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Original Research Article

Determination of Significant Baseline Widal Titre amongst Health Personnel & General Population of Ajmer City

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ABSTRACT

Background: Enteric Fever is the most infectious disease on global scale and endemic in all parts of India. In a developing country like India laboratory diagnosis of enteric fever relies heavily on serological tests such as widal test. Knowledge of prevalent endemic titre in a region needs to be periodically updated to interpret the significance of the titre as to patient is indeed suffering from enteric fever or not. The present study has been undertaken to evaluate the baseline antibody titre for O, H antigens of *Salmonella Enterica*, serovar Typhi and AH, BH antigen of *Salmonella Enterica*, serovar Paratyphi among health personnel and general adult population of Ajmer (Rajasthan).

Aim and objectives: To evaluate the significant endemic baseline titre of Ajmer city using quantitative Widal test to suggest the cut off value for interpreting a positive widal test. To compare the endemic baseline titre amongst the Health care providers and General Population.

Material and methods: A total number of 600 healthy volunteers and 200 medical personnel working in Jawahar Lal Nehru Medical College, Ajmer were screened during period of study from 1st April 2014 to 31st December 2015. Non repetitive samples were collected from healthy adults aged 18 year to 70 year.

Results and conclusions: Out of 600 screened samples for healthy volunteers 61% were males and 39% were females. The frequency of positive results was 43.67%. The agglutinins to S. Typhi were the most prevalent among the sera of various dilutions (33.16% for 'O' antigen and 43.33% for 'H' antigen) which was tested. The levels of agglutinins for Salmonella paratyphi AH and paratyphi BH were low (only 1.16% for AH and 1.83% for BH antigen respectively).Out of 200 samples of health personnel screened 69% were males and 31% were females. The frequency of positive results was 45.5%. The agglutinins to S. Typhi were the most prevalent among the sera of various dilutions (33.16% for 'O' antigen and 44.5% for 'H' antigen) which were tested. The levels of agglutinins for Salmonella paratyphi AH and paratyphi BH were low (only 4.0% for AH and 1.5% for BH antigen respectively). Based on the above results, it has been recommended that cut-off titre of 1:80 for anti-O antibodies and of 1:120 for the anti-H antibodies may be considered as diagnostic for enteric fever in Ajmer region of Rajasthan, India.

Keywords: Enteric fever, Salmonella, Widal titre.

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INTRODUCTION

Enteric Fever is one of the most infectious diseases on a global scale and endemic in all parts of India. Typhoid fever has important socio-economic impact because, most of the time, several months are necessary for a patient to recover and be able to work again. So accurate diagnosis of typhoid fever at an early stage is important not only for etiological diagnosis, but also to identify individuals that may serve as a potential carrier, who may be responsible for acute typhoid fever outbreaks.

In India the disease is endemic with an incidence which ranges from 102 -2219 /100,000 population.[1] Hospital based studies and outbreak reports from India indicates that occurrence rate of enteric fever has risen sharply in recent years.[2] Enteric fever is endemic in developing countries like India and it continues to be one of the major public health problems.[3] Widal test is an alternative to the Microbial culture, which is commonly used for the diagnosis of enteric fever ever since its introduction 100 years back.[4]

In the endemic areas, the healthy people may contain antibodies which are capable or reacting up to a variable titre in the Widal test, due to a past exposure, TAB vaccination and cross reacting antigens. Therefore it varies widely from place to place and is referred to as the baseline titre of that area. The isolation of the organism from blood, bone marrow or stool is required to confirm the diagnosis.[5-7]

Although the isolation of Salmonella Typhi on blood culture remains the gold standard for diagnosis of typhoid fever, it is time consuming, requires elaborate laboratory equipment and a level of technical expertise which may not be present in resource-poor laboratories.

Even under the best conditions, there may be failure to isolate the organism, especially after antimicrobial treatment has been started. Culture of bone marrow is more sensitive, but the procedure is invasive, stool cultures are positive in only 30% of patients with the acute illness.[7,8]

The illness begins with mounting fever, headache, vague abdominal pain and constipation, which may be followed by appearance of rashes.[9] During the third week, the patient reaches a state of prolonged apathy, toxemia, delirium disorientation and/or coma followed by diarrhea. If left untreated, it can lead to complications affecting various organ systems.[10]

Typhoid fever is a major health problem in developing countries where safe water supplies and adequate sewage disposal are often lacking.[11] For the diagnosis of typhoid fever during first week blood culture is most reliable test, but the blood culture is expensive, time consuming, cumbersome, lack of facilities for routine culture unavailable, inhibition of growth due to improper & inadequate medication/Self-medication and minimum culture positivity rate.[12]

An optimal diagnosis of enteric fever on clinical ground alone is very difficult because of the nonspecific sign & symptoms of the disease. In a developing country like India the bacterial culture facilities are often unavailable. The Widal test continues to be the most simple and popular test in the presumptive diagnosis of typhoid fever.[13] The Widal test measures agglutinating antibodies against the lipo-polysaccharide O, flagellar proteins H antigen of Salmonella Typhi & flagellar proteins H antigen of paratyphi A & B.[13,14] In endemic countries like India, interpretation of Widal test depends upon the baseline titre which is prevalent amongst the healthy individuals in a particular geographical area.[15] The baseline titre among the healthy population of different areas differs substantially & this depends upon the endemicity of the enteric fever cases in each area which keeps on changing over time.[8] Knowledge of prevalent endemic titre in a region needs to be periodically updated to interpret the significance of the titre as to the patient is indeed suffering from enteric fever or not.[3] Antibody titre beyond the cut off value should be regarded as significantly elevated titers which may be used for diagnosis in an appropriate clinical setting.[16]

Few limitations of Widal test are; low reliability, low sensitivity & specificity even it can't distinguish between current infection/past infection / vaccination. False positive result may lead to over diagnosis / over treatment of typhoid fever, furthermore antibody titre may be high in healthy individuals in the presence of cross reacting antigens such as brucellosis, chronic liver disease, dengue fever, endocarditis, healthy carrier state, malaria and other Enterobacteriaceae infections (>40%).[17-19]

AIM AND OBJECTIVES

Our aim was to evaluate the significant endemic baseline titre of AJMER city using quantitative Widal test to suggest the cut off value for interpreting a positive Widal test. To compare the endemic baseline titre amongst the Health care providers and General Population.

MATERIAL AND METHODS

This was a community based, cross sectional study; conducted in the Department of Microbiology, Jawahar Lal Nehru Medical College and Associated Group of Hospitals, Ajmer from 1st April 2014 to 31st December 2015. The study protocol and objectives were duly explained to the volunteers of both sexes and of the respective age groups which ranged from 18-70 years, non-repetitive blood samples were collected.

The population of Ajmer city according to 2011 census was 5, 42,580 Approximately 5, 50,000. The incidence of enteric fever was 1767 (calculated from April 2013 to March 2014). A total of 600 healthy and 200 medical volunteers were screened for the period of one year i.e. from 1st April 2014 to 31st March, 2015 for the agglutinins against the Salmonella enterica, subspecies enterica serotypes, Typhi, paratyphi A and paratyphi B by the Widal Agglutination Test.

Inclusion Criteria

- They should be Residents of Ajmer since last three years.
- They should have no history of Fever since last six months.
- They should not have been immunized against enteric fever recently.

Exclusion Criteria

- Immunized for enteric fever.
- Above or below the considered age group.

We used the standard laboratory method i.e. serial dilution of tube agglutination for Widal test.

Table A: For Healthy Volunteers: Base Line Demographics of Selected Cases (N = 600)

VARIABLE		NUMBER	%
	1. AGE DISTRIBUTION		
A.	18-40 (YEAR)	395	65.83%
B.	>40 (YEAR)	205	34.17%
	2. SEX DISTRIBUTION		
A.	MALE	366	61%
B.	FEMALE	234	39%
	3. WIDAL STATUS		
A.	POSITIVE AGGLUTININS (≥1:20)	262	43.67%
B.	NEGATIVE AGGLUTININS (≤1:20)	383	56.33%
	4. DISTRIBUTION OF SAMPLES		
A.	**S.TYPHI**		
	ANTI 'O' ANTIGEN	199	33.16%
	ANTI 'H' ANTIGEN	260	43.33%
B.	**PARATYPHI A**		
	ANTI 'H' ANTIGEN	25	4.16%
C.	**PARATYPHI B**		
	ANTI 'H' ANTIGEN	11	1.83%

Table B: Results of Widal Test in Selected Cases of Healthy Volunteers

Antigen	No.of positive samples (%)	Dilution (1:20)	Dilution (1:40)	Dilution (1:60)	Dilution (1:80)
S.Typhi 'O'	199 (33.16%)	51 (8.5%)	130 (21.66%)	16 (2.66%)	2 (0.4%)
S.Typhi 'H'	260 (43.4%)	62 (10.33 %)	18 (3%)	172 (28.66%)	8 (1.4%)
S.Paratyphi AH	25 (4.2%)	17 (2.9%)	8 (1.33%)	-	-
S.Paratyphi BH	11 (1.9%)	11 (1.9%)	-	-	-

Table C: For Medical Personnel: Base Line Demographics of Selected Cases (N = 200)

VARIABLE		NUMBER	%
	1. AGE DISTRIBUTION		
A.	18-40 (YEAR)	165	82.5%
B.	>40 (YEAR)	35	17.5%
	2. SEX DISTRIBUTION		
A.	MALE	138	69%
B.	FEMALE	62	31%
	3. WIDAL STATUS		
A.	POSITIVE AGGLUTININS (≥1:20)	91	45.5%
B.	NEGATIVE AGGLUTININS (≤1:20)	109	54.5%
	4. DISTRIBUTION OF SAMPLES		
A.	**S.TYPHI**		
	ANTI 'O' ANTIGEN	68	34.0%
	ANTI 'H' ANTIGEN	89	44.5%
B.	**PARATYPHI A**		
	ANTI 'H' ANTIGEN	8	4.0%
C.	**PARATYPHI B**		
	ANTI 'H' ANTIGEN	3	1.5%

Table D: Result of Widal Test in Selected Cases for Medical Personnel

Antigen	No.of positive samples (%)	Dilution (1:20)	Dilution (1:40)	Dilution (1:60)	Dilution (1:80)
S.Typhi 'O'	68 (34%)	18 (9.0%)	45 (22.5%)	4 (2%)	1 (0.5%)
S.Typhi 'H'	89 (44.5%)	22 (11 %)	10 (5%)	54 (27%)	3 (1.5%)
S.Paratyphi AH	8 (4%)	5 (2.5%)	3 (1.5%)	-	-
S.Paratyphi BH	3 (1.5%)	3 (1.5%)	-	-	-

OBSERVATIONS

As mentioned in Table-A, 65.83% of study participants belong to age group 18-40 years and 34.17% were in age group of >40 years. Youngest volunteer was 18 years of age and oldest volunteer was 69 years of age. Out of 600 participants, 366 (61.0%) were male and 234 (39%) were females. Out of 600 healthy volunteers 262 (43.67%) samples were positive for the agglutinins (≧ 1:20) whereas 338 (56.33%) samples did not show aggluntinins (≦1:20). The agglutinins to S. Typhi were the most prevalent among the sera of various dilutions (33.16% for 'O' antigen and 43.33% for the 'H' antigen) which were tested. The levels of agglutinins for Salmonella paratyphi AH and paratyphi BH were low (only 4.16% for AH and 1.83% for BH antigen respectively).

Table-B depicts the distribution of 199 samples with the anti O titre of ≥ 1: 20 to the Salmonella enterica serotype, Typhi showed that 51 samples (8.5%) had a titre of 1: 20, 130 samples (21.66%) had a titre of 1:40 and 16 samples (2.66%) had a titre of 1:60, while only 2 samples (0.4%) had the highest titre of 1: 160. Similarly, among the 260 samples which showed the anti H titre to the Salmonella enterica serotype, Typhi 62 samples (10.33%) had a titre of 1:20, 18 samples had a titre of 1: 40, 172 samples (28.66%) had a (3.0%) titre of 1:60 and only 8 samples (1.4%) showed the highest dilution titre. Altogether, 25 samples (4.2%) showed agglutination titre against the H antigen of the Salmonella enterica serotype paratyphi A among which 17 samples (2.9%) had a titre of 1:20 rest of the 8 samples (1.33%) had a titre of 1:40. Only 11 samples (1.9%) had an anti H-titre of 1:20 for the *Salmonella Enterica* serotype, paratyphi B.

200 medical personnel were only screened in this category as the cut-off titre was nearly similar to that of the healthy population and also the test was to be conducted within a period of one year i.e. from 1st April 2014 to 31st December 2015, therefore considering time limit of study only 200 samples were screened. As mentioned in table 82.5% of patients belong to age group 18-40 years. 17.5% were in age group of ≥40 years. Youngest individual was 20 years of age and oldest individual was 65 years of age. Out of 200 cases, 138 (69%) were male and 62 (31%) were females. Out of 200 medical personnel 91 (45.5%) samples were positive for the agglutinins (≥ 1:20) whereas 109 (54.5%) samples did not show aggluntinins (≤1:20). Table-C shows the distribution of Salmonella agglutinins titre in the 91 sera of the medical personnel. The agglutinins to S. Typhi were the most prevalent among the sera of various dilutions (33.16% for 'O' antigen and 44.5% for the 'H'

antigen) which were tested. The levels of agglutinins for Salmonella paratyphi AH and paratyphi BH were low (only 4.0% for AH and 1.5% for BH antigen respectively).

Table-D depicts the distribution of 68 samples with the anti O titre of ≥ 1: 20 to the *Salmonella Enterica* serotype, Typhi showed that 18 samples (9.0%) had a titre of 1: 20, 45 samples (22.5%) had a titre of 1:40 and 4 samples (2.0%) had a titre of 1:60, while only 1 sample (0.5%) had the highest titre of 1: 160. Similarly, among the 89 samples which showed the anti-H titre to the *Salmonella Enterica* serotype, Typhi 22 samples (11.0%) had a titre of 1:20, 10 (5.0%) samples had a titre of 1: 40, 54 samples (27.0%) had a titre of 1:60 and only 3 samples (1.5%) showed the highest dilution titre. Altogether, 8 samples (4.0%) showed agglutination titre against the H antigen of the Salmonella enterica serotype paratyphi A among which 5 samples (2.5%) had a titre of 1:20 and the rest of the 3 samples (1.50%) had a titre of 1:40. Only 3 samples (1.5%) had an anti H-titre of 1:20 for the Salmonella enterica serotype, paratyphi B.

DISCUSSION

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. For practical purposes, the treatment decision must be made on the basis of the results which are obtained with a single acute phase sample. The cut off titre in a particular population depends on the background level of the typhoid antibodies and the level of the typhoid vaccination, which may vary with time.[14]

Out of total 800 serum samples, about 44.12% sample showed antibodies against Salmonella antigens with varying antibody titre. This may be due to the higher endemicity of enteric fever in Ajmer. This may also be due to the repeated subclinical infections with either of Escherichia, Shigella, Citrobacter or Proteus species which shared common O or H antigens with Salmonella spp. Variable titre with both O, H and AH or BH antigens was found, this might be due to the cross infection of *Salmonella Enterica* serotype Typhi and *Salmonella Enterica* serotype paratyphi A and /or B. Lower antibody titre against AH and BH antigens highlighted the lower endemicity of paratyphi infection compared with Typhi infection, and/or low antibody response against paratyphi infection.

This study clearly showed that in an endemic area such as Ajmer, *S. Enterica* serotype Typhi agglutinins against both H and O antigens may be present in the normal population at titers up to or greater than 1:80. Out of the total serum samples, we found O and H agglutinin titre of ≥1:120 in 10.4% and 10.6% of blood donors respectively. H agglutinin at a titre of 1:240 was found in 4.9% of blood donors. This presence of significant agglutinin titre in healthy individuals decreases the specificity of the Widal test, leading to misdiagnosis and mismanagement of the patient. In a similar type of study, 15% of the individuals had anti-O antibody titers of ≥1:80 and 16% had anti-H antibody titers of ≥1:160.

A study by Pang and Puthucheray[2] found, O and H agglutinins titers of ≥ 1:160 in 5% and 2% of non-infected individuals, they found geographical variation of both O and H Widal agglutinin titre. H agglutinin titer varied greatly compared with O agglutinin titre. The currently used cutoff value for anti-H titre against S. Enterica serotype paratyphi A in Ajmer is ≥ 1:80. Our study found that 3.7% of individuals had anti-H agglutinin titre of ≥ 1:80 to Salmonella Enterica serotype paratyphi A. Our study found that AH agglutinin titre is increasing in the population as compared with the similar study done in Nepal.[20] This may be due to an increase in general population antibody levels caused by the changing pattern of Salmonella Enterica serotype paratyphi A in the community. The seroprevalence of Salmonella enterica serotype paratyphi B was found quiet low in the whole studied population.

The percentage of samples showing agglutination with both O and H antigens at a titre of 1:80 were 8.2%, whereas only 2.7% of the total samples were positive with both O and H agglutinin at a titre of 1:160. This showed that when both O and H antigens are considered together, titre of 1:160 is sufficient to make the presumptive diagnosis of enteric fever. From the study done in *Kathmandu* by Pokhrel et al.[20] found that 12% of individuals had an anti- O titre of 1:80 and anti- H titre of 1:160. Based on this they recommended cut off levels against S. Enterica serotype Typhi to >1:80 for anti - O and >1:160 for anti- H titres for Nepal. From a similar type of study by *Zailani* et al[21] the baseline and significant titre to S. Typhi/paratyphi for both O and H antibody is 1:80 and ≥1:160 respectively in another study in Nigeria.[22] In this study we found that about 10.5% of individuals had both O and H agglutinin titre of 1:120. Based on this finding we recommend that it will be more appropriate to change currently used cut off titre levels against *S. Enterica* serotype Typhi for O (1:80) and H (1:120) titers for Ajmer.

A study in the Garhwal region[23] concludes that the current baseline titre for the diagnosis of typhoid fever is 1:40 for the anti-O agglutinins and that it is 1:80 for the anti-H agglutinins. Based on this finding, we have set our own laboratory guidelines of the H and O agglutinin Widal titres of 1:120 and 1:80 as being of diagnostic significance. The baseline anti-H agglutinin titre of the paratyphoid A and B groups was found to be 1:20 in 2.5 % and 1.3 % of the healthy population respectively, which suggested that the paratyphoid groups were less prevalent in this area as compared to S. Typhi.

Our results were in concordance with those of the studies which were reported by some researchers in other endemic states of India.[24-27] For the anti-TH antibodies, an agglutinin titre of up-to 1:60 was discovered in the apparently healthy study population, whereas for the anti-TO antibodies, our result was lower (a titre of 1:40), which was in contrast to the reports of some other workers but it was in accordance with the reports of some previous studies. Several factors may have contributed to this discrepancy, because the differences in the antibody response may be due to the poorly standardized antigen preparation and the sharing of the antigen determinants with other Salmonellae. A widespread antibiotic abuse can dampen the antibody response, giving a low titre in the Widal test and a previous immunization with the TAB vaccine and technical differences may be the other contributory factors. It has been evident from the various studies which have been conducted across our country that the baseline titre is subjective variations, depending on the geographical area and the sanitary conditions of the region. Hence, the baseline titre of a particular area should be known. The probable reason for the low

titre in our study could be the better health and hygiene conditions.

CONCLUSION

Based on the results of our study, it has been recommended that cut-off titre of 1:80 for anti-O antibodies and of 1:120 for the anti-H antibodies may be considered as diagnostic for enteric fever in Ajmer region of Rajasthan, India. So knowledge of prevalent endemic titre in a region needs to be periodically updated to interpret the significance of the titre as to the patient is indeed suffering from enteric fever or not.

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