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Widal test: Diagnostic tool for typhoid and paratyphoid diseases

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Abstract
Typhoid is a severe, infectious and life-threatening disease. Typhoid disease mainly hit on children who are in school-going age. It is not very common in adults and older people. In developing countries, facilities for isolation and culture are often not available especially in smaller hospitals and diagnosis relies upon the clinical features of the disease and detection of agglutinating antibodies to *Salmonella typhi* and salmonella paratyphi by the Widal test. A total of 1461 blood samples received in the lab over a period of two years were tested by conventional rapid slide agglutination using commercially available antigens. In the present study the prevalence of seropositivity was 24.36%. With 59% of the samples from males and 41% from females and maximum positive cases were from males 13.27%. Most of the positive cases in the present study were from the age group of 05-10 years 39.01%. Amongst all antigen, O antigen was found to be maximum positive antigen i.e18.75%. The elevated levels may have been due to cross-reacting antigens or an anamnestic response. There are more than 40 cross-reacting antigens between *S. typhi* and other *Enterobacteriaceae*. The disease remains an important public health problem in developing countries. Proper sanitation, public health education and vaccination are the long term preventive measures to decrease or control the disease.

Keywords: *Salmonella*, Widal, Typhoid, Antigen, Antibody

1. Introduction
Typhoid is a severe, infectious and life-threatening disease. Typhoid disease mainly hit on children who are in school-going age. It is not very common in adults and older people. Typhoid is a communicable disease and it is transmitted in many ways in India. Bacteria of typhoid are survived in unhygienic conditions. These bacteria are spread by typhoid patients and carriers in large quantities through stools and vomit. The bacteria then travel to food, drinks and water through house-flies and other insects. A person infected with *Salmonella typhi* may infect others, as the bacteria remain in the body for months. There are 107 different strains of this bacterium. Paratyphoid is caused by *Salmonella enteritis* paratyphi A, B or C. It is generally a less common infection than typhoid [1, 2].

In India, due to population explosion, water is polluted and this disease is matter of worry especially, where water supply and sewage disposal are disrupted. Raw vegetables grown on sewage fields also spread infection. The bacteria can survive in soil and water for several months. They grow rapidly in milk and milk-products.

Typhi is found to be associated with over 90% cases of enteric fever [1]. The definitive diagnosis of typhoid fever requires the isolation of *Salmonella typhi* from the blood, faeces, urine or other body fluids. In developing countries, facilities for isolation and culture are often not available especially in smaller hospitals and diagnosis relies upon the clinical features of the disease and detection of agglutinating antibodies to *Salmonella typhi* by the Widal test [3]. Over 100 years since its introduction, the Widal test has been and is still being widely used for the diagnosis of typhoid fever, simply owing to the fact that no other sero - diagnostic test of sufficient sensitivity and specificity along with cost effectiveness has been developed, especially in typhoid endemic regions [4].

The real concern is that though the gold standard technique of culture isolation of Salmonella typhi provides a definitive diagnosis in 73- 97% of cases prior to medications, excessive antibiotic use have reduced this isolation rate to 40-60%. Isolation of Salmonella typhi or paratyphi is time taking and the facilities for blood cultures are not always feasible in resource poor regions. All these limitations have made

Widal test (a rapid slide agglutination test) the most utilized test for enteric fever [5].

In India the disease is endemic with an incidence ranging from 102 to 2219 per 100,000 populations. It results in considerable morbidity, absenteeism and resource utilization [6]. Typhoid fever occurs worldwide, primarily in developing nations whose sanitary conditions are poor. Typhoid fever is endemic in Asia, Africa, Latin America, the Caribbean, and Oceania, but 80% of cases come from Bangladesh, China, India, Indonesia, Laos, Nepal, Pakistan, or Vietnam. Within those countries, typhoid fever is most common in underdeveloped areas. Typhoid fever infects roughly 21.6 million people (incidence of 3.6 per 1,000 populations) and kills an estimated 200,000 people every year [7].

The World Health Organization (WHO) has deemed typhoid fever a serious problem in endemic areas (India, Southeast Asia, Africa, Central and South America) where there are between 16 and 33 million cases each year that result in over half a million deaths [8].

However, some industrial nationals are experiencing worrying rises in typhoid fevers; mainly among people who visited parts of the world where it is endemic.

The Widal test is one of the most utilized diagnostic tests for typhoid fever in developing countries. This test demonstrates the presence of somatic (O) and flagellar (H) agglutinins to Salmonella typhi in the patient's serum using suspensions of O and H antigens. The rapid slide test was developed which is now the most commonly used technique in local Laboratories because of its convenience.Therefore, a fast, reliable, and easy to perform Serodiagnostic test with a higher sensitivity and specificity than Widal test is required for rapid diagnosis and management of typhoid cases [9].

In this study more than 1461 samples of patients with enteric fever over a period of 2 years were analysed for seropositivity of Typhoid and paratyphoid infection. The assessment was based on slide agglutination test to confirm presence of antibody against 4 antigens namely O, H, AH and BH. The trends in seropositivity differentiated in factors like age, gender and antibody present.

2. Materials and methods

The present study was conducted in the serology section of Clinical Pathology department, Haffkine institute Parel, Mumbai. The samples were received from different peripheral hospitals and tertiary care centres. A total of 1461 blood samples received in the lab from January 2014 to August 2016 were tested by conventional rapid slide agglutination using commercially available antigens (Tydal tulip diagnostic private limited, Mumbai, India). Atleast 3ml of venous blood collected in clot activator vacutainers were received. These were subjected to centrifuge at 3000rpm for 5 mins for separation of serum. Approx 0.4 ml of two fold serially diluted patients sera (dilution from 1:80 to1:320) in 0.9% normal saline were tested by adding an equal volume of antigen. Positive and negative controls were included in each batch of the test. Diagnostic titres of 1:80 and above were taken as positive. Interpretations of the results were done as per the kit literature. The positive reaction is agglutination of antigen – antibody is critically read in short duration of time so as to avoid any false positive reporting [5].

3. Results

A total of 1461 serum samples received over a period of two years from July 2014 to August 2016 in the department of Clinical Pathology, Haffkine Institute were assessed for typhoid and paratyphoid antibodies using popular serological diagnostic method Widal test.

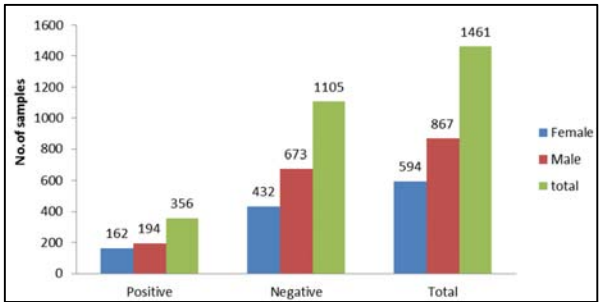

Fig 1: Gender wise distribution of seropositive samples

Of the total 1461 samples 594 belonged to female patients and 867 to male patients. Amongst the 594 female patient samples 162 (27.27%) were positive, whereas in case of 867 male patient samples only 194 (22.37%) were found to be positive.

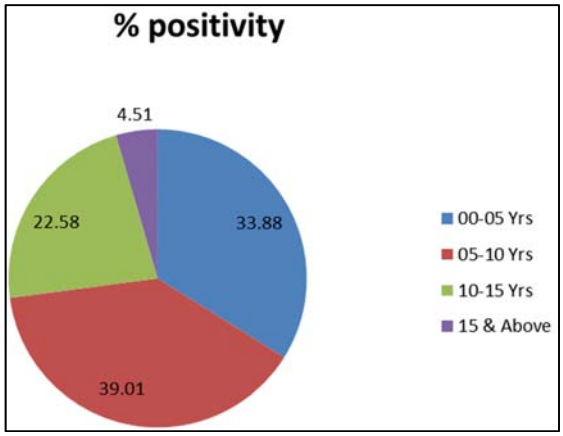

Fig 2: Age wise distribution of seropositive samples

The samples were divided in four major age groups, in case of 00-05 yrs 33.88 % samples were found to be positive, similarly in case of 05-10 yrs 39.01%, 10-15 yrs 22.58% and in age group 15 yrs and above only 4.51% samples were found to be positive.

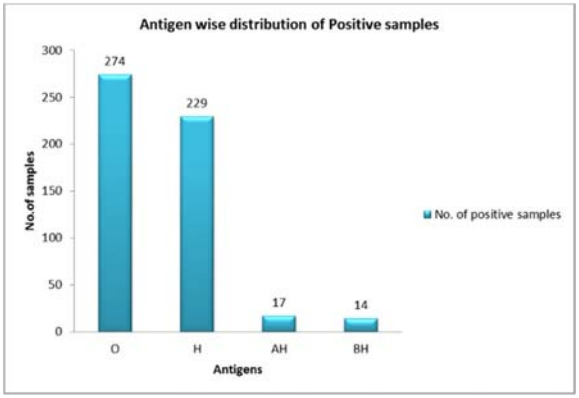

Fig 3: Antigen wise distribution of Positive samples

Out of 1461 patient sera 356 were found to be positive in all. The patient sera were found to be positive for different antigens assessed in WIDAL. 276 samples were found to be positive for O antigen, 229 for H antigen, 17 for AH and 14 for BH.

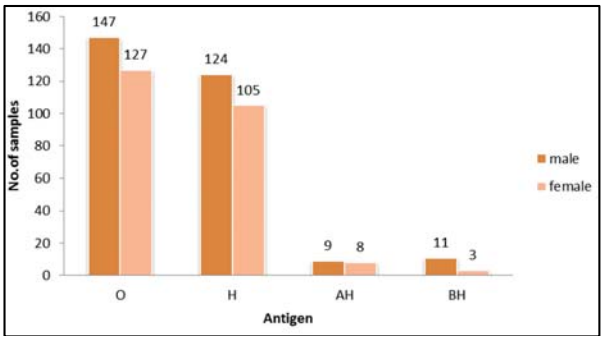

Fig 4: Gender distribution of samples present for various antigens

Each antigen had almost equal number of male and female patient positive samples as seen in case of O antigen 147 male and 127 female, similarly in case of H antigen 124 male and 105 female were found to be positive. Presence of AH and BH positive samples was lower compared to O and H antigens in case of both male and female patient sera.

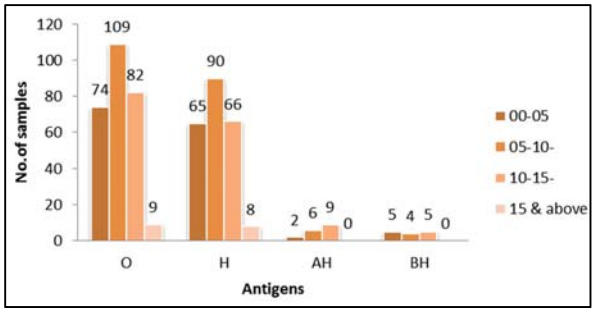

Fig 5: Distribution of antigen in different age groups

Maximum positive sample were found in age groups 00-05, 05-10 and 10-15 whereas in case of age 15 and above least number of positive samples were obtained. O and H antigens prevailed in age group 05-10, no sample were found to be positive for Ah and BH antigens in case of age group of 15 and above.

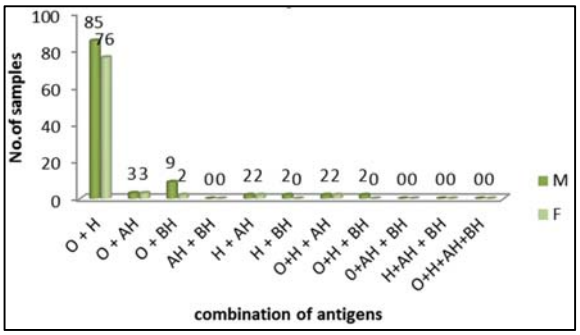

Fig 6: Gender wise distribution of multiple antigens in patient samples

In most patient sera presence of two or more than two antigens was observed. The combination of O and H antigen was found to be maximum in both male and female with 85 and 76 samples respectively. No sample was found to be positive for the combination of AH + BH, O + AH + BH, H + AH + BH and O + H + AH + BH.

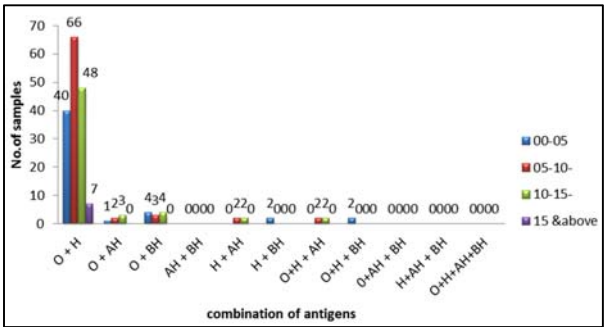

Fig 7: Age wise distribution of multiple antigens in patient samples

Presence of two or more than two antigens was observed in various patient sera. The combination of O and H antigen was found to be maximum in the age group of 05-10 with 66 samples. No sample was found to be positive for the combination of AH + BH, O + AH + BH, H + AH + BH and O + H + AH + BH in any age group.

4. Discussion

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. Isolation of *Salmonella enteric* serotype typhi from blood, urine or stool is the most reliable means of confirming an infection. Most serotype typhi infections are diagnosed purely on clinical grounds and treated presumptively. As a result, the diagnosis may be delayed or missed while other febrile illnesses are considered and patients without typhoid fever may receive unnecessary and inappropriate antimicrobial therapy [10].

In the present study the prevalence of seropositivity was 24.36% which correlates with Bharadwaj *et al* who reported 27.3 %, M.A. Isa *et al* reported 20.6% whereas Akanksha Sharma *et al* reported 12.1% and R Shyamala *et al* reported 8.57% in their studies [11-14].

In our study 59% of the samples were from males and 41% were from females whereas R Shyamala *et al* reported 59% from males and 41% from females. In our study maximum positive cases were from males 13.27% followed by females 11.08% which correlates with Vallab Ganesh Bharadwaj B *et al*. Most of the positive cases in the present study were from the age group of 05-10 years (39.01%) followed by 00-05 years (33.88%) which correlates with Vallab Ganesh Bharadwaj B *et al* who reported 36 % in the age group 21-40 years followed by 30.4% in < 20 years age group [11, 14].

Amongst all antigen, O antigen was found to be maximum positive antigen i.e. 274 samples (18.75%) as compared to H antigen i.e. 229 samples (15.67%) in case of typhi. In the assessment of paratyphi AH antigen i.e. 17 (1.16%) and BH antigen i.e. 14 (0.95%) were found. In case of typhi both O &H antigen were found maximum positive in male patients amongst as against females. Combination of O+H antigen is showing the highest positive samples amongst all the combination of antigens. Seropositivity for both antigens of typhi are commonly observed, while that of typhi and paratyphi are reasonably lesser in number. Combination of O+H antigen shows maximum no of positive cases in the

age group of 05-10 yrs.as compared to other age groups of 00-05 yrs., 10-15yrs, and 15yrs and above.

The elevated levels may have been due to cross-reacting antigens or an anamnestic response. There are more than 40 cross-reacting antigens between *S. typhi* and other *Enterobacteriaceae* [15]. One of the reasons for this high rate of seropositivity against serotype Typhi is the widespread presence of salmonella infections in the community. The other factors for the seroepidemiologic datavaries the cross-reactivity of serotype Typhi antigens with other salmonella infections and the longevity of these antibodies in the serum. Due to the rapid growth in population, inadequate human waste disposal, limited water supply and overburdened heath care systems have made all disease difficult to control and made it contribute to the endemicity. The role of Widal test has been debated widely, because the sensitivity, specificity, and predictive values of this widely used test vary considerably among various areas [16].

One of the limitations of this study was that we could not include blood culture technique for all the samples and therefore, could only predict the epidemiological trends rather than the exact incidence of the disease. Further, a prospective approach rather than a retrospective one could have yielded a better insight. Widal selected as a diagnostic tool for typhoid and paratyphoid infection is a screening test rather than a confirmatory test. The positive sera in case of Widal have to be asserted with the clinical manifestation of the disease as well as confirmed by blood culture, which is gold standard for identification of salmonella infections. The possibility of false positive, false negative and variability in antigens of Widal test cannot be ruled out. Thus, the result of Widal test for salmonella infections is just indicative of the presence of antibodies and not the disease in the patient. The present study revealed the changing trends of typhoid fever as obtained by an inexpensive accessible, easily performed screening test During a 10 yr period in north India. The findings indicate towards a need to review the vaccination policy for typhoid fever in an endemic set up.

6. Conclusion

The disease remains an important public health problem in developing countries. Proper sanitation, public health education and vaccination are the long term preventive measures to decrease or control the disease. Updated data on the incidence or prevalence of typhoid fever is essential before introducing the vaccines into regular programmes.

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