Original Research Article

Study to determine the distribution pattern of agglutinating antibodies to Salmonella enterica serotype: typhi, paratyphi A and paratyphi B antigens in normal healthy individuals and individuals with afebrile illness in pediatric population

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Abstract

Background: Enteric fever continues to be a major health problem in developing countries including India. The serological test, Widal test, is a well known test, used as an indirect test to detect the serological evidences of presence of Salmonella groups. 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) in normal healthy individuals and individuals with afebrile illness in paediatric population coming to our hospital (both IPD and OPD). **Materials & Methods**: The Widal test was performed with serial serum dilutions: 1:20, 1:40, 1:80, 1:160 and 1:320 by using isotonic normal saline. 0.5ml of each of the antigen suspension was added to corresponding tubes, mixed well and incubated at 37^{C} for 24 hours. The tubes were then examined visually for agglutination. A 50% agglutination is considered and recorded as the titre of antibodies present in the individual against Salmonella. Results: Positive agglutination in Widal test was observed to be lowest among under-fives as compared to older children (46% vs 62%), but the results were not significant (p=0.06). No significant association was observed between Positive agglutination in Widal test and gender (p=0.31). **Conclusion**: It is clear that Salmonella agglutinins are common among apparently healthy people and as endemicity of typhoid in an area may change over time, more studies should be carried out to determine Salmonella agglutinin titre in apparently healthy populations, so that a better judgment which is based on the prevailing agglutinin titres can be made.

Keywords: Agglutination, Salmonellosis, Typhoid fever, Salmonella typhi, Paratyphi A, Paratyphi .

Introduction

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Salmonella enterica subsp. enterica serotype typhi is the aetiological agent of typhoid fever. In India, disease is endemic with an incidence which ranges from 102 to 2219 per 100,000 populations [1]. It results in considerable morbidity, mortality, absenteeism and resource utilization [2]. 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 [3, 4] and the demonstration of the four 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. Antibody titer may be high in healthy individuals in the presence of cross reacting antigens, such as malaria, brucellosis, dengue fever, healthy carrier state, chronic liver disease, endocarditis or other enterobacteriaceae infections [6]. There are more than 40 cross-reacting antigens between *S. typhi* and other enterobacteriaceae [7]. Persons who had past enteric infection or vaccinated with the old typhoid vaccine (TAB) may develop transient anamnestic reaction during an unrelated febrile illnesses, such as malaria [8]. Epidemiology of cross-reacting antigens determines the baseline titer of Widal test as antibody produced in these diseases may cross-react with Salmonella antigens. Therefore, a four-fold rise in antibody titers between acute and convalescent phases is considered as a significant change in a given person. Since this type of comparison is not practically helpful in establishing diagnosis of an acute illness, a single cutoff value is widely used. In a given population, interpretation of a single Widal test result needs to be based on average baseline titer among the healthy individuals. Antibody titers beyond a cut off value should be regarded as significantly elevated titers which may be used for diagnosis in an appropriate clinical setting.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 [8-12]. 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) in normal healthy individuals and individuals with afebrile illness in paediatric population coming to our hospital (both IPD and OPD).

Material and methods

A hospital based prospective observational study at Department of Paediatrics, Dr. Babasaheb Ambedkar Memorial Central Railway Hospital, Byculla, Mumbai between June 2014 to May 2016. Consecutive type of non-probability sampling was used for selection of study subjects. A total of 300 children satisfying the eligibility criteria were taken in the study after informed consent from their parents. Patil AM et al. [9] conducted cross sectional study in Devengere on 250 healthy children to determine baseline widal titre in year 2007. Gunjal SP et al. [13] conducted cross sectional study in Ahmednagar to determine baseline widal titre on 103 healthy individuals from 2011 to 2013. Ibekwe AC, et al. [14] studied 200 normal healthy population in Nigeria in 2008.

Inclusion criteria

1. Children with afebrile illness coming to the hospital
2. Apparently healthy children.
3. Age 1-12 years of age

Exclusion criteria

1. Persistent fever > 7 days in past 6 months
2. Febrile patients
3. Children with grade iii, iv, malnutrition (IAP)
4. Children with immunocompromised status
5. Vaccinated with any available typhoid vaccine

A detailed history was taken from each participant with special reference to source of water and method used for purification. Widal titres were done with tube agglutination method for all the patients. A widal antigen kit (Antigen suspension of Salmonella enterica serotype typhi, paratyphi A and B) was used. Venous blood sample was collected from each participant, left to clot for 15 minutes in the room temperature then Sera was separated by using micropipette. The separated sera was properly labelled and stored in -20^{C} for further study.The Widal test was performed with serial serum dilutions: 1:20, 1:40, 1:80, 1:160 and 1:320 by using isotonic normal saline. 0.5ml of each of the antigen suspension was added to corresponding tubes, mixed well and incubated at 37^{C} for 24 hours. The tubes were then examined visually for agglutination. A 50% agglutination is considered and recorded as the titre of antibodies present in the individual against Salmonella. Routine care and

treatment was given to all the afebrile patients as per respective conditions.

Statistical Analysis

All the collected data was entered in Microsoft Excel sheet and then transferred to SPSS software ver. 21 for analysis. Qualitative data was presented as frequency and percentages and analyzed using chi-square test of fisher's exact test (in case of 2x2 contingency tables). Quantitative data was presented as mean and SD. P-value < 0.05 was taken as level of significance.

Results

Table 1: Age distribution among study subjects

Age group (yrs)	N	%
</= 5	63	21.0%
6 to 10	145	48.3%
> 10	92	30.7%
Total	300	100.0%

Most of the study subjects were between 6-10 years of age with mean age of 7.8 +/- 3.2 years [Table 1].

Table 2: Distribution of subjects based on gender

Gender	N	%
Male	201	67.0%
Female	99	33.0%
Total	300	100.0%

In our study, numbers of males were more than females with male to female ratio of 2:1 (67% males vs 33% females) [Table 2].

Table 3: Distribution of subjects based on for agglutination in Widal test

Widal Status	N	%
Agglutination (>/ = 1:20)	177	59.0%
No agglutination (< 1:20)	123	41.0%
Total	300	100.0%

Agglutination in Widal test with titre ≥ 1:20 was seen in 59% of the healthy subjects [Table 3].

Table 4: Association between agglutination in Widal test and age

Age Group	**Widal**		**Total**
	Agglutination (>/ = 1:20)	**No Agglutination (< 1:20)**	
</= 5	29	34	63
	46.0%	54.0%	100.0%
6 - 10	91	54	145
	62.8%	37.2%	100.0%
> 10	57	35	92
	62.0%	38.0%	100.0%
Total	177	123	300
	59.0%	41.0%	100.0%
p- value = 0.062			

Positive agglutination in Widal test was observed to be lowest among under-fives as compared to older children (46% vs 62%), but the results were not significant (p=0.06) [Table 4/Fig.1]. The results can be attributed to the relatively lower frequency of eating outside food by under-fives, which makes one prone to salmonella infection.

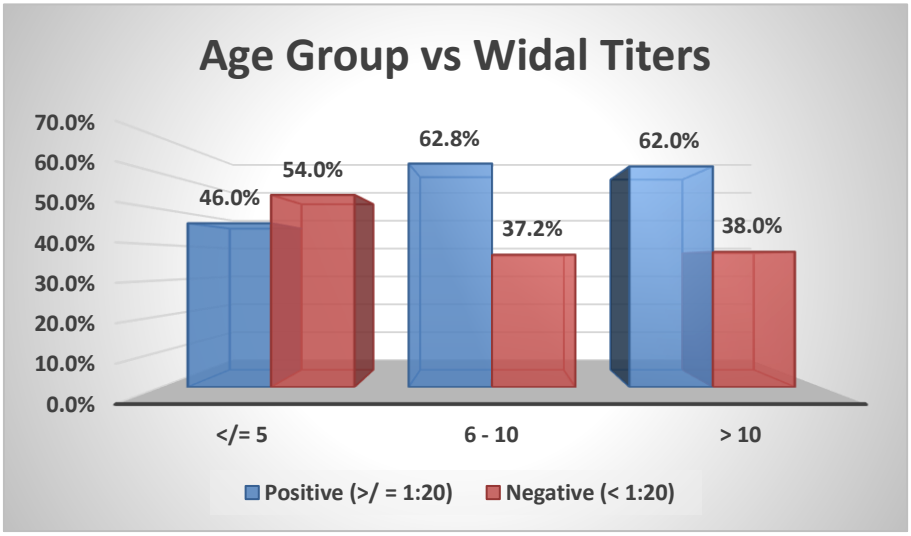

Fig 1: Association between agglutination in Widal Test and Age

Table 5: Association between agglutination in Widal test and gender

Gender	Widal		Total
	Positive Agglutination (>/ = 1:20)	Negative Agglutination (< 1:20)	
Male	123	78	201
	61.2%	38.8%	100%
Female	54	45	99
	54.5%	45.5%	100%
Total	177	123	300
	59.0%	41.0%	100%
p- value=0.31			

No significant association was observed between Positive agglutination in Widal test and gender (p=0.31) [Table 5].

Table 6: Distribution of samples with antibody titre>1:20 against different serotypes of Salmonella enterica subspecies enterica (n-300)

Serotype	Antibody	N	%
S. Typhi	O	109	36.3%
S. Typhi	H	134	44.7%
S. Paratyphi A	H	13	4.3%
S. Paratyphi B	H	6	2.0%

The agglutinins to S. typhi were the most prevalent among the sera of various dilutions (44.7% for the H antigen and 36.3% for the O antigen) which were tested. The levels of the agglutinins for Salmonella paratyphi AH and paratyphi BH were low (only 4.3% and 2% for the AH and the BH antigens respectively) [Table 6].

Discussion

A hospital based prospective observational study was conducted with the aim of determining the distribution pattern of agglutinating antibodies to Salmonella enterica serotype: typhi, paratyphi A and paratyphi B antigens in normal healthy individuals and individuals with afebrile illness in pediatric population coming to our hospital. Most of the study subjects were between 6- 10 years of age with mean age of 7.8 +/- 3.2 years. In our study, numbers of males were more than females with male to female ratio of 2:1 (67% male vs 33% females). Positive agglutination in Widal test was observed to be lowest among under-fives as compared to older children (46% vs 62%), but the results were not significant (p=0.06). The results can be attributed to the relatively lower frequency of eating outside food by under-fives, which makes one prone to salmonella infection. While, no significant association was observed between Positive agglutination in Widal test and gender (p-0.31). A significant association was observed between Positive agglutination in Widal test and consumption of tap water supplied from municipal sources and with lower social-economic class. Enteric fever has continued to pose serious challenge to public health especially in economically developing and under developed countries, where level of hygiene is below standards and sanitary conditions are poor [15,16]. Ajayi et al in their study observed the influence of water source on typhoid fever prevalence in the study areas. The study revealed that patients who sourced their water from well had the highest frequency (76%) of typhoid fever while the least frequency (3%) was obtained from patients using treated water [17]. Similarly in another study by Udeze et al., significant titre of antibody to salmonella was detected among subjects who drank sachet water while the least was detected in subjects drinking treated water [18].

Widal tube agglutination test

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 [19]. The serological diagnosis relies classically on the demonstration of the rising titre of the antibodies in paired samples, 10 to 14 days 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 [20]. 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 [21]. 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 [22]. The variation depends on the degree to which typhoid is endemic in each area, a fact which may change over time [9]. So, each country or region should have a baseline titre of their healthy population, which should be updated with time [3, 9, 19-22]. Thus present study was done in the Central Mumbai, 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.

Positive agglutination

Among the 300 samples of the normal healthy individuals and individuals with afebrile illness in pediatric population, 59% were positive for the agglutinins against various serotypes of Salmonella enterica. Our findings were in accordance with those which were reported by other investigators. A comparative analysis of samples which were positive for agglutinins for *Salmonella* serotypes has been presented below:

Table 7: Comparison of different studies with present study

Author	Positive samples
Achary et al., [10]	50.6%
Shekhar Pal et al., [19]	42.6%
Prashant Peshattiwar [11]	55.1%
Shukla S et al., [23]	62.0%
Gunjal et al. [13]	81.5%
Kogekar et al. [24]	49.7%
Present study	59.0%

Conclusion

Most of the study subjects were between 6- 10 years of age with mean age of 7.8 +/- 3.2 years. More males were observed among study subjects with male to female ratio of 2:1 (67% males vs 33% females). Positive agglutination in Widal test i.e titre ≥ 1:20 was seen in 59% of the healthy subjects. Positive agglutination in Widal test was observed to be lowest among under-fives as compared to older children (46% vs 62%), but the results were not significant (p=0.06). No significant association was observed between Positive agglutination in Widal test and gender (p=0.31). The agglutinins to S. typhi were the most prevalent among the sera of various dilutions (44.7% for the H antigen and 36.3% for the O antigen) which were tested. The levels of the agglutinins for Salmonella paratyphi AH and paratyphi BH were low (only 4.3% and 2% for the AH and the BH antigens respectively).The baseline titre for antibodies to "O" and "H" antigens of Salmonella enterica serotype typhi was 1:40 and 1:80. Hence, based on the above results, it could be recommended to use a cut-off level of 1:80 for the S. Typhi anti-O antibodies and of 1:160 for the anti-H antibodies as diagnostic for enteric fever in the study area. Similarly, baseline titre for antibody to H antigen of Salmonella enteric serotype paratyphi A and paratyphi B was 1:20 and the cut-off level was ≥1:40 for a single antibody test titre. It is clear that Salmonella agglutinins are common among apparently healthy people and as endemicity of typhoid in an area may change over time, more studies should be carried out to determine Salmonella agglutinin titre in apparently healthy populations, so that a better judgment which is based on the prevailing agglutinin titres can be made.

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