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RESEARCH ARTICLE

A STUDY ON THE BASELINE OF WIDAL TITER OF THE APPARENTLY HEALTHY STUDENTS OF A PRIVATE MEDICAL COLLEGE OF DHAKA CITY

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ABSTRACT

Typhoid is endemic in almost all parts of Bangladesh. Widal test is one of the easily available tools in many areas of the developing countries for the diagnosis of typhoid fever. The interpretation of Widal test depends upon the baseline titer which is prevalent amongst healthy individuals in a particular geographical area. In the endemic areas, the healthy people may contain antibodies which are capable of reacting up to a variable titer in the Widal test due to a past exposure, vaccination and cross reacting antigens. Therefore, it varies widely from place to place and is referred to as the baseline titer of that area. The objectives of this study were to determine the average baseline titer of the apparently healthy students of a private medical college of Dhaka and to find out the correlation among age; sex and the history of typhoid fever, antibiotics and vaccines against typhoid in regard to the Widal titers. The blood samples were collected from 100 apparently healthy students over the period of five months. After collecting the blood, sera were separated and analyzed for the presence of the Salmonella antibodies using the Widal tube agglutination test. Among the 100 serum specimens which were tested, 59 were positive for TO from 1:20 to 1:80 and 27 were positive for TH from 1:20 to 1:80. Based on the above results of the study, it has been revealed that the cut-off titers of 1:80 for the TO and of 1:80 for the TH may be considered as diagnostic for typhoid fever in Dhaka, Bangladesh. Besides, the study showed that there is no correlation among the age; sex and the history of typhoid fever, antibiotics and vaccines against typhoid in regard to Widal titers.

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INTRODUCTION

Typhoid fever still continues to be one of the major public health problems particularly in developing countries. It remains a global health problem due to *Salmonella typhi*. Typhoid fever is a severe multisystemic illness which is characterized by the classic prolonged fever, sustained bacteremia without endothelial or endocardial involvement and bacterial invasion of and multiplication within the mononuclear phagocytic cells of the liver, spleen, lymph nodes and Peyer's patches (Brusch, 2016).

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Typhoid fever is potentially fatal when it is untreated. It is caused by *Salmonella typhia* Gram negative bacilli (Kidgell *et al.,* 2002). Typhoid fever is considered as a global health problem. Incidence of typhoid fever has been estimated as approximately 22 million cases with at least 200,000 deaths occurring worldwide annually (Crump *et al.,* 2004). The disease is endemic in the Indian Subcontinent including Bangladesh. A recent epidemiologic study showed that South-East and South-Central Asia are the regions of highest endemicity with rates greater than 100/100,000 cases per year (Crump *et al.,* 2004). In Bangladesh, the overall incidence of typhoid fever is 390 cases per 100,000 population per year. The incidence among people greater than 5 years of age is 210 per 100,000 population per year and among children less than 5 years of age, the rate is 1870 per 100,000 children per year

that is 8.9 times greater when compared with all other age groups (Brooks *et al.,* 2001). In the wake of emerging multidrug-resistant strains of bacteria causing typhoid fever, the disorder is known to be associated with significant morbidity and mortality (Rahman *et al.,* 2006; Bhutta, 1996). It is also recognized that a delay in diagnosis and administration of appropriate therapy may significantly increase the risk of adverse outcome and mortality (Bhutta, 1996). Therefore, an accurate diagnosis of typhoid fever at anearly stage is important not only for aetiological diagnosis, but also to identify individuals that may serve as a potential carrier, who may be responsible for acute typhoid fever outbreaks (Parker, 1990).Despite improved methods of bacteriologic isolation, there is a real need for rapid serologic diagnostic tests for typhoid fever (Bhutta and Mansurali, 1999). The Widal test has been used for almost 100 years. It is widely available in the developing countries and is still regarded as a useful test in endemic areas (Pang and Puthucheary, 1983). In Bangladesh, the diagnosis of typhoid fever is still based on clinical presentation. Here facilities for isolation and culture are often not available especially in smaller hospitals, and diagnosis relies upon the clinical features of the disease and the detection of agglutinating antibodies to *S. typhi* and *S. paratyphi* by the Widal test (Shahidulalam *et al.,* 2011). In endemic country like Bangladesh, sera of proportion of healthy individuals contain antibodies capable of reacting to variable titer in Widal test. The knowledge of local baseline titer in healthy individuals will definitely help for the appropriate interpretation of Widal test. Literature reveals that no such research work has been conducted yet so far. Therefore, the present research work was undertaken with the following objectives:

- To determine the average baseline titer of the apparently selected healthy students of a private medical college of Dhaka city.
- To find out the correlation among age; sex and the history of typhoid fever, antibiotics and vaccines against typhoid on the basis of the Widal titers.

METHODS

A cross-sectional study was conducted in the Department of Microbiology, Ibrahim Medical College, situated at Shahbag in Dhaka city. The study was conducted from September 2016 to January 2017. The apparently healthy students of Ibrahim Medical College were the population of this study. Total 100 apparently healthy medical students were selected for this study. The health screening was done by using a semi structured survey questionnaire. Those who suffered from enteric fever during last one month or were vaccinated for enteric fever in last one month were excluded from the study. Blood was collected from those 100 apparently healthy students and the serum was separated from the blood. Commercially available antigens which contained the *Salmonella enterica subspecies enteric* serovar Typhi O and H antigens, the *Salmonella enterica subspecies enteric* serovar *Paratyphi* AH antigen and the *Paratyphi* BH antigen were used in this study. For a single widal test, twenty test tubes were arranged in four rows for four antigens. Each row has five tubes and the terminal tube of each row is for normal control. At first 1.9 ml normal saline was added to the first tube. Then 1 ml normal saline was added to second, third, fourth and the fifth tube (normal control) each. Then 0.1 µl serum was added to the first tube and serial dilution was carried out except the tube of normal control. One drop of antigen was added to the five tubes each and the tubes are incubated at 37^{O}C for exact 24 hours. 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 100 sera were tested. Among these, 90 (90%) showed agglutinations at a titre of ≥1:20 for TO, TH, AH and BH. The highest frequency of TO titer (25) was found in both 1:40 and 1: 80 dilutions. The highest frequency of TH (17) was found in 1:40 dilution, the highest frequency of AH (1) was found in 1:40 dilution and the highest frequency of BH (2) was also found in 1:40 dilution (Table-1). There in not any correlation between age and Widal titers of the respondents (Table-2 & 3). No statistically significant difference was found between male and female respondents when the frequency of the Widal titers was distributed according to the sex of the respondents (Table-4 & 5).

Table 1. Frequency distribution and the percentage of the antibody titers against the antigens of *S. typhi* and *S. paratyphi* in the respondents

Antigens	Frequencies and percentages of the dilutions		
	Dilution (1:20)	Dilution (1:40)	Dilution (1:80)
*S. typhi*O	9 (9.0)	25 (25.0)	25 (25.0)
*S. typhi*H	6 (6.0)	17 (17.0)	4 (4.0)
*S. paratyphi*AH	0 (0.0)	1 (1.0)	0 (0.0)
*S. paratyphi*BH	1 (1.0)	2 (2.0)	0 (0.0)

Table 2. Frequency distribution of antibody titers against*Salmonella typhi* antigen TO according to the age of the respondents

Age Group (Year)	*Salmonella typhi* antigen TO				P value
	0	1:20	1:40	1:80	
21-22	19	8	14	15	0.129
23-24	22	1	11	10	

Table 3. Frequency distribution of antibody titers against*Salmonella typhi* antigen TH according to the age of the respondents

Age Group (Year)	*Salmonella typhi* antigen TH				P value
	0	1:20	1:40	1:80	
21-22	37	4	11	4	0.189
23-24	36	2	6	0	

Table 4. Frequency distribution of antibody titers against*Salmonella typhi* antigen TO based on the sex of the respondents

Sex	*Salmonella typhi* antigen TO				P value
	0	1:20	1:40	1:80	
Male	22	4	10	7	0.231
Female	19	5	15	18	

Table 5. Frequency distribution of antibody titers against*Salmonella typhi* antigen TH based on the sex of the respondents

Sex	*Salmonella typhi* antigen TH				P value
	0	1:20	1:40	1:80	
Male	34	3	4	1	0.511
Female	39	3	12	3	

In our current study the past history of typhoid fever had not any effect on the Widal titers (Table-6 & 7). No correlation was found between the history of antibiotic against typhoid

and the Widal titers (Table-8 & 9) and between the history of vaccines against typhoid and the Widal titers (Table-10 & 11).

Table 6. Frequency distribution of antibody titers against*Salmonella typhi* antigen TO based on the history of typhoid of the respondents

History of Typhoid	*Salmonella typhi* antigen TO				P value
	0	1:20	1:40	1:80	
Suffered	11	2	5	10	0.431
Did not suffer	30	7	20	15	

Table 7. Frequency distribution of antibody titers against*Salmonella typhi* antigen TH based on the history of typhoid of the respondents

History of Typhoid	*Salmonella typhi* antigen TH				P value
	0	1:20	1:40	1:80	
Suffered	19	3	4	2	0.438
Did not suffer	54	3	13	2	

Table 8. Frequency distribution of antibody titers against*Salmonella typhi* antigen TO based on the history of antibiotic against typhoid of the respondents

Antibiotic against typhoid	*Salmonella typhi* antigen TO				P value
	0	1:20	1:40	1:80	
Used	4	0	1	4	0.365
Did use	37	9	24	21	

Table 9. Frequency distribution of antibody titers against*Salmonella typhi* antigen TH based on the history of antibiotic against typhoid of the respondents

Antibiotic against typhoid	*Salmonella typhi* antigen TH				P value
	0	1:20	1:40	1:80	
Used	6	1	1	1	0.586
Did not used	67	5	16	3	

Table 10. Frequency distribution of antibody titers against*Salmonella typhi* antigen TO according to the history of vaccines against typhoid of the respondents

Vaccine against typhoid	*Salmonella typhi* antigen TO				P value
	0	1:20	1:40	1:80	
Vaccinated	4	0	4	5	0.390
Not vaccinates	37	9	21	20	

Table 11. Frequency distribution of antibody titers against*Salmonella typhi* antigen TH according to the history of vaccines against typhoid of the respondents

Vaccine against typhoid	*Salmonella typhi* antigen TH				P value
	0	1:20	1:40	1:80	
Vaccinated	11	0	2	0	0.616
Not vaccinated	62	6	15	4	

DISCUSSION

The results of our current study are almost similar to the results of some other similar studies like – a cross-sectional study conducted by Shraddha Prasad Gunjal and his co-workers during 2012-13 in Maharashtra, India (Gunjal *et al.,* 2013) and a community based study carried out by Gufran Ahmed and his co-workers from March 2012 to June 2012 at Kannur Medical College (Gufran *et al.,* 2014). But there are variations of the frequencies of the titers of our current study and the frequencies of the titers of the similar studies carried out by other investigators like – A community based, cross-sectional study was conducted by Shekhar Pal and his co-workers from February 2011 to January 2012 in Uttarakhand, India (Pal *et al.,* 2012). These variations in the frequencies could be small sample size and limited time duration.It was observed that there are some variations in the results regarding the frequencies of the titers of our present study compared to the results regarding the frequencies of the titers frequencies of the author's studies based on the age groups, for example - a cross-sectional study, conducted by Saleel V Maulingkar and his co-workers from August 2013 to December 2013 in Wayanad, Kerala, India (Maulingkar *et al.,* 2015) and another cross-sectional study conducted by Jeyakumari and her co-workers during 2014 in Puducherry (Jeyakumari *et al.,* 2015). The possible cause of such variations could be the number of sample size. The sample size of our present study was smaller compared to other previous studies. Limitation of time and the limited access to the students could be the other reasons of such variations. The results of our current study showed that the titer based frequencies of males and females of this study is less than the titer based frequencies of males and females of the previous similar studies based of gender, such as - a PhD dissertation, study by Sydney Chikukwa in September 2012 in Namibia (Chikukwa, 2012). The small sample size of our present study could be one of the reasons of this particular variation. Other major cause of this variation may be the socio-demographic conditions of the respondents. In the previous similar studies, respondents from all the socio-demographic levels participated in the study. However in our present study the respondents, both males and females were medical students and so they are concern about their health and sanitation status.
A retrospective audit of the Widal tube agglutination test was carried out by RalteLalremruata and his co-workers from August 2012 to June 2013 at Delhi, India. In that study a total of 100 febrile children were enrolled for this study. All of them were started on ceftraixone and five of these patients became afebrile within 5 days of the drug treatment. The highest frequency of TO was 8 in both 1:40 and 1:80 dilutions and the highest frequency of TH was 5 in both 1:40 dilution and 25 in 1:80 dilution (Lalremruata *et al.,* 2014). The most probable reasons of the variations in the obtained results could be the sample size and the time of antibiotic consumption. In our present study those 9 respondents who received antibiotic against typhoid, received those antibiotic in their childhood.
In 1987, Milhomem conducted a study in Brazil. In that study, a group of respondents was observed before and after vaccination. It may be mentioned that, before vaccination the frequency of TO was not within the 1:40 and 1:80 dilutions. After the first dose of vaccination the frequency of TO was 8 in 1:40 dilution and 6 in 1:80 dilution; after the second dose of vaccination the frequency of TO was 12 in 1:40 dilution and 13 in 1:80 dilution. Before vaccination, the frequency of TH was found 8 in 1:40 dilution and 1 in 1:80 dilution. After the first dose of vaccination the frequency of TH was 9 in 1:40 dilution and 15 in 1:80 dilution, whereas after the second dose of vaccination the frequency of TH was 6 in 1:40 dilution and 10 in 1:80 dilution (Milhomem *et al.,* 1987). The most probable reasons of the variations in the obtained results could be the sample size and the time of vaccination. In this study those 13 respondents who was vaccinated against typhoid fever, was actually vaccinated in their childhood.

Conclusion

This research work has been done with a view to measure the base line titer of *Salmonella typhi* and *Salmonella paratyphi* of the selected medical students to measure the TO, TH, AH and BH titer levels in order to diagnose the typhoid fever. The findings of the present study reveal that the frequency of TO titer was 25 in 1:40 and 1:80 dilution which indicates that the

experimental human subjects (Medical student) were highly exposed by the TO antigen of *Salmonella typhi*. On the other hand, the frequency of TH titer was 17 in 1:40 which indicates that the experimental human subjects (Medical student) were less exposed by the TH antigen of *Salmonella typhi*. The correlation among age; sex and the histories of typhoid fever, antibiotic against typhoid and vaccines against typhoid with the titer was investigated. There was no correlation among the variables and the titer. It can be expected that the result obtained in this study will contribute regarding proper diagnosis of typhoid fever prevailing in our country. It will also help to build up awareness among policy makers and general people to uptake the preventive measures against typhoid fever.

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