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An investigation into the correlations among the Widal titers and some diseases (other than typhoid) that are responsible for the false positive results of Widal test

[*1] Arrafy Rahman, [2] Sinthia Tahmina, [3] Md. Imran Hossain, [4] Md. Zakir Hosen, [5] Khondoker Moazzem Hossain, [6] Hasina Momtaz

[1-5] Biotechnology and Genetic Engineering Discipline, Life Science School, Khulna University, Khulna, Bangladesh
[6] Community Medicine Department, Ibrahim Medical College, Shahbag, Dhaka, Bangladesh

Abstract
Typhoid fever is one of the most common infectious diseases. The Widal test is one of the extensively used and only available sero-diagnostic test of typhoid fever in developing countries. But the main fault of Widal test is it often produces false positive results. For this reason it is no more used in the developed countries. The objectives of this study were to find out the correlation of diarrhea, dengue and cold fever with the Widal titers; to identify the correlation between typhoid fever and diarrhea as well as between typhoid fever and cold fever and to find out the correlation between Widal titers and cold fever treatments. Blood samples of this study were collected from 100 apparently healthy medical students for a period of about seven months. The sera were separated and the presence of the *Salmonella* antibodies was analyzed by Widal test. Among the 100 samples, 59 were found positive for TO and 27 were found positive for TH. No correlation was found between Widal titers and diarrhea, dengue and cold fever. There was not any correlation between typhoid and diarrhea as well as typhoid fever and cold fever. Treatments of cold fever had no effect on the Widal titers of the students.

Keywords: typhoid, infectious, Widal test, Sero-diagnostic, false positive, diarrhea, dengue, cold fever, Widal titers

Introduction
Typhoid fever is a major health problem in developing countries and its diagnosis on clinical ground is difficult [Andualem *et al.,* 2014] [1]. Typhoid fever is a systemic prolonged febrile illness caused by certain *Salmonella* serotypes including *Salmonella typhi*, *S. paratyphi* A, *S. paratyphi* B and *S. paratyphi* C. We, the humans are the only reservoir host for the serotypes of *Salmonella*. The pathogens of typhoid are transmitted by faecally contaminated water and food in endemic areas. The World Health Organization (WHO) estimates about 21 million cases of typhoid fever with more than 600,000 deaths annually. The cases are more likely to be seen in India, South and Central America, Africa and Bangladesh i.e. in areas with rapid population growth, increased urbanization, and limited safe water, infrastructure, and health systems [Willke *et al*., 2002 and Crump *et al.* 2004] [12, 5]. The incident rate of typhoid in Asia-pacific region is estimated at more than 100 cases/100,000 population per year. The highest burden of disease has been observed in children [Bhutta *et al.,* 1996] [3]. Accurate diagnosis of typhoid fever at an early stage is essential for etiological diagnosis and to identify individuals that may serve as potential carriers, who may be responsible for acute typhoid fever outbreaks [Mengist and Tilahun, 2017] [8]. Widal test is a serological diagnosis test for enteric fever was founded by Georges Fernand Isidore Widal in 1896 [Sridhar Rao, 2009] [10]. It is an agglutination reaction that indicates the existence of the lipopolysaccharide (LPS) somatic (O) and fla-gella (H) agglutinins to *Salmonella typhi* in the blood serum of a patient using suspensions of O and H antigens [Tupasi *et al.,* 1991] [11]. The Widal test is being used widely and extensively in the serodiagnosis of typhoid fever and so remains the only practical test available in most third world countries [Kulkarni *et al.* 1994] [7]. But one of the major drawback of Widal test is cross-reactivity due to which some other bacteria of same genus often produces false positive results [Aziz and Haque, 2012] [2]. As there is variation in the level of antibodies detectable in apparently healthy population of different areas and this variation can effected by cross infection with other *Salmonella* spp. False positive results can be obtained in healthy individuals by the presence of cross reacting antigens for example malaria, brucellosis, dengue fever and other enterobacteriaceae infections, vaccinated individual (Colle *et al.,* 1996) [4].

Objectives
1. To find out whether there is any correlation of diarrhea, dengue and cold fever with the Widal titers.
2. To find out the correlation between typhoid fever and diarrhea as well as typhoid fever.
3. To find out the correlation between Widal titers and cold fever treatments.

Methodology
This was a cross-sectional study which was conducted from August 2016 to February 2017 in the laboratory of Microbiology Department of Ibrahim Medical College and BIRDEM Hospital, Shahbag, Dhaka. Total 100 apparently

healthy students of Ibrahim Medical College were selected for this study. The health screening of the respondents was done by using a questionnaire. The exclusion criteria were – (a) enteric fever during last one month and (b) vaccination for enteric fever in last one month. Blood was collected serum was separated using centrifugation. For a single Widal test, we used 20 Widal test tubes, arranged in 4 rows for 4 antigens (TO, TH, AH and BH). Each row had 5 tubes and the fifth tube of each row was set as control. Each Widal test was done as follows –

1. All the reagents were brought to room temperature and mixed well
2. Four sets of test tubes were prepared for individual antigen, each containing 1- 5 tubes.
3. 1.9 ml of 0.85% physiological saline was added to tube no. 1 of each antigen set.
4. To tube no. 2-5 of all sets 1 ml of physiological saline was added.
5. To tube no. 1 of all sets 0.1 ml of test sample was added and mixed well by pipetting.
6. Then serial dilution of the serum was carried out from tube no. 1 to tube no. 4 of all sets.
7. 1 ml of the diluted serum was discarded from tube no.4 of each set.
8. 1 drop of S. typhi 'O' antigen was added to the first set i.e. from tube no.1- 5
9. 1 drop of S. typhi 'H' antigen was added to the first set i.e. from tube no.1- 5
10. For third and fourth sets, 'AH' and 'BH' were added to all tubes from 1-5.
11. These tubes were mixed well, covered and incubated at 37^0C for about 24 hours.
12. After incubation the sediment was dislodged and observed for agglutination.

Result

Among 100 respondent, agglutination has occurred in case of 90 respondents. 16 respondents showed agglutination in 1:20 dilution, 45 respondents showed agglutination in 1:40 dilution and 29 respondents showed agglutination in 1:80 dilution. [Table No. 01]

Table 1: Frequency distribution 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	25	25
S. typhi H	6	17	4
S. paratyphi AH	0	1	0
S. paratyphi BH	1	2	0

The correlation among the Widal titers and the history of the diarrhea of the respondents was observed and no statistical significance was found. So there is no correlation among Widal titers and the history of diarrhea and it is found for both antibody titers against *Salmonella typhi* antigen TO and antibody titers against *Salmonella typhi* antigen TH. [Table No. 02 and Table No. 03]

Table 2: Frequency distribution of the antibody titers against *Salmonella typhi* antigen TO based on the history of diarrhea of the respondents

History of Diarrhea	*Salmonella typhi* antigen TO				P value
	0	1:20	1:40	1:80	
Suffered	2	0	4	2	0.321
Did not suffer	39	9	21	23	

Table 3: Frequency distribution of the antibody titers against *Salmonella typhi* antigen TH based on the history of diarrhea of the respondents

3	*Salmonella typhi* antigen TH				P value
	0	1:20	1:40	1:80	
Suffered	7	0	1	0	0.747
Did not suffer	66	6	16	4	

No effect of the dengue fever was found on the antibody titers against *Salmonella typhi* antigen TO and TH. [Table No. 04 and Table No. 05]

Table 4: Frequency distribution of the antibody titers against *Salmonella typhi* antigen TO based on the history of dengue of the respondents

History of Dengue	*Salmonella typhi* antigen TO				P value
	0	1:20	1:40	1:80	
Suffered	2	0	1	3	0.486
Did not suffer	39	9	24	22	

Table 5: Frequency distribution of the antibody titers against *Salmonella typhi* antigen TH according to the history of dengue of the respondents

History of Dengue	*Salmonella typhi* antigen TH				P value
	0	1:20	1:40	1:80	
Suffered	4	1	1	0	0.682
Did not suffer	69	5	16	4	

Again, there was no correlation between typhoid fever and the history of diarrhea as well as there was no correlation between typhoid fever and the history of cold fever of the respondents. [Table No. 06 and Table No. 07]

Table 6: Correlation between typhoid and diarrhea in past life of the respondents

Typhoid in past life	Diarrhea		P value
	Suffered	Did not suffer	
Suffered	1	27	0.309
Did not suffer	7	65	

Table 7: Correlation between typhoid and cold fever in past life of the respondents

Typhoid in past life	Cold fever		P value
	Recently suffered	Suffered long ago	
Suffered	5	23	0.102
Did not suffer	5	67	

Again, the cold fever of the respondents has no correlation with the Widal titers whether the respondents recently suffered or suffered long ago. It is true for both antibody titers against *Salmonella typhi* antigen TO and the antibody titers against *Salmonella typhi* antigen TH. [Table No. 08 and Table No. 09]

Table 8: Frequency distribution of the antibody titers against *Salmonella typhi* antigen to base on the history of cold fever of the respondents

History of cold fever	*Salmonella typhi* antigen TO				P value
	0	1:20	1:40	1:80	
Recently suffered	5	1	2	2	0.929
Suffered long ago	36	8	23	23	

Table 9: Frequency distribution of the antibody titers against *Salmonella typhi* antigen TH on the history of cold fever

History of cold fever	*Salmonella typhi* antigen TH				P value
	0	20	40	80	
Recently suffered	8	0	2	0	0.742
Suffered long ago	65	6	15	4	

Paracetamol and antibiotic, used against fever, have no effect on antibody titers against *Salmonella typhi* antigen TO. [Table No. 10]

Table 10: Frequency distribution of the antibody titers against *Salmonella typhi* antigen TO based on the treatment of cold fever of the respondents

Treatment of cold fever	*Salmonella typhi* antigen TO				P value
	0	1:20	1:40	1:80	
Paracetamol	2	1	2	1	0.098
Antibiotics	5	0	0	0	
No treatment	0	0	0	1	

Discussion

In this study out of 100 respondents 8 suffered from diarrhea and only 92 did not suffer. In case of the respondents who suffered from diarrhea, the highest frequency of TO titer was 4 in 1:40 dilution and the highest frequency of TH titer was 1 in 1:40 dilution. In case of those who did not suffer from diarrhea, the highest frequency of TO antigen titers was 23 in 1:80 dilution the highest frequency of TH antigen titers was 16 in 1:40 dilution. The results of this study vary form the results of other similar studies. The major cause of variation may be, the other studies did not included diarrhea as a variable. Even, so far, no study related to the measurement of titer of antibody against *S. typhi* antigens included diarrhea as a variable. Another cause may be the respondents of the study were not the patients of diarrhea. However, they may have been suffered from diarrhea since long ago.

In our present study out of 100 respondents 6 suffered from dengue fever and rest 94 did not suffer. In case of the respondents who suffered from dengue, the highest frequency of TO titer was 3 in 1:80 dilution and the highest frequency of TH titer was 1 in 1:40 dilution. In case of those who did not suffer from dengue, the highest frequency of TO antigen titers was 24 in 1:40 dilution and the highest frequency of TH antigen titers was 16 in the same dilution. During 1974-82, Tikky Ping and Savithri Puthucheary carried out a cross sectional study in Kualalumpur, Malaysia. Here the frequency of TO titer was 26 in 1:40 dilution and 34 in 1:80 dilution and the frequency of TH titer was 14 in 1:40 dilution and 5 in 1:80 dilution [Ping and Puthucheary, 1983] [9]. Another cross-sectional study was conducted by Kulkarni and Rego during 1993 at Davangere. It was observed that, in case of normal children, the frequency of TO titer was 5 in 1:40 dilution and 2 in 1:80 dilution. In case of children suffering from non-typhoidal fever, the frequency of TO titer was 11 in 1:40 dilution and 5 in 1:80 dilution. In case of normal children suffering from typhoidal fever, the frequency of TO titer was 6 in 1:40 dilution and 2 in 1:80 dilution. In case of normal children the frequency of TH titer was not found. In case of children suffering from non-typhoidal fever the frequency of TH titer was 4 in 1:40 dilution and 1 in 1:80 dilution. In case of normal children suffering from typhoidal fever the frequency of TH titer was 9 in 1:40 dilution and 2 in 1:80 dilution. [M.L. Kulkarni and S.J. Rego, 2016] The results related to the titers of our present study vary from the results related to the titers obtained from the similar cross-sectional studies mentioned above. The possible reasons of this variation may be that the respondents of the study were not the patients of dengue. However, they might suffer from dengue since long ago. The smaller sample and time limitation could be other reasons for such variations. Variations in the respondent's life styles may also be considered as other parameters for such variations.

Conclusion

The study was conducted to find out the correlation between Widal titers and the history of diarrhea as well as the correlation between Widal titers and the history of dengue. No correlation was found between Widal titers and the history of diarrhea as well as the correlation between Widal titers and the history of dengue. The correlation between Widal titers and the history of cold fever as well as between Widal titers and fever treatment was also investigated and no correlation was found between Widal titers and the history of cold fever as well as between Widal titers and fever treatment in this study. We expect that the results of our study will play an important role in the diagnosis of typhoid through the Widal test, especially for the third world countries where Widal test is still popular. The study will make general people aware of typhoid fever and also make them interested in healthy lifestyle. Conducting the study in broader range would be worthy to investigate.

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