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Suchitra Sharma, Dr. Anuradha Singh, Dr. Geeta Parihar , Aagosh Verma

Address B-343, Janta Colony, Adarsh Nagar, Jaipur, India

THE BASELINE WIDAL TITRE AMONG THE HEALTHY VOLUNTEERS IN JAIPUR REGION RAJASTHAN, INDIA.

ABSTRACT

Introduction Typhoid fever is endemic in all parts of India and the Widal test is widely used for its diagnosis. In the endemic areas, the healthy people may contain antibodies which are capable of 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 aim of this study was to determine the average baseline titre of the apparently healthy population in the Jaipur region of Rajasthan, India.

Material and Methods Blood samples were collected from healthy volunteers over the period from June 2009 to September 2009 and they were analyzed for the presence of the Salmonella antibodies by carrying out the Widal tube agglutination test.

Results Among the 2164 serum specimens which were tested, 922 (42.6%) sera were found to be positive for the Widal test and 1242 were negative. The most frequently recorded titre of the reactive sera was 1:40 for the anti-O antibodies and it was 1:80 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 1:80 for the anti-O antibodies and of 1:160 for the anti-H antibodies may be considered as diagnostic for enteric fever in the Jaipur region of Rajasthan, India.

Key Words Baseline titre, Widal agglutination test, Typhoid fever, *Salmonella*.

INTRODUCTION

Salmonella enterica subsp. enterica serotype Typhi is the etiological agent of typhoid fever. In India, the disease is endemic with an incidence which ranges from 102 to 2219 per 100,000 populations [1]. It results in considerable morbidity, absenteeism and resource utilization [2]. 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 [3]. The social factors that add to the enigma are the pollution of the drinking water supplies due to open air defecation, urination, sub-standard food, personal hygiene habits and health ignorance. The definitive diagnosis of enteric fever in the patients with a compatible clinical picture are the isolation of the Salmonellae from blood, bone marrow, stool or urine [4] and the demonstration of the 4 fold rise in the antibody titre to both the O and the H antigens of the organism between the acute and the convalescent phase sera [5]. Apart from being costly, the culture facilities are limited outside the teaching hospital and they are not employed on a routine basis. Moreover, the isolation and the identification of organism may take several days. Also, many patients engage in antibiotic self-medication, which limits the number of positive cultures which are reported. Many laboratories also use suboptimal culture methods such as an inappropriate blood to broth-medium ratio, whilst at the same time, ignoring the fact that their media could contain substances which could inactivate the anti-bacterial agents in the blood. Even when growth is obtained, the facilities for the biochemical and the serological identification of the isolates may be inadequate [5]. In these settings, the Widal test, a serological test which was developed by Georges Fernand Isidore Widal in 1896, is an alternative to the microbial culture, which is commonly used for the diagnosis of enteric fever ever since its introduction 100 years back [6]. To provide its aid in the diagnosis of typhoid fever, the Widal test utilizes a suspension of killed Salmonella Typhi as the antigen to detect typhoid fever in the serum from suspected S. typhi infected patients who present with a febrile illness [6]. 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. Updating the baseline Widal titre is mandatory for the proper interpretation of the Widal test [4-10]. Hence, 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 Jaipur region in Rajasthan state, 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 ANDMETHODS

This was a community based, cross-sectional study which was conducted in the Professor and Head of the Department of Microbiology, S.M.S. Medical College, Jaipur from June 2009 to September 2009.

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 Jaipur region. The study protocol and objectives were duly explained and after obtaining a written consent from the apparently healthy volunteers of both the sexes and of the age groups which ranged from 18 to 50 years, non-repetitive blood samples were collected (n= 2164).

The health screening was done by using a semi structured survey questionnaire. Those who were vaccinated for enteric fever in the preceding 3 years and those with a recent history of fever were excluded from the study.

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 1:320) 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 2164 healthy volunteers were screened for the agglutinins against the Salmonella enterica subspecies enterica serotypes, Typhi, Paratyphi A and Paratyphi B by the Widal tube agglutination test. 922 (42.60%) samples were positive for the agglutinins (≥1in 20) whereas 1242 (57.40%) samples did not show agglutinins (≤ 1in 20) [Table/Fig-1]. [Table/Fig-2] shows the distribution of the Salmonella agglutinin.

Widal Status	Frequency	Percentage
Positive Agglutinins(≥1:20)	922	42.60
Negative Agglutinins(≥1:20)	1242	57.40
Total Participants	2164	100%

[Table/Fig-1]: Distribution of samples for agglutination in Widal Test

Serotype	Antibody type	Frequency	Percentage(%)
Typhi	Anti O antigen	698	32.25
Typhi	Anti H antigen	922	42.6
Paratyphi A	Anti H antigen	066	3.04
Paratyphi B	Anti H antigen	029	1.3

[Table/Fig-2]: Distribution of samples with Antibody titre>1:20 against different serotypes of Salmonella enterica subspecies enterica. (Total no of samples n=2164)titre in the 922 sera of the healthy volunteers. The agglutinins to S. typhi were the most prevalent among the sera of various

dilutions(42.6% for the H antigen and 32.25% for the O antigen) which were tested. The levels of the agglutinins for Salmonella paratyphi AH and paratyphi BH were low (only 3.04% and 1.3% for the AH and the BH antigens respectively).

Antigen	No.of positive Samples(%)	Dilution (1:20)	Dilution (1:40)	Dilution (1:60)	Dilution (1:80)
S.typhi O	698 (32.25)	182 (8.4)	465 (21.5)	46 (2.1)	5 (0.2)
S.typhi H	922 (42.6)	213 (9.8)	78 (3.6)	590 (27.3)	41(1.9)
S.paratyphi AH	66 (3.04)	54 (2.5)	12 (0.6)	-	-
S.paratyphi BH	29 (1.3)	29(1.3)	-	-	-

[Table/Fig-3]: Number & percentage of sera with end titres in healthy volunteers

[Table/Fig-3] depicts that the distribution of 698 samples with the anti-O titre of ≥1:20 to the Salmonella enterica serotype, Typhi showed that 182 samples (8.4%) had a titre of 1:20, 465 samples (21.5%) had a titre of 1:40 and 46 samples (2.1%) had a titre of 1:80, while only 5 samples (0.2%) had the highest titre of 1:160.

Similarly, among the 922 samples which showed the anti-H titre of ≥1:20 to the Salmonella enterica serotype, Typhi, 213 samples (9.8%) had a titre of 1:20, 78 samples had a titre of 1:40 and 590 samples (27.3%) had a titre of 1:80. The highest titre of 1:160 was found in 41 samples (1.9%).

Altogether, 66 samples (3.04%) showed an agglutination titre of ≥1:20 against the H antigen of the Salmonella enterica serotype, Paratyphi A, among which 12 samples (0.6%) had a titre of 1:40 and the rest of the 54 samples (2.5%) had a titre of 1:20. Only 29 samples (1.3%) had an anti-H titre of 1:20 for the Salmonella enterica serotype, Paratyphi B.

DISCUSSION

The isolation of the various strains of Salmonella enterica subspecies enterica from blood remains the gold standard for the diagnosis of enteric/typhoid fever. However, 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, which 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 14days apart. In typhoid fever, however, such a rise is not always demonstrable, even in the blood culture confirmed cases. This situation may occur because of the acute phase sample which is obtained late in the natural history of the disease, because of the high levels of the background antibody in a region of endemicity or because in some individuals, the

antibody response is blunted by the early administration of an antibiotic[11]. Furthermore, the patient treatment cannot wait for long. For practical purposes, the treatment decision must be made on the basis of the results which are obtained with a single acute phase sample [12]. 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 [13].

The variation depends on the degree to which typhoid is endemic in each area, a fact which may change over time [7]. So, each country or region should have a baseline titre of their healthy population, which should be updated with time [4-10]. This was the first study which was done in the Jaipur region of Rajasthan, India, to estimate the baseline antibody titre in the healthy population against various serotypes of S. enterica by using the Widal tube agglutination test. The results of this study showed that the sera of a significant proportion of healthy individuals in this area contained antibodies which were capable of reacting to the variable titres in the Widal test. Among the 2164 samples of the healthy volunteers among the local population, 42.6 % were positive for the agglutinins against various serotypes of Salmonella enterica. The agglutinins to S. typhi were the most prevalent among the sera which were tested at various dilutions. The most frequently recorded titre of the reactive samples was found to be 1:40 and this was considered as the cut off titre, as 21.5% of the study population had provided samples which were reacting at this level. For the H agglutinins, we observed that a majority of the study population (27.3%) had a titre of 1:80 and this was taken as the cut off titre. This study concludes that the current baseline titre for the diagnosis of typhoid fever in the Jaipur region 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 1:160 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 [8,14,7]. For the anti-TH antibodies, an agglutinin titre of up to 1:80 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 other workers [8,14, 7,15] but it was in agreement with the reports of some previous studies [9,4]. 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 [7]. 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. With respect to the last point, the Widal test which was performed on the same serum specimen in four laboratories gave widely different results [4]. The countries which have enough resources to support highly developed national typhoid fever surveillance systems generally experience a low incidence of typhoid fever as compared to the developing countries and they contribute little to the global burden of typhoid fever [16,17]. Proper hygiene and sanitation are the keys to a low prevalence of enteric fever in the developed countries, which can result in a low antibody titre [3].

It has been evident from the various studies which have been conducted across our country [Table/Fig-4] that the baseline titre is subject to 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.

Several studies have highlighted the limitations of using the Widal serological test in the laboratory diagnosis of Salmonella, the worst being it's no specificity. Despite this fact, considering the low cost and the absence of comparatively cheap tests, the Widal tube agglutination test is likely to remain the test of choice in many developing countries, as of ours, provided a baseline antibody titre of healthy individual in the population, is known.

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