTAR PRADESH, INDIA

Microbiology

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ABSTRACT

Background: Enteric fever is a major public health problem in India. Widal test is a cheap, affordable and easily available and the most commonly used test for the serodiagnosis of enteric fever. **Aims:** The purpose of the present study is to evaluate the baseline titre for O and H antigens of Salmonella enterica subspecies enteric serotype Typhi and H antigens of Salmonella enteric subspecies enterica serotype Paratyphi A and Paratyphi B among normal healthy adult population in Meerut, Uttar Pradesh, India. **Material and Methods:** Blood samples were collected from healthy volunteers over the period from February 2019 to January 2020 and they were analyzed for the presence of the Salmonella antibodies by carrying out the Widal tube agglutination test. **Results:** Among the 2646 serum specimens who were tested, 1021 (38.6%) sera were found to be positive for the Widal test and 1625(61.4%) were negative. The most frequently recorded titre of the reactive sera was 1:80 for the anti-O antibodies and it was 1:40 for the anti-H antibodies and this was the baseline titre for this region. **Conclusion:** Based on the above results of our study, it has been recommended that the cut-off titre of 1:160 for the anti-O antibodies and of 1:80 for the anti-H antibodies may be considered as diagnostic for enteric fever in the Meerut region of Uttar Pradesh, India.

KEYWORDS

INTRODUCTION

Enteric fever is a systemic infection caused by the human adapted pathogens Salmonella enterica serotype Typhi(S. Typhi) and Salmonella enterica serotype Paratyphi (S.Paratyphi) A, B & C1. It continues to be a global health problem with over 21.6 million cases and at least 2,50,000 deaths occurring annually. Almost 80% of the cases and deaths are in Asia, the rest occur mainly in Africa and Latin America. In developing countries such as India, the disease occurring with an incidence ranging from 102 to 2,2190/100,000 of the population2. The diagnosis of typhoid fever on clinical grounds is difficult, as the presenting symptoms are diverse and similar to those observed with other febrile illnesses3. The Widal test, a serological test which was developed by George Fernand Isidore Widal in 1896, is an alternative to the microbial culture, which is commonly used for the diagnosis of enteric fever since its introduction 100year back4. 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. Regular updating of the baseline titer is a must for the proper interpretation and utilization of the Widal test in diagnosis of enteric fever5,6. The following study was undertaken to determine the baseline Widal titre (the titre of the antibodies to the O and the H antigens of S. typhi and to the H antigens of S. paratyphi A and B amongst the apparently healthy individuals of the Meerut region in Uttar Pradesh, India. It was also aimed to define the significant titre for the Widal agglutination test for the diagnosis of enteric fever in an endemic area in a single serum test.

MATERIALS AND METHODS

This was a community based, cross-sectional study which was conducted in the Department of Microbiology and Immunology, Mulayam Singh Yadav Medical College and Hospital, Meerut, India from February 2019 to January 2020.

Our aim was to determine the average baseline antibody titre against the Salmonella enterica serotypes among the healthy people of various age groups in the Meerut region. The study protocol and objectives were duty explained and after obtaining a written consent from the apparently healthy volunteers of both the sexes and of the age groups which ranged from 14 to 50 years, non-repetitive blood samples were collected (n= 2646).

Commercially available antigens which contained the Salmonella enterica subspecies enterica serovar Typhi O and H antigens, the Salmonella enterica subspecies enterica serovar Paratyphi AH antigen and the Paratyphi BH antigen were used (Span Diagnostics Ltd). Briefly, 0.5 ml of the 2 fold serially diluted sera (dilutions from 1:20 to 1:640) in 0.9% normal saline were tested by adding an equal amount of antigen and the tubes were than incubated overnight at 37°C in a water bath. The results were interpreted and analyzed as per the standard guidelines. A negative control was included in each batch of the tests. The Widal anti-O agglutinin (TO) and the anti-H agglutinin (TH) titres were taken as the highest dilutions of serum with a visible agglutination.

RESULTS

A total of 2624 healthy volunteers were screened for the agglutinins against the Salmonella enterica subspiecies enterica serotype Typhi, Paratyphi A and Paratyphi B by standard widal tube agglutination test. Out of 1021 (38.6%) serum samples were positive for one or more type of agglutinins ≥1:20 and 1625(61.4%)serum samples were negative for agglutinins(<1:20) (Table1).

Table 1: Results of Widal test

Widal Status	Frequency	Percentage
Positivefor (≥1:20) Agglutinin	1021	38.6%
Negative for(1:20) Agglutinin	1625	61.4%
Total Sample	2646	100%

The distribution of the samples with an antibody titre of ≥1:20 against different serotypes of Salmonella enterica subspecies enterica showed an antibody to the anti 'O' antigen in 1712(65.24%) samples, an antibody to the anti 'H' antigen in 1550(59.1%), an antibody to the anti AH antigen in 375(14.3%) samples and an antibody to the anti BH antigen in 564(21.4%) samples (Table 2).

Table 2: Distribution of the samples with antibody titer ≥1:20 against different serotypes of Salmonella enterica

Serotype	Antibody type	Frequency	Percentage
Typhi	Anti 'O' antigen	1712	65.24
Typhi	Anti 'H' antigen	1550	59.1
Paratyphi A	Anti 'H' antigen	375	14.3
Paratyphi B	Anti 'H' antigen	564	21.4

Among the 1712(65.24%) samples which showed the anti 'O' titre of ≥1:20 to the Salmonella enterica subspecies enterica serotype Typhi, 625(23.61%) samples had a titre of 1:20, 713(27.17%) samples had a titre of 1:40 and 314(11.9%) samples had a titre of 1:80.The highest titre of 1:160 was found in 60(2.3%) (Table 3).

Similarly, among the 1550 samples showing anti 'H' titres of ≥1:20 to Salmonella enterica subspecies enterica serotype Typhi, 764 (29.1%) samples were positive at titre of 1:40, 234(8.9%) had a titre of 1:80 and 127(4.8%) samples had a titre of 1:160 (Table 3).

Altogether 375(14.3%) samples showed agglutination titre of ≥1:20 against anti 'H' antigen of Salmonella enterica subspecies enterica serotype Paratyphi A. Among which 143(5.46%) had a titre of 1:40 and the rest of the 232(8.84%) samples had a titre of 1:20 (Table 3).

Among the 564(21.4%) samples with anti BH titre against Salmonella ser.Paratyphi B a titre of 1:40 was seen in 134(5.1%) samples (Table 3).

Table: 3 Number & Percentage of sera with end titres in healthy volunteers

Antigen	No. of positive samples (%)	Dilution (1:20)	Dilution (1:40)	Dilution (1:80)	Dilution (1:160)
S. typhi O	1712 (65.24)	625 (23.61)	713 (27.17)	314 (11.9)	60 (2.3)
S. typhi H	1550(59.1)	425(16.2)	764(29.1)	234(8.9)	127(4.8)
S. paratyphi AH	375(14.3)	232(8.84)	143(5.46)	–	–
S. paratyphi BH	561 (21.4)	430 (16.39)	134 (5.1)	–	–

DISCUSSION

Enteric fever afflicts the local community and the travelers to the endemic areas, the incidence being on upsurge during the rainy season due to water logging and the contamination of the water with faecal material. The social factors that add to enigma are the pollution of the drinking water supplies due to open air defaecation, urination, substandard food, personal hygiene habits and health ignorance [7,8].

The isolation of the various strains of Salmonella enterica subspecies enterica from blood remains the gold standard for the diagnosis of enteric/typhoid fever. in the modern era, there is an alarming upsurge in the empirical use of broad spectrum antibiotics, the practice of self-medication and the lack of proper timing for the specimen collection, that attributes to the reduced productivity of the blood culture technique. Also, in the developing countries, such as the Indian subcontinent, many clinics and hospitals do not have a ready access to the blood culture method, thus making the Widal tube agglutination test the most common alternative laboratory procedure for the diagnosis of enteric fever. The serological diagnosis relies classically on the demonstration of the rising titre of the antibodies in paired samples, 10 to 14 days apart. There are several difficulties associated with evaluation of the Widal test. These include high endemicity, nonavailability of paired sera for the demonstration of rising titres, poorly standardized antigens, the sharing of effects of treatment with antibiotics and previous immunisation with TAB vaccine 9,10. False-positive Widal results have been reported for patients with nonenteric fever salmonellae infections, malaria, typhus, Cryptococcus neoformans meningitis, chronic liver disease, collagenous and immunological diseases. There are more than 40 cross reacting antigens between S.typhi and other Enterobacteriaceace [11,12].

The level of titre detectable in healthy population of different area vary considerably. Frequency of antibodies in normal population reported by various workers from different parts of India ranges from 1:20 to 1:160 13. In the present study, the baseline titre for the 'O' and 'H' antibodies of Salmonella ser. Typhi was found to be 1:80. Similarly the baseline titre for the H antigen of Salmonella ser.Paratyphi A and Salmonella ser.Paratyphi B was found to be 1:[40].

The results which were obtained in the present study were in accordance with the results of previous studies which were done by Sneha AJ in Puducherry (Table 4). Studies support that reevaluation of the Widal baseline titre for healthy individuals should be done at regular intervals.

Table 4:- Comparative analysis of Baseline titre of O and H agglutinins in different regions of India

AUTHOR	PLACE	YEAR	TO	TH	AH	BH
A.J Sneha14	Puducherry	2011	1:80	1:80	1:40	1:40
Punia JM et al.,15	Chandigarh	2003	1:80	1:160	1:20	1:20
Seema Mittal et al6	Rohtak(Haryana)	2014	1:40	1:80	1:20	0
Shukla S et al.,16	Central India	1997	1:80	1:80	0	0
Present study	Meerut	2020	1:80	1:80	1:40	1:40

CONCLUSION

According to results of our study, it has been recommended that the significant titre of the 'O' and the 'H' agglutinins of Salmonella enterica subspecies enterica serotype Typhi was ≥1:160.While the significant titre of the 'H' agglutinin of Salmonella enterica subspecies enterica serotype Paratyphi A and Paratyphi B was ≥1:80 for the diagnosis of enteric fever in Meerut, Uttar Pradesh.

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