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Seroprevalence of Antibodies Against Salmonella Enterica in a Healthy Population

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ABSTRACT

Background: In poor nations like India, enteric fever is a severe health issue. For the purpose of diagnosing enteric fever, the Widal test is frequently utilized. In the Western Region of India, this study attempted to measure the baseline antibody titers for Salmonella typhi and paratyphi A, B in healthy persons.

Methods: A total of 150 blood samples from healthy persons were taken, and the pattern of antibody titer was determined using the conventional quantitative tube method.

Results: Among 150 blood samples, 103 had shown significant antibody titers (≥ 1:20). The significant proportion (10.7%) of the individuals had anti-O titer ≥ 1:80. Similarly, 86 had anti-H titers of ≥ 1:20 to *S. enteric* serotype *typhi*, 23 had a titer of ≥1:80 and 4 had a titer of ≥1:160 respectively. We found 10% and 1.3% for *paratyphi A* and *B*, anti-H titers of ≥ 1:20respectively.

Conclusion: The study's findings suggest that the Western Development Region of India should modify the threshold values for antibody titers against S. typhi to > 1:80 for both anti-O and anti-H titers.

INTRODUCTION

With an annual burden of almost 16 million cases worldwide, enteric fever is a serious public health issue in many developing nations, including India. Typhoid fever is caused by S. typhi and S. paratyphi serotypes A and B globally. [2,3,4] Consuming tainted food or water that contains S. typhi and/or S. paratyphi results in illness. [5] The major area where illness outbreaks occur in India is due to insufficient sanitation and hygiene. [6,7] Typhoid, which is mostly brought on by Salmonella typhi and Salmonella paratyphi A and paratyphi B, is an endemic disease in India. [8,9] Somatic (O) antigen, surface (H) antigen, and flagellar (O) antigen are the three different antigens found in S. typhi. [10,11,12] The O and H antigens serve as the foundation for numerous Widal agglutination tests. [13]

Clinical symptoms differ from person to person and might take longer in immunocompromised people. Clinical diagnosis and typhoid fever symptoms range from vague symptoms like non-severe dengue fever and malaria to asymptomatic; for this reason, the confirmative diagnosis can be made using blood and/or bone-marrow culture, which is ultimately time consuming, expensive, and labor-intensive. [14,15]

The Widal test is a serological test that is only performed in a small number of hospitals, private clinics, and primary health care facilities in India because district laboratories and lower district level health care centres lack the laboratory facilities for blood culture for the confirmation of typhoid fever. [12,16,] The Widal test results are used to make the diagnosis. [17]

Only one research, which looked at the pattern of antibody titer against Salmonella enterica over the entire nation19, was undertaken addressing the assessment of baseline Widal titer in the Kathmandu valley. Furthermore, this is the first investigation of the pattern of Widal reactivity in supposedly healthy people to be done outside the Kathmandu Valley. The hospital laboratories and healthcare facilities have not, to now, complied with the reference cut off value, which is not considered to be a standard. Our study's objective is to identify the Widal agglutination reaction pattern in a sample of seemingly healthy individuals in India's western development zone.

METHOD

Setting and Design

In Gujarat, Western India, a tertiary care hospital's department of microbiology carried out prospective cross-sectional research in 2021. Random volunteers were chosen from among the families of the hospital visitors who appeared to be in good health. All participants gave their agreement after being given questionnaires to complete the study.

All the blood donors appeared to be in good health and were eligible for this study once the physical examination was completed. This research had 150 participants in all, ranging in age from 17 to 64. In this study, physiological saline from Nirlife, India, and the Widal Antigen set, which includes ready-to-use concentrated and smooth antigen suspensions of the following bacilli: S typhi O, S typhi H, S paratyphi AH, S paratyphi BH, Poly specific positive control by Tulip diagnostics (Pvt) LTD., India, were both used.

Sample collection

The 2.0 ml of whole blood from each blood bag's tubes that had not already been diluted by the CPDA-1 (Citrate Phosphate Dextrose Adenine) in the blood bags was taken. The samples were subsequently brought to the lab in sterile condition for further processing within 1-2 hours. The serum was immediately separated, put in a cryovial with a code number on it, and kept at -20°C for later processing.

Assay Procedures

Appropriate number of sets of 8 test tubes with antigen suspension was obtained and tube numbers were labelled from 1-8. In the first tube of each set, 1.9 ml of normal saline was added, and 1 ml was added to the remaining tubes. First tubes received 0.1 ml of sample serum, which was well mixed in. Then 1 cc of thoroughly mixed serum solution was transferred from tube 1 to tube 2, then from tube 2 to tube 3, and so on until tube 7. Each set's tube 7 was used to dispense 0.1 ml of the mixed serum solution to create the following dilutions: 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, and 1:1280. [1]

All of the sets utilized tube no. 8 as a saline control. The tube was well mixed after the TYDAL antigen suspension had been added to the appropriate antigen. The agglutination was seen when all test tubes were covered and incubated at 37°c for 18 hours. [2]

Interpretation of the observation

By looking at the test tubes for "O" antigen and "H" antigen for bigger, looser, flocculent agglutination, the findings were evaluated. The test's outcomes were graded as follows: There are four possible outcomes: 0, 1 + (25 percent agglutination), 2, 50 percent agglutination, 3, and 4+ (75 percent agglutination) (100 percent agglutination). [2] There must be at least 50% agglutination in the visualization and test for it to be considered positive. After that, the screening of positive tests was further diluted to detect antibodies at dilutions of 1:40, 1:80, 1:100, 1:160, and 1:320. The observation of 2+ or 50% agglutination served as the final marker for the serum antibody titer end point. [2]

Quality Control of the Test

A variety of standard operating procedures were used to preserve the quality of each test. Positive controls using the polyspecific positive antigen and negative controls using saline were preserved for each pair of sera. To keep dust out of the mixture, cotton plugs were placed on top of each test tube. Every day, the water bath's temperature was checked. Prior to usage, all test tubes underwent sterilization. After spending the night in the disinfectant, the micropipettes tubes were cleaned and reused. Every day, each lot of reagents had its expiration date, colour, and clumping verified. On the work surface, disinfectants were used, and sterile conditions were maintained during sample processing. As a result, aseptic conditions were preserved during the course of all treatments.

Statistical Analysis : Microsoft Excel 2007 and the Statistical Package for Social Science (SPSS) version 16.0 (SPSS Inc., Chicago, IL, USA) were used for all statistical analyses. The descriptive statistics were conducted using SPSS version 16 software. The findings were presented using pie charts, bar graphs, tables, etc.

RESULTS

The bulk of the population was between the ages of 21 and 30. Only 24 blood samples from women were taken since so few women provided blood. Table 1 displays the subjects' age and sex distributions.

On the isolated serum, the antibody titers against several Salmonella enterica serotypes were measured. 150 samples were examined, and 103 (68.7%) of those samples exhibited agglutination for the O or H antibodies against Salmonella enterica serotypes Typhi, Paratyphi A, or Paratyphi B in a titration of less than one minute. The remaining 47 samples were deemed negative since they did not exhibit any agglutination.

H antibody was the most often positive, followed by O antibody, paratyphi AH antibody, and only 2 samples

were positive for S. paratyphi BH antibody. Among the normal healthy individuals, there was a higher prevalence of H antibody titer compared to O antibody titer (Table 2).

Twenty samples (13.3%) out of a total of 70 samples (1.20%) had an end titer of 1:40. Additionally, end titers of 1:80 were discovered in 9 samples (6 percent), which is a large part of the population (Table 3).

Table 1: Profile of study participants

Profile	Participants (%)
Age group	
<20 year	12 (8%)
20-30 years	80 (53.33%)
30-40 years	33 (22%)
40-50 years	21 (14%)
>50 years	4 (2.67%)
Gender	
Male	126 (84%)
Female	24 (16%)
Proportion of Antibody titer	
<1:20	46 (30.67%)
≥1:20	104 (69.33%)

Table 2: Distribution of *Salmonella* agglutinin titers of ≥ 1:20 in apparently normal individuals

Salmonella antigen	Cases (n=150) (%)
S. typhi O	70 (46.67%)
S. typhi H	86 (57.33%)
S. paratyphi AH	15 (10%)
S. paratyphi BH	2 (1.33%)

Table 3: Pattern of titer (≥1:20) in various Serotype

Titer (≥1:20) in various Serotype	Cases (%)
Anti-O against *S. Typhi*	
1:20	34 (48.57%)
1:40	20 (28.57%)
1:80	9 (12.86%)
1:100	3 (4.29%)
1:160	3 (4.29%)
1:320	1 (1.43%)
Anti-H against *S. Typhi*	
1:20	25 (29.07%)
1:40	38 (44.19%)
1:80	13 (15.12%)
1:100	6 (6.98%)
1:160	3 (3.49%)
1:320	1 (1.16%)
Anti 'H' against S. *Paratyphi A*	
1:20	3 (20%)
1:40	10 (66.67%)
1:80	2 (13.33%)
Anti 'H' against *S. Paratyphi B*	
1:20	1 (50%)
1:40	1 (50%)

DISCUSSION

A substantial proportion of 10 (6.6 percent) of the 15 samples that tested positive for the AH antibody (1:20) had an end titer of 1:40, and 3 samples (2 percent) had an end titer of 1:20. Additionally, it was discovered that 2 samples (1.3%) had an end titer of 1:80. (Table 3).

Major epidemics of typhoid have occurred in India, despite the fact that the entire nation of India is classified as an endemic location for the disease. Major outbreaks are caused by sewage pipeline breaks and the resulting faecal pollution of the water supply. The majority of India's rural communities lack such close contact between drinking water supply and sewage pipelines, rendering them less vulnerable to typhoid epidemics. The Widal agglutination reaction pattern was examined in this study for the first time in Western India in order to determine the average baseline antibody titer in healthy persons against different serotypes of S. enterica.

We discovered that healthy people in this area have a wide range of antibody titers against Salmonella enterica. A considerable percentage of people had high antibody titers , which forced authorities to reconsider the diagnostic titer's current cut-off levels. [9]

Our research also reveals that a sizable number of healthy people have some degree of agglutination titer. It was discovered that against Salmonella enterica serotype typhi, 10.7% of these samples exhibited anti-O antibody titers of 1:80 and 15.3% had anti-H antibody titers of 1:80. Additionally, the anti-AH antibody titers in 8.0 percent of the total samples were less than 1:40, and the anti-BH antibody titers were 1.3 percent. For both O and H agglutination, the diagnostic baseline titer of the Widal agglutination test for typhoid fever in India is 1:80. [9] In contrast, we discovered higher levels of Salmonella typhi agglutinins in healthy people than those who were utilised to diagnose typhoid illness in India. As a result, the findings of this study recommend raising the cutoff values for anti-O and anti-H to more than 1:80 for a likely diagnosis of typhoid fever.

Similar findings were made in Vietnam, India, and Kathmandu. [9,18] Nevertheless, Pokhrel et al. (2009) discovered that only 2.7 percent of patients had H antibody titers that were less than 1:160 and 12 percent of cases had anti-O titers that were larger than 1:80. This discrepancy might be the result of low standard antigen preparation, variations in titer levels in endemic areas, and technical and manual differences. [19] When non-typhoid antigen specific antibody interacts with typhoid specific antigen, cross reactions may also occur. Numerous other illnesses (military TB, chronic liver disease, endocarditis, brucellosis, malaria, and dengue) brought on by non-Salmonella species can also contribute to cross reactivity, which can lead to inaccurate Widal test findings. [17] We discovered that the antibody titer may differ between endemic locations. In addition, regardless of immune status, higher salmonella antibody titer in this group may be explained by life style and oth-

er social factors, such as limited access to clean drinking water. Baseline titer may also vary in healthy populations of different regions, and it should be updated over time.

CONCLUSION

The Widal agglutination test used in this investigation demonstrated that Salmonella enterica agglutinins are present in seemingly healthy individuals at various levels. In the Western Region of India, we discovered that the sole Widal test for the diagnosis of typhoid fever had no diagnostic value. Additionally, it shouldn't be utilized as a screening test for those who are asymptomatic. The findings of this study also recommend increasing the existing cutoff limits of antibody titer against S. enterica serotype typhi for the Western Development Region of India to > 1:80 for both anti-O and anti-H titers in order to reduce the likelihood of erroneous diagnoses of enteric fever. To comprehend the long-term effects of titer against Typhoid antibodies in various regions of India, more research is required.

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