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Prevalence of Positive Widal test in comparison with clinical request in a tertiary care teaching hospital, West Bengal

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Abstract:
Introduction*: Enteric fever which includes typhoid and paratyphoid fever is a systemic febrile illness caused by the bacterium Salmonella entericaserovartyphi and paratyphi A, B, C respectively. Widal test is almost the most widely used method for diagnosis of typhoid fever in many developing countries.****Objectives****: 1. To know the percentage of positivity of Widal test. 2. To know the common age group affected. 3. To know the titres of S.typhi 'O' and 'H', S. paratyphiA 'H' and S. paratyphi B 'H' antibodies.* ***Methodology:*** *It is a prospective study conducted at the Central laboratory of Department of Microbiology, Malda Medical College, West Bengal. We haveanalysed all requests during last one year from September 2017 to August 2018. Sera, obtained from patient, we have done widal tube agglutination test.* ***Results:*** *Total 10267 Widal test conducted from September 2017 to August 2018, in one year. 1576 subjects were found Widal test positive. Positivity rate of widal test found 15.35%. 759 (48.16%) positive cases were male and 817 (51.84%) positive cases were female. Male: Female ratio was 0.92: 1. Common age group affected amongst both male and female was 21 to 30 years. Maximum positive antibody titre seen against S.typhi 'O' antigen was (65.4%). 1: 80 titre, 1: 160 titre and 1: 320 antibody titre against Salmonella typhi 'O' antigen were found in 739 (71.6%), 249 (24.1%) and 44(4.3%) patients respectively. No antibody titre positivity seen in 1: 640 dilution.* ***Conclusion:*** *Widal test is very cheap, simpler, widely used tool for diagnosis of typhoid fever. Typhoid fever remains an important public health problem in developing countries. Proper sanitation, public health education, awareness and vaccinations are the long term preventive measures to decrease or control the disease.*

Keywords: *Prevalence, Widal test, Enteric fever, Malda.*

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I. Introduction

Enteric fever which includes typhoid and paratyphoid fever is a systemic febrile illness caused by the bacterium Salmonella entericaserovartyphi and paratyphi A, B, C respectively. S.entericaserovartyphi is found to be associated with more than 90% cases of enteric fever. Paratyphoid fever is clinically similar but milder than typhoid fever.[1]This highly adapted human specific pathogen has evolved remarkable mechanisms for its persistence in its host that help the organism to ensure its survival and transmission.[2]Human beings are the only reservoir and host for typhoid fever, the disease is transmitted by faecally contaminated water and food in endemic areas. The signs and symptoms of uncomplicated typhoid fever are nonspecific and an accurate diagnosis only clinically is difficult.[3]Symptoms may vary from mild to severe and include long term high fever, weakness, headache, stomach pain, loss of appetite and rose coloured rash. Typhoid is mainly associated with low socioeconomic status and poor hygiene.[4]These fevers are considered as a major cause of morbidity and mortality in developing countries with more than 90% of cases found in Asia only.[1]WHO estimate of nearly 17 million infections and more than 1,50,000 deaths occurs each year worldwide.[5] The absolute diagnosis of typhoid fever is by isolation of bacteria from blood, stool, urine or bone marrow cultures. However the isolation of the bacteria by culture methods is very limited for various reasons including non availability of infrastructure for culture, time consuming, improper techniques used and also due to the wide spread misuse and self administration of antibiotics by patients.[2,5,6]Widal test is the most widely used method for diagnosis of typhoid fever in many developing countries.[5]Widal test is the agglutination test which detects the IgM and IgG class of antibodies to Salmonella typhi and paratyphi in the patient's serum from the second week of the onset of the symptoms of typhoid fever.[7]Typhidot is another rapid test used to ascertain the diagnosis of typhoid fever but not as cost effective as widal. Nowadays a rapid slide agglutination widal test is most commonly used technique

in laboratories because of its convenience. The recommended method of performing the widal test is by the tube agglutination technique where serial two fold dilutions of the patient's serum are tested.[8]

II. Objectives

1. To know the percentage of positivity of Widal test.
2. To know the common age group affected.
3. To know the titres of S.typhi 'O' and 'H', S. paratyphiA 'H' and S. paratyphi B 'H' antibodies.

III. Methodology

It is a prospective study conducted at the Central laboratory of Department of Microbiology, Malda Medical College. We haveanalysed all requests during last one year from September 2017 to August 2018. We have looked at age, gender and clinical symptoms which led to the request.

All requests from both inpatients and outpatients for Widal test was included in this study and we have excluded incomplete data, especially those in which age, clinical symptoms are missing.

2 ml. of venous blood was collected from each subject with proper aseptic precautions. It was allowed to clot at room temperature for about 30 to 60 minutes. Serum was separated by centrifugation for 5 minutes. The standard widal tube agglutination test was done. Four rows were set for each serum sample to be tested. The first row contained 6 Felix tubes and the remaining 3 rows of 6 Dreyer's tubes each. Serial two fold dilutions of the test serum i.e. 1:10, 1:20, 1:40, 1:80, 1:160, 1:320 were prepared in all the rows. So that each tube contained 0.5 ml. of the diluted serum. In the first and the second rows, 0.5 ml. of Salmonella typhi- O and H antigens were added respectively. In the third and fourth rows, 0.5 m.l. Salmonella paratyphi- AH and BH antigens were added respectively. So the final serum dilutions were obtained for each antigen 1:20, 1:40, 1:80, 1:160, 1:320 and 1:640. Appropriate positive and negative controls were put up for each test. The test tube racks were placed in water bath at 37^0C for overnight incubation. The Widal TO, TH, AH, BH antibody titres were taken as the highest dilutions of serum with a visible agglutination.[6, 9, 10]

IV. Results

Total 10267 Widal tests were conducted from September 2017 to August 2018 in Central Laboratory, in the Department of Microbiology.

Table 1 shows the distribution of positive and negative samples of Widal test. Out of 10267 samples**,** 1576 subjects were found Widal test positive. Positivity rate of widal test were found 15.35%. 759 (48.16%) positive cases were male and 817 (51.84%) positive cases were female. Male: Female ratio was 0.92: 1.

Table 2 shows age wise distribution of Male and female patients in positive Widal tests. In both male and female patients, maximum positive Widal tests were found in the age group 21 to 30 years. In this age group, 188 patients (24.8%) were male and 208 patients (25.5%) were female, followed by 11 to 20 years age group, 175 patients (23.0%) were male and 177 patients (21.7%) were female respectively.

Table 3 shows, distribution of positive samples against different serotypes of Salmonella. Maximum positive antibody titre seen against S.typhi 'O' antigen was (65.4%). Antibody titres against S. typhi 'H' antigen (19%), S. paratyphiA 'H' (21%) and S. paratyphi B 'H' (4.1%) were found in this study.

Table 4 shows, number and percentage of reactive sera in various titres in study population. 1: 80 titre, 1: 160 titre and 1: 320 antibody titres against Salmonella typhi 'O' antigen were found in 739 (71.6%), 249 (24.1%) and 44(4.2%) patients respectively. Similarly, against Salmonella typhi 'H' antigen 1: 80, 1: 160 and 1: 320 antibody titres were found in 163 (54.3%), 117 (39%), 20 (6.6%) patients respectively. Against Salmonella paratyphi A 'H' antigen 1: 80, 1: 160 and 1: 320 antibody titres were observed in 204 (61.4%), 104 (31.3%), 24 (7.2%) patients respectively. Also against Salmonella paratyphi B 'H' antigen 1: 80, 1: 160 and 1: 320 antibody titres were found in 47 (72.3%), 18 (27.7%), 0 (0%) patients respectively. No antibody titre positivity seen in 1: 640 dilution.

Diagram 1 shows, total antibody titres of reactive sera comparatively represented by bar diagram.

V. Discussion

Typhoid fever has an important socio-economic impact, so accurate diagnosis of the disease at an early stage is important not only for etiological diagnosis but also for identifying individuals that may serve as potential carrier who may be responsible for acute typhoid fever outbreak.[11] The widal test has been used very extensively in the diagnosis of typhoid fever and in developing countries; it remains as the only practical test available.[12] The present study focused on the utility of the widal test. In a developing country like India, widal test has been used extensively in the serodiagnosis of typhoid fever. Our study shows, out of 10267samples**,** 1576 subjects wereWidal test positive. Positivity rate of widal test was found 15.35%. 759 (48.16%) positive cases were male and 817 (51.84%) positive cases were female. Male: Female ratio was 0.92: 1. It correlates well with other studies.[3, 8, 12, 13]

Our study shows that both male and female patients are affected mostly in the age group of 21 to 30 years followed by 11 to 20 years age group. These findings are consistent with other studies.[3, 7, 8, 12] Since the young adults and adolescents are more infected in this study, the reason could be their more outdoor activities and junk food eating habits, poor personal hygiene and may be lack of administration of vaccine or booster doses.[14]

In this study, maximum positive antibody titre seen against S.typhi 'O' antigen was (65.4%). That is similar with other studies.[2, 14, 15, 16, 17, 18] Antibody titre against S. typhi 'H' antigen (19%), S. paratyphiA 'H' (21%) and S. paratyphi B 'H' (4.1%) was found in this study. From our data it is evident that paratyphoid fever is slightly predominant over typhoid fever in this region which corresponds with other studies.[5, 6, 7, 12]A possible explanation for the increasing prevalence of paratyphoid fever is consumption of food from street vendors. Because paratyphoid require a higher infective dose which is more likely to be present in food from street vendors.[12, 15]

Our study reflects, highest 1: 320 antibody titre against Salmonella typhi 'O' antigen, Salmonella typhi 'H' antigen, Salmonella paratyphi A 'H' antigen, Salmonella paratyphi B 'H' antigen was found in 4.2%, 6.6%, 7.2% and 0% patients respectively, out of each positive antibody titre group. This observation is more or less correlates with other studies.[5, 19, 20] More than 1:320 antibody titre seen in some studies.[3, 17] But, in the present study no antibody titre positivity seen in 1: 640 dilution, possibly patient seek advice from health care personnel earlier as compared to other.

The updated data for prevalence of typhoid fever would help to know the present scenario.

Early diagnosis of typhoid fever from a single specimen is also of therapeutic value as early diagnosis is most vital in typhoid; otherwise if the treatment is delayed it would result in fatal complications such as perforation or haemorrhage of the small bowel. Thus widal test in situation where blood culture is not feasible, can be of diagnostic value in the early stage of the disease and thus help in reducing morbidity and mortality.

Researchers must continue to search for the ideal rapid test to diagnose acute typhoid fever. Several urine assays have been developed but none has proved to up to the mark. More sophisticated molecular techniques for diagnosis such as PCR are also being studied.[21, 22]

VI. Figures and Tables

Table 1: Distribution of positive and negative samples of Widal test

Widal reactivity	Frequency	Percentage
Positive (≥ 1:20)	1576	15.35%
Negative < 1:20	8691	84.65%
Total	10267	100%

Table 2:Age and Gender wise distribution of positive samples of Widal tests (n=1576)

Age	Male	%	Female	%
0-10	87	11.5%	75	9.2%
11-20	175	23.0%	177	21.7%
21-30	188	24.8%	208	25.5%
31-40	109	14.4%	156	19.1%
41-50	76	10.0%	109	13.3%
>50	124	16.3%	92	11.2%
Total	759	100%	817	100%

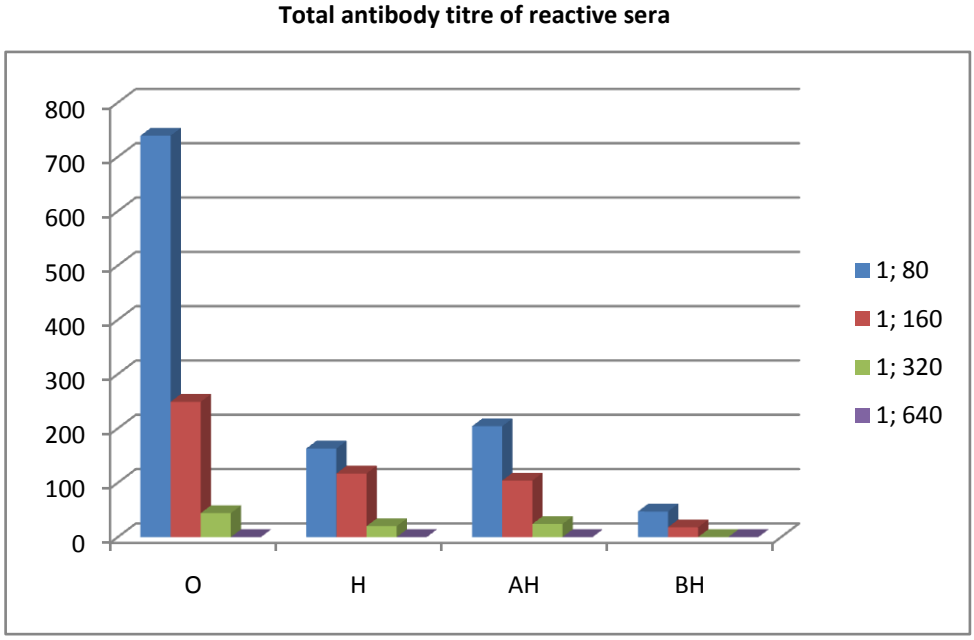
Table 3: Distribution of positive samples against different serotypes of Salmonella.

Serotype	Antibody type	Frequency of agglutinating sera (n= 1576)	Percentage (%)
S. typhi	Anti O antigen	1032	65.4%
S. typhi	Anti H antigen	300	19.0%
S. paratyphi A	Anti H antigen	332	21.0%
S. paratyphi B	Anti H antigen	65	4.1%

Table 4: Number and Percentage of reactive sera in various titres in study population.

Antigen	No. of Positive samples	Dilution (1: 80)	Dilution (1: 160)	Dilution (1: 320)	Dilution (1 : 640)
S. typhi 'O'	1032 (65.4%)	739 (71.6%)	249 (24.1%)	44 (4.3%)	0 (0%)
S. typhi 'H'	300 (19.0%)	163 (54.3%)	117 (39%)	20 (6.6%)	0 (0%)
S. paratyphi A'H'	332 (21.0%)	204 (61.4%)	104 (31.3%)	24 (7.2%)	0 (0%)
S. paratyphi B'H'	65 (4.1%)	47 (72.3%)	18 (27.6%)	0 (0%)	0 (0%)

Diagram 1. Bar diagram shows total antibody titre of reactive sera.



VII. Conclusion

Widal test is very cheap, simpler, widely used tool for diagnosis of typhoid fever.It should be remembered that, a 'negative' widal test maynot always rule out the diagnosis of typhoid fever in patients with sign and symptoms of the diseasebecause a 'negative' widal testmay be seen early in the course of illness. Enteric fever remains an important public health problem in developing countries. Proper sanitation, public health education, awareness and vaccination are the long term preventive measures to decrease or control the disease.

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