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A brief study of diagnosis and frequency of typhoid fever incidence by Widal test

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ABSTRACT

Laboratory diagnosis of typhoid fever requires isolation and identification of Salmonella enterica serotype Typhi. In many areas where this disease is endemic, laboratory capability is limited. We studied the value of the Widal tube and slide agglutination test for the diagnosis of typhoid fever. Blood donor controls were screened with a slide agglutination test for the Salmonella enterica serotype Typhi O and H antigens, and positives were then tested with the Widal test. We concluded that the majority tested (51%) had an H agglutinin titre of <1/20 with 2% having a titre of 1/160. For 0 agglutinins, 31% had a titre of 1/80 and 2% had a titre of 1/160 that O and H agglutinin titers of >1/200 is of diagnostic significance. Out of 10 samples subjected to 2-mercapto-ethanol test 4 showed reductions indicating the presence of IgM antibodies and 6 sera negative for the test. Widal test can be of diagnostic value in the early stage of disease and thus help in reducing morbidity and mortality from typhoid. A false-positive may be the result of past infection with serotype Typhi or another nontyphoidal Salmonella serotype that shares common antigens.

Keywords: Widal test, *Salmonella typhi*, 2-mercapto-ethanol.

INTRODUCTION

Typhoid fever is a common illness in developing countries like India [1] and is a potential threat to developed nations, in an era of increasing air travel and global operations [2]. It is a systemic infectious disease characterized by an acute illness, the first typical manifestations of which are fever, headache, abdominal pain, relative bradycardia, splenomegaly, and leukopenia. *Salmonella enterica* subspp. *Enteric* serotype Typhi is the etiological agent of typhoid fever. Typhoid fever is an important cause of morbidity in many regions of the world, with an estimated 12 to 33 million cases occurring annually [3] and 600,000 deaths annually [5] the first immunodiagnostic test in the world—the Widal test [4] was developed for typhoid, a test that is still widely used today. Typhoid has remained a major health threat globally, affecting some 20 million people annually [6]. The signs and symptoms of uncomplicated typhoid fever are nonspecific, and an accurate diagnosis on clinical grounds alone is difficult [7]. Although a definitive diagnosis can be made by isolation of *Salmonella typhi* from blood or bone marrow [8], and the Widal test is the only specific diagnostic investigation tool available. The Widal test has been in use for more than a century as an aid in the diagnosis of typhoid fever in many countries [9]. In the absence of appropriate chemotherapy, typhoid fever was often a fatal illness and introduction of effective antibiotic therapy in 1950s led to

a sharp decline in the rates of complications and mortality due to typhoid fever [10]. However, in early 1990s multidrug-resistant strains of *Salmonella enterica* serotype *typhi* (MDR-ST) that were resistant to all the three first-line drugs then in use, namely chloramphenicol, amoxycillin and co-trimoxazole emerged, and sooner MDR-ST became endemic in many areas of Asia, including India [11].

The definitive diagnosis of the disease requires the isolation of *Salmonella typhi* from the blood, feces, urine or other body fluids. In the 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 the detection of agglutinating antibodies to *S.typhi*. Sero diagnosis of typhoid fever has been attempted since the late 19th century when Widal and Sicard showed that the serum of patients with typhoid fever agglutinated typhoid bacilli. Unfortunately, neither the Widal test, which remains in widespread use in the developing world, nor any of the serodiagnostic tests that have since been developed has proven sufficiently sensitive, specific, and practical value added in areas where this disease is endemic [12]. Recent advances in molecular immunology have led to the identification of potentially more sensitive and specific markers in the blood and urine of patients with typhoid fever and have enabled the manufacture of practical and inexpensive kits for their detection. A related disease, paratyphoid fever A, that resembles typhoid fever clinically and which is probably under-diagnosed, has recently emerged to be as dangerous as typhoid fever [13]. The propensity and curability is still a challenge for Salmonella Typhi infection in developing countries hence determining the frequency of incidence for this study is important.

MATERIALS AND METHODS

Isolation and processing sample

Isolation of *Salmonella typhi* from the blood and feces was carried out using standard procedures. Blood specimens were cultured on blood and chocolate agar following overnight incubation at 37°C in Robertson's cooked meat medium and Liquid broth. Fresh feces were plated directly on MacConkey and deoxycholate-citrate agar (DCA) and also into selenite F broth which was sub cultured after overnight-incubation at 37°C. Further identification was based on biochemical reactions and agglutination with specific anti sera.

Widal test

Donor sera were screened by slide agglutination with *Salmonella enterica* serotype Typhi O and H antigens (Difco). The positive donor sera and all patients' sera were serially diluted in tubes with 08.5% NaCl from 1/50 to 1/6,400, and antigens (H and O) were added. The tubes were incubated at 37°C for 2 h and then at room temperature overnight and examined for agglutination by an agglutinoscope. The Widal test was performed for all the symptomatic patients; an acute-phase serology was done when the patients were asymptomatic and a second (follow-up) serology done 7 to 10 days later.

Confirmation and antimicrobial susceptibility testing of isolates

Antimicrobial susceptibility testing was done by using the Kirby- Bauer disk diffusion method. The following antimicrobial agents (zone size for resistance) were used: ampicillin 30 µg (> 17 mm), tetracycline 30 µg (> 19 mm), chloramphenicol 30 µg (> 18 mm), ceftriaxone 30 µg (> 21 mm), ciprofloxacin 5µg (> 21 mm), ofloxacin 10 µg (> 16 mm), norfloxacin 10 µg (> 17 mm), nalidixic acid 30 µg (> 19 mm), and gentamicin 10 µg (> 15 mm) (HiMedia Laboratories Ltd., India). Antibiotic susceptibility of the isolates was determined by disc diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines [14].

Slide test for the detection of IgM antibodies

Positive sera which showed a titer of 1:320 for '0' and 1:160 for 'H' agglutination were subjected to a simple test using 2-mercapto-ethanol treatment to detect for the presence of the IgM antibodies. Two sets of dilutions were prepared, first one 100mcl of serum was added with 100mcl of '0' antigen, in second set 100mcl of serum was added with 100mcl of 0.2 M of 2-mercapto-ethanol and left for 10 min after that 100µl of '0' antigen was added. Use of2-mercapto-ethanol selectively destroys IgM and causes a fall in titre.

RESULTS AND DISCUSSION

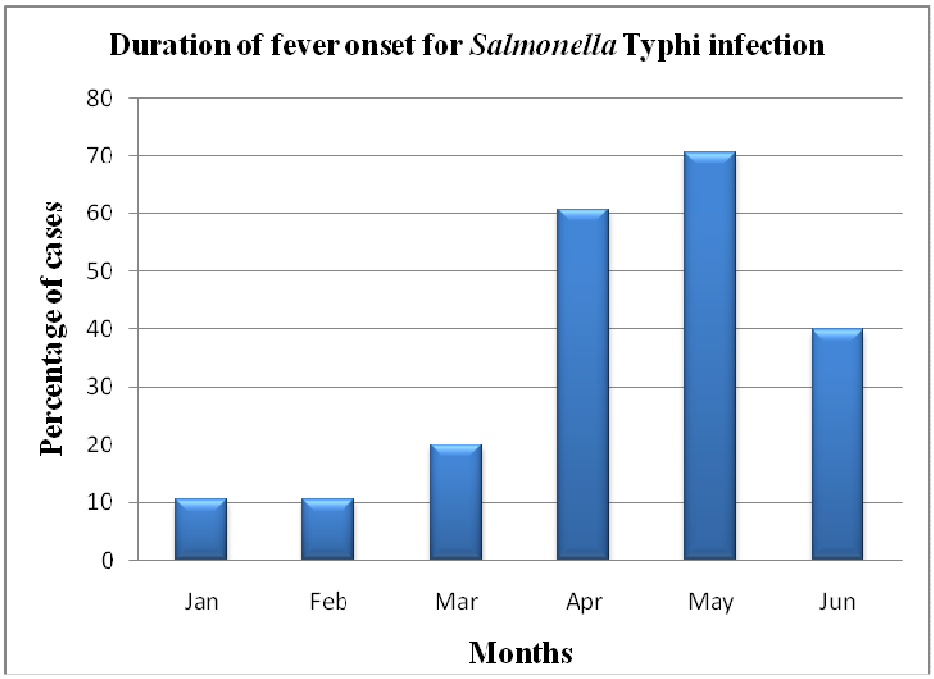
The dates of fever onset in patients with serotype Typhi ranged from February to March 2002, peaking in April and May. A total of 58 serotypes of Typhi isolates were available for testing. Of the 58 isolates tested, (26%) were pansensitive. All of the remaining 34 isolates were resistant to nalidixic acid; 30 were also resistant to

chloramphenicol and tetracycline, and 27 of these were also resistant to ampicillin. Two isolates were also resistant to cefotaxime, one of which was also resistant to norfloxacin. Among the 57 cases with serologic results, there was no statistically significant difference in the typhoid assay results by sensitivity as defined by pansensitivity or resistance to at least one antimicrobial agent. For *S typhi* it can be seen that the majority tested (51%) had an H agglutinin titre of <1/20 with 2% having a titre of 1/160. For 0 agglutinins, 31% had a titre of 1/80 and 2% had a titre of 1/160. Based on these data it was decided that an H and/or 0 agglutinin titre of: 1/320 would be significant and indicative of typhoid fever. Using such criteria the levels of H and 0 agglutinins in bacteriologically proven typhoid cases and cases of non-typhoidal fever was determined.

Out of 10 samples subjected to Mercapto ethanol test 4 showed reductions and 6 sera were negative for the test. About 84% of typhoid fever cases had a significant Widal test result. The test was more sensitive for children than for adults at each cutoff for both O and H antigens but was less specific at O- and H-antigen titers of 100. In adults, the sensitivity for both O and H antigens increased with the duration of illness before admission In contrast, only 2.8% of patients with non-typhoidal fever showed a significant Widal reaction. Of the 19 typhoid fever cases which gave a normal Widal test result, five had been treated with full courses of antibiotics (chloramphenicol), four patients remained febrile even after 10 days of fluoroquinolone therapy but were observed sensitive to chloramphenicol. There was no significant relationship between the Widal test results and a history of prior antibiotic therapy.

Researchers continue to search for the ideal rapid test to diagnose acute typhoid fever. Several urine assays have been developed [15], although none has proved optimal. Salmonellae are divided into serological groups on the basis of O or somatic antigens. Group D organisms, including *S.typhi*, possess O-antigen 9. Cross-reactions, producing a false-positive O-antigen titer in the Widal test, can therefore occur with any of these serotypes [16, 17]. *S typhi* agglutinins against both H and 0 antigens may be present in the normal population at titres of up to 1/160. This finding is in agreement with a study carried out in Sri Lanka, another endemic area, where agglutinin titres of up to 1/80 were discovered even in the normal population. The earliest serological response in acute typhoid fever is on rise in the titer of the O antibody, with an elevation of the H-antibody titer developing more slowly but persisting longer than that of the O-antibody cutoff titer value.

Figure: 1



In this study, 17% of patients with blood culture-positive typhoid fever had no detectable O antibodies at a cutoff titer of 100, and 33% had any detectable H antibodies at a cutoff titer of 100. Although these patients may have had antibodies at a lower titer, it is well recognized that patients with confirmed typhoid fever may have a negative Widal test throughout the course of their illness, although the proportion has varied in different reports [18]. This lack of antibody response among patients with blood culture-positive typhoid fever has been attributed to undefined host or bacterial factors or prior antibiotic treatment. Mercapto ethanol test was performed to detect IgM antibodies in Widal positive sera, where the titre is on the border line between significant titre and normal local population due to various reasons [19, 20]. The presence of IgM is an evidence of recent infection and the 6 sera negative for the test had only IgG antibodies indicating past infection [21]. With the recent sequencing of the entire serotype Typhi genome, it may now be possible to identify other antigens, such as fimbrial antigens, that may produce an antibody response specific to serotype Typhi [22]. More sophisticated molecular techniques for diagnosis, such as PCR, are also being explored and implemented.

CONCLUSION

Several factors have contributed to this uncertainty of exactly identifying Typhi infections. These include poorly standardized antigens, the sharing of antigenic determinants with other Salmonellae serovars and the effects of treatment with antibiotics and previous immunization with TAB vaccine. It is a tube dilution test which measures agglutinating antibodies against the lipopolysaccharide O and protein flagellar H antigens of *S. typhi*. The value of the test for the diagnosis of typhoid fever has been debated for as many years as it has been available. It is clear that any interpretation as to the significance of a Widal test result must be made against these "baseline" information titres of H and 0 agglutinins and need to be interpreted against a baseline titre in normal individuals living in the same geographical location. A result that appears to be a false-positive test compared to a blood culture may in fact be completely positive. A false-positive may be the result of past infection with serotype Typhi or another non typhoidal *Salmonella* serotype that shares common antigens. This situation may occur because the acute-phase sample was obtained late in the natural history of the disease, because of high levels of background antibodies in a region of endemicity, or because in some individuals the antibody response is blunted by the early administration of an antibiotic course. This change in pattern of susceptibility was reflected even in places far away, such as the United Kingdom and the United States of America. The ability to diagnose typhoid fever on an early, single specimen is also of therapeutic value as early diagnosis is vital in typhoid; delay in starting treatment may result in complications such as perforation or hemorrhage of the small bowel, which may be fatal. Thus the test can be of diagnostic value in the early stage of disease and thus help in reducing morbidity and mortality.

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REFERENCES

[1] Sinha A, Sazawal S, Kumar R, Sood S, Reddaiah VP, Singh B, Rao M, Naficy A, Clemens JD, Bhan MK: *Lancet*, **1999**, 354,734-737.
[2] Mermin JH, Townes JM, Gerber M, Dolan N, Mintz ED, Tauxe RV: *Arch Intern Med*, **1998**, 158,633-638.
[3] Pang, T., Z. A. Bhutta, B. B. Finlay, and M. Altwegg. *Trends Microbiol*, **1995**, 3,253–255.
[4] Pang, T., M. M. Levine, B. Ivanoff, J. Wain, and B. B. Finlay. *Trends in Microbiology*, **1998**,6,131–133.
[5] Widal, F. Serodiagnostic de la fie`vre typhoide a`-propos d'une modification par M. M. C. Nicolle et A. Halipre. *Bull. Mem. Soc. Med. Hop. Paris*, **1896**,13,561–566.
[6] Crump JA, Luby SP, Mintz ED. *Bull World Health Organ*, **2004**,5, 346–353.
[7] Hoa, N. T. T., T. S. Diep, J. Wain, C. M. Parry, T. T. Hien, M. D. Smith, A. M. Walsh, and N. J. White. *Trans. R. Soc. Trop. Med. Hyg*, **1998**,92:,03–508.
[8] Hoffman, S. L., N. H. Punjabi, R. C. Rockhill, A. Sutomo, A. R. Rivai, and S. P. Pulungsih. *J. Infect. Dis*, **1984**,149,157–161.
[9] Grunbaum, A. S. *Lancet*, **1896**,806–807.
[10] van den Bergh ET, Gasem MH, Keuter M, Dolmans MV: *Trop Med Int Health*, **1999**, 4,211-215.
[11] Rowe B, Ward LR, Threlfall EJ: *Clin Infect Dis*, **1997**, 24(Suppl 1),106-S109.
[12] Levine, M. M., and W. A. Orenstein. Typhoid fever vaccines, *In* S. A. Plotkin (ed.), Vaccines, 3rd ed. W. B. Saunders Co., Philadelphia,Pa, **1999**, 781– 814.

[13] Vollard AM, Ali S, Widjaja S, Asten HA, Visser LG, *et al*. *Trans R Soc Trop Med Hyg*, **2005**,6, 440–450.
[14] National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing; twelfth informational supplement. M100-S12. National Committee for Clinical Laboratory Standards. **2002**,Wayne, PA.
[15] 203. Fadeel, M. A., J. A. Crump, F. J. Mahoney, I. A. Nakhla, A. M. Mansour, B. Reyad, et al., *Am. J. Trop. Med. Hyg*, **2004**,70,323–328.
[16] Pang, T., and S. D. Puthucheary. *Southeast Asian J. Trop. Med. Public Health*, **1989**, 20,163–164.
[17] Reynolds, D. W., L. Carpenter, and W. H. Simon. *J. Am. Med. Assoc*, **1970**, 204, 2192–2193.
[18] Saha, S. K., M. Ruhulamin, M. Hanif, M. Islam, and W. A. Khan. *Ann. Trop. Paediatr*, **1996**, 16, 75–78.
[19] Sommerville, P. C., M. Lewis, H. J. Koornhof, M. Alberts, H. W. Alberts, and R. Raymond. *S. Afr. Med. J*, **1981**, 59,851–854.
[20] Lakshminarayan, C *et al. J Mediscope*, **1976,** (8)8.
[21] Banatavala, J.E. *et al*., *BMJ*, **1967**, (3)285-286.
[22] Wain, J., D. House, J. Parkhill, C. Parry, and G. Dougan. *Lancet Infect. Dis*, **2002**,2,163–170.