Al Ameen J Med Sci 2014; 7(1):72-77 ● US National Library of Medicine enlisted journal ● ISSN 0974-1143

ORIGINAL ARTICLE CODEN: AAJMBG

A study to determine significant titre-values of widal test in the diagnosis of enteric fever for a population of north Kerala, India

Gufran Ahmed M. Bijapur[1*], Shamshuddin R. Kakkeri[2], Raysa N.P[1] and Shaik Mohammed Usman[1]

[1]Department of Microbiology, Kannur Medical College, Anjarakandy, Kannur District-670612, Kerala, India and [2]Department of Forensic Medicine, Al Ameen Medical College, Athani Road, Bijapur-586108, Karnataka, India

Abstract: *Objectives*: To determine the baseline Widal titres of a study population and to propose titre-values of significance in the diagnosis of enteric fever. *Background*: Enteric fever is endemic in the Indian subcontinent with high prevalence rates. Etiological diagnosis of the condition is problematic due to several limitations implicit in the region. Singly run Widal test is often the only means available for a workable diagnosis. A knowledge of prevalent baseline titres is essential for its interpretation. *Method*: We performed the standard Widal tube agglutination test on the serum samples of 250 individuals of the local population selected by using appropriate criteria of inclusion and exclusion criteria. *Results*: A majority (74.8%) of the subjects were non-reactive. Prevalence of anti-TO antibody was highest (25.2%) followed by that of anti-TH antibody (15.2%), anti-AH antibody (6.8%) and anti-BH antibody (0.8%). Based on the distribution of titre values, the baseline titres determined were 40 for anti-TO and anti-TH antibodies and <20 for anti-AH and anti-BH antibodies. Any value obtained in Widal test over and above these values, i.e., ≥80 for anti-TO and anti-TH antibodies can be considered as significant. Similarly, for anti-AH and anti-BH antibodies, values of ≥20 can be considered as significant for diagnosis in an appropriate clinical setting. *Conclusion:* The baseline Widal titres of the local population were lower than those found in most other parts of India.
Keywords: Widal, Baseline Titres, Typhoid, Kerala.

Introduction

Enteric fever is a serious public health problem in developing countries [1-2]. The Indian subcontinent is a hotspot of typhoid activity [3-9]. Clinical diagnosis of typhoid may be difficult. The presentation may be altered or at times, atypical [10-11]. Although the mainstay of diagnosing typhoid fever is a positive blood (and less definitively urine and stool) culture, the test is positive in only 40-60% of cases [12] usually early in the course of the disease. In developing countries, sensitivity of blood cultures is lower still. Patients visit the hospital late in the course of disease. They frequently take antibiotics as self medication or upon unauthorized prescription before visiting the hospital. Culture facilities for an etiological diagnosis are mostly unavailable. Where available, these methods are frequently sub-optimal. Other demerits of the test are its cost and relatively long turnaround time. As such, blood culture is a grossly under-utilized and under-performed test in the developing world and thus the definitive diagnosis of enteric fever is problematic. Recourse is made to the use of Widal test which offers a simpler, rapid and cost-effective alternative.

It is a classic serologic reaction developed by F Widal in 1896 [13] that results in clumping of a suspension of killed Salmonella cells as antigen by a specific antibody expected to be present in the serum of a patient suspected to be infected with the organism. It involves the use of bacterial suspensions of *S typhi* and *S paratyphi* 'A' and 'B', treated to retain only the 'O' and 'H' antigens. The IgM somatic O antibody appears first, while the IgG flagella H antibody usually develops more slowly but persists for longer [14-16]. The slide test is rapid and is used as a screening procedure. The tube agglutination test [17-18] serves as a means of confirming the results and clarify erratic or equivocal reactions of the slide test. It is essentially an indirect evidence of infection where antibody response rather than

the pathogen or its components is detected. But the antibody detected and its titre has to be 'meaningful' for it to be considered as evidence of 'current infection'. The recommended definitive interpretation of the Widal test is a 4-fold rise in agglutinins taken 7-10 days apart [19]. Clinically however, this is rarely demonstrated and 2-3 fold rises are commonly seen [20] probably due to the fact that titres are already raised when the patient's serum was first tested [21]. Towards the end of first week, titres of either O or H or both may rise to as high as 1:160 [22]. The titres rise for the first four weeks followed by fall thereafter. Once elevated above the baseline, the titres remain high for a period of 6 months. In some cases due to severe hypoproteinemia [21] or early administration of antibiotics [23] the titres may not rise at all. But generally, the lack of paired sera may lead to erroneous interpretation of test results [24-25].

Non-feasibility of obtaining the second serum sample from patients makes it practically unhelpful in establishing diagnosis. Hence a single cut-off value is widely used. Indeed, in many developing countries the use of a single Widal test appears to be the norm as it often is the only laboratory means employed in the diagnosis of typhoid [22]. In endemic areas anti-salmonella antibody is almost invariably present in the community in varying titres due to continuous exposure in the form of clinical and subclinical infections. These 'normal' O and H agglutinin titres in the population – 'the baseline titres' vary with region and with time. Apart from clinical typhoid infections, the Widal titres are elevated in healthy carrier statuses, TAB vaccination, asymptomatic infections and non-typhoid salmonella infections. Epidemiological studies in an endemic country have shown that at least 7 subclinical cases occur for each clinical case [26]. Furthermore, there are many other conditions such as malaria, brucellosis, dengue fever, chronic liver disease, endocarditis and infections due to other enterobacteriaceae, where the Widal titres may be elevated due to antigenic cross reactions [27]. There are more than 40 cross reacting antigens between *S. typhi* and other enterobacteriaceae [28]. Most of the above conditions are also endemic in typhoid-endemic regions. Therefore the baseline Widal titres are high in endemic compared to non-endemic areas – the level of elevation corresponding to the degree of endemicity and that spiking of titres in febrile illnesses could be due to infections other than typhoid owing to anamnestic responses. The titre-rise in most of these conditions is said to be only transient or non-progressing compared to that in enteric fever where the rise is sustained and progressive [23]. The value of Widal test also depends upon the standardization and maintenance of the antigens to produce consistent results. Lack of standardization of antigens compromises the technique [29] accounting for poor reproducibility and erroneous results.

Due to the multiple reasons given above, both positive as well as negative results of the test may be open to several different interpretations and hence wrong diagnoses. According to Welch in 1936, no Widal test, regardless of the composition and standardization of the antigens used, is infallible [30]. Schroeder [31] concluded that the test is nonspecific, poorly standardized, confusing and difficult to interpret. Many authors have similarly disputed the usefulness of a single Widal test result [32-35]. In contrast to this, a number of reports from developing countries have concluded that even today, it is still a useful diagnostic tool and that with relevant clinical findings the test results were found to be highly suggestive of a diagnosis of typhoid fever [36-41]. Due to these contradicting reports, the Widal test – and its interpretation – is mired in controversy about its usefulness. Going by the latter premise – that the test is still very relevant and useful – we undertook this study seeking to determine the baseline Widal titres in the local population because, interpretation of a single Widal test result needs to be based on the average baseline titres among the healthy individuals in a given population. Antibody titres beyond a cut-off value should be regarded as significantly elevated which may be used for diagnosis in an appropriate clinical setting.

Material and Methods

This was a community based, cross-sectional study which was conducted in the Department of Microbiology, Kannur Medical College from March 2012 to June 2012. Our aim was to determine the average baseline antibody

titre against the Salmonella enterica serotypes among the healthy people of various age groups in the North Malabar region. A total of 250 subjects of both the sexes and of ages between 18 and 50 years, attending out-patient departments with noninfectious causes and having been living in Kannur District (North Kerala) for at least 5 years were enrolled in the study. Individuals who gave a history of suffering from fever during the last three months and those who have taken typhoid vaccine in past were excluded from the study. Clearance from ethics committee of Kannur Medical College was taken. After explaining the study protocol and objectives, a written consent was obtained from the participants.

Two milliliters of venous blood was collected from each subject with aseptic precautions. It was allowed to clot at room temperature for about 30-60 minutes. Serum was separated by centrifugation for 5 minutes and whenever required was stored at 4°C and tests were performed within 48hrs. Four rows were set for each serum sample to be tested. The first row consisted of 5 Felix tubes and the remaining 3 rows of 5 Dreyer's tubes each. Serial 2-fold dilutions of the test serum i.e., 1:10, 1:20, 1:40, 1:80 and 1:160 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 ml of *Salmonella paratyphi*–AH and BH antigens (Span Diagnostics Ltd) were added respectively. So the final serum dilutions obtained for each antigen were 1:20, 1:40, 1:80 1:160 and 1:320. Appropriate positive (positive polyspecific control) and negative (physiological saline) controls were put up for each test. The test tube rack was placed in a water bath at 37 ˚C for overnight incubation. 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

The sample size of our study was 250 who were screened for the presence of anti-TO, anti-TH, anti AH and anti-BH agglutinins by the Widal tube agglutination test. Sixty three (25.2%) samples were positive (i.e., showed a titre of ≥ 20) whereas 187 (74.8%) samples did not show agglutination [Table 1].

Table-1: Distribution of positive and negative samples for agglutination in Widal Test

Widal reactivity	Frequency	Percentage
Positive (≥ 1:20)	63	25.2
Negative (<1:20)	187	74.8
Total	250	100

Table 2 shows the relative reactiveness of the subjects to the various salmonella antigens used in the Widal test. The agglutinins to S. typhi were the most prevalent (25.2% for the TO antigen and 15.2% for the TH antigen). Positive agglutinations for AH antigen were found to be less (only 6.8%) and those for BH were found to be meager (only 0.8% with only 2 subjects reacting).

Table-2: Distribution of positive samples (with Antibody titres >20) against different serotypes of Salmonella

Serotype	Antibody type	Frequency of agglutinating sera (n=250)	Percentage (%)
Typhi	Anti O antigen	63	25.2
Typhi	Anti H antigen	38	15.2
Paratyphi A	Anti H antigen	17	06.8
Paratyphi B	Anti H antigen	02	00.8

Table-3: Number & percentage of reactive sera in various titres in the study population.

Antigen	No. of +ve samples (%)	Dilution (1:20)	Dilution (1:40)	Dilution (1:80)	Dilution (1:160)
S. typhi O	63(25.2)	30(12)	25(10)	07(2.8)	1(0.4)
S. typhi H	38(15.2)	21(08.4)	08(03.2)	08(03.2)	1(0.4)
S. paratyphi AH	07(02.8)	06(02.4)	01(0.4)	00(0%)	00(0%)
S. paratyphi BH	02(0.8)	01(0.4)	01(0.4)	00(0%)	00(0%)

Table 3 reveals the distribution of titres in positive samples. Among the 63 sera which showed agglutination to TO, 30(12% of the study population) had a titre of 20, 25(10%) had a titre of 40 and 07(2.8%) had a titre of 1:80, while only 1 sample (0.4%) had the highest titre of 1:160. Similarly, among the 38 samples which showed the anti-TH titre of ≥ 20, 21 samples (8.4%) had a titre of 20, 8(3.2%) had a titre each of 40 and 80. One agglutination (0.4%) was seen in a dilution of 1:160 which was the highest titre for anti-TH agglutinin. As expected, agglutination titres for anti-AH anti-BH were low. A total of 7 samples (2.8%) showed an agglutination titre of ≥1:20 for anti-AH. This comprised of 6 samples (02.4%) with an agglutinating titre of 20 and one (0.4%) that of 40. As for anti-BH, the titres were extremely low – only 2 samples were positive – one each with titres of 20 and 40.

Discussion

It is ironical that the best practices of diagnosis are observed in regions of the world where enteric fever is far less prevalent and in countries where the disease is rampant and where such practices are more acutely needed, it often goes undiagnosed or overdiagnosed. Due to reasons elucidated in the introduction, physicians in the developing world have to make do with a 100 year old procedure which is fraught with many inadequacies and which has largely been abandoned in most western countries [22]. Between the two extreme opinions regarding the clinical utility of Widal test, the present authors believe that although it is unscientific to base the diagnosis of enteric fever solely on the results of Widal test, it can be used with benefit to suggest such a diagnosis particularly when there is no other confirmatory supportive test, such as positive culture, available.

Indeed it is imperative to make use of this test in resource-constrained countries where the prospects of improving upon the practices relating to making of an accurate diagnosis of typhoid seem too unrealistic to be realized in the immediate future. Interpretation of a single Widal test demands a knowledge of the prevalent baseline titres in the community. Based on this, a cut-off titre would be assigned for the community the finding of which in a single Widal test in a febrile patient would serve as presumptive evidence of infection. Thus, each geographical area needs to have its own titre-values – the baseline and significant titres. To fulfill this need we undertook this study. We selected 250 individuals belonging to age groups between 18 and 50 representing the local population for the study. Inclusion and exclusion criteria were designed to ascertain they were not in a state of current ongoing infection of any kind or of artificial immunity to typhoid. Their sera were tested with the Widal tube agglutination test for the presence of antibodies to *S. typhi* and *S. paratyphi* serotypes A and B.

The results showed a certain degree of sero-prevalence (25.2%) of salmonella agglutinins among the members of the community. But the vast majority of the subjects (74.8%) were nonreactive. In those who had the antibody, the titres were not very high – only 8 (3.2% of the subjects) and 9 (3.6%) sera agglutinated TO and TH antigens respectively in dilutions > 1:80. The highest titre found for both anti-TO & anti-TH was 160 (1 subject each). Only 7 (02.8%) subjects were reactive to AH antigen and 2 (0.8%) to BH antigen. Collard et al offered an interesting proposal for arriving at the so called significant Widal titers for a population.

They proposed the value that the titer of agglutinins to be considered of significance should be such as would not be expected in more than 5% of normal population [42]. It follows from this premise that, the highest value found in the remaining 95% of the studied healthy population can still be taken as baseline titer. Applying the same premise to our findings, < 5% of the subjects had the highest titres of ≥80 for both anti-TO (3.2%) and anti-TH (3.6%) and >95% of the subjects had the titres of ≤40 for anti-TO and anti-TH agglutinins. Hence the baseline titre for both anti-O and anti-H Widal agglutinins in our population is 40 and any value found in Widal test for TO and TH antigens in titres of ≥80 can be considered significant in the diagnosis of typhoid fever.

Similarly in agglutinations with AH & BH antigens, >95% (97.2% & 99.2% respectively)

showed no agglutination at all. Hence significant titre for anti-AH and anti-BH is ≥20 along with a finding of significantly raised anti-TO and a clinically relevant situation. These findings are not entirely consonant with those found in studies conducted on populations of other regions of our country [43-46] where higher baseline titres are reported. Probably this is a reflection of a higher level of health awareness and better public health engineering and sanitation facilities found in this region.

Conclusion

Baseline Widal titres vary from region to to region and with time to time. It is essential to know the levels of seroprevalence of the community to interpret the results pf Widal test. In the local population we studied (North Malabar region, Kerala, India), the baseline titres of anti-TO and anti-TH were found to be 40 and those of anti-AH & anti-BH, <20. Any titres obtained in a single Widal test over and above these values i.e., ≥80 for anti-TO & anti-TH and of ≥20 for of anti-AH & anti-BH can be considered to be significantly raised to suggest a diagnosis of enteric. Considering the high endemicity and seroprevalence of typhoid in most parts of India, these values seem considerably low.

References

1. Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. *Bul World Health Organ* 2004;82:346-53
2. Bhutta ZA. Current concepts in the diagnosis and treatment of typhoid fever. *BMJ* 2006; 333:78-82.
3. Ochiai RL, Acosta CJ, Danovaro-Holliday MC, et al. A study of typhoid fever in five Asian countries: disease burden and implications for controls. *Bull World Health Organ* 2008; 86:260.
4. Ochiai RL, Acosta CJ, Danovaro-Holliday MC et al. A study of typhoid fever in five Asian countries: disease burden and implications for control. *Bull World Health Organ* 2008; 86(4):260-68.
5. Chuttani CS, Prakash K, Vergese A et al. Effectiveness of oral killed typhoid vaccine. *Bull World Health Organ* 1971; 45:445-450.
6. Chuttani CS, Prakash K, Vergese A et al. Ineffectiveness of an oral killed typhoid vaccine in a field trial. *Bull World Health Organ* 1973; 48:756-757.
7. Chuttani CS, Prakash K, Gupta P et al. Controlled field trial of a high- dose oral killed typhoid vaccine in India. *Bull World Health Organ* 1977; 55(5):643-644.
8. Sinha A, Sazawal S, Kumar R et al. Typhoid fever in children aged less than 5 years. *Lancet* 1999; 354: 734-737.
9. Ochiai RL, Wang XY, Von Siedlein L et al. Salmonella Paratyphi A rates, Asia. *Emerg Infect Dis* 2005; 11(11): 1764-1766.
10. Hathout S el-D. Relation of schistosomiasis to typhoid fever. *J Egypt Public Health Assoc* 1970; 45:145-56.
11. Nsutebu EF, Martins P, Adiogo D. Prevalence of typhoid fever in febrile patients with symptoms clinically compatible with typhoid fever in Cameroon. *Trop Med Int Health* 2003; 8:575-8.
12. World Health Organization Department of Vaccines and Biologicals. Background document: the diagnosis, prevention and treatment of typhoid fever.Geneva: *WHO* 2003; 19-23. *(www.who.int/entity/vaccine_ research/documents/en/typhoid_diagnosis.pdf.)*
13. Widal FM. Serodiagnostic de la fiévre typhoid a-propos d'uve modification par MMC Nicolle et al. Halipie. *Bull Soc Med Hop Paris* 1896; 13:561-6.
14. Manson-Bahr PEC, Bell DR. In: Manson's tropical diseases, 19th edn. London: *Bailliere-Tindall* 1987; 194-206.
15. Anon. Typhoid and its serology. *BMJ* 1978; 18:389-90.
16. Hoffman SL, Flanigan TP, Klancke D, et al. The Widal slide agglutination test, a valuable rapid diagnostic test in typhoid fever patients at the infectious disease hospital in Jakarta. *Am J Epidemiol* 1986; 123:869-75.
17. Welch H, Mickle FL. A rapid slide test for the serological diagnosis of typhoid and paratyphoid fevers. *Am J Public Health* 1936; 26:248-55.
18. Gaultney JB, Wende RD, Williams RP. Microagglutination procedures for febrile agglutination tests. *Appl Microbiol* 1971;22:635-40.
19. Somerville PC, Lewis M, Koomhof HJ, et al. The Widal test in the diagnosis of typhoid fever in the Transvaal. *South Afr Med J* 1981; 59:851-4.
20. Satma VNB, Malaviya AN, Kumar R, et al. Development of immune response during typhoid fever in man. *Clin Exp Immunol* 1977; 28:35-39.
21. Chessbrough M: Medical Laboratory Manual for Tropical Countries Cambridge *University Press* 2000; 186.
22. Olopoenia LA, King AL. Widal agglutination test - 100 years later: still plagued by controversy. *Postgraduate Medical Journal.* 2000; 76(892):80-4. Epub 2000/01/22.
23. Ananthanarayan R, Paniker CKJ: Textbook of Microbiology Hyderabad, *Orient Longman* 2013; 298.
24. Hoffman SL, Flanigan TP, Klancke D, et al. The Widal slide agglutination test, a valuable rapid diagnostic test in typhoid fever patients at the infectious disease hospital in Jakarta. *Am J pidemiol* 1986; 123:869-75.
25. Olopoenia L, Oyewole F, Onafowokan R, et al. Widal agglutination in malaria infection in Nigeria (abstract). *Clin Immunol Immunopathol* 1995; 75:314.

26. Murphy HR, et al. Characteristics of humoral and cellular immunity to Salmonella typhi in residents of typhoid endemic and typhoid free regions. *J Infect Dis* 1987; 156:865-67.
27. Colle JG, Frason AG, Marmion BP, Simmons A: Mackie and Mc Cartney Practical Medical Microboiology New York, *Churchill Livingston* 1996; 38.
28. Parry CM, Hoa NT, Diep TS, Wain J, Chinh NT, Vinh H, Hien TT, White NJ, Farrar JJ: Value of a single-tube widal test in diagnosis of typhoid fever in Vietnam. *J Clin Microbiol* 1999; 37:2882-6.
29. DeVillier AB, Deupree RH, Dickinson C, Beeler MF. Comparative study of 'O' antigens. *Am J Clin Pathol* 1965; 44:410-2.
30. Welch H, Mickle FL. A rapid slide test for the serological diagnosis of typhoid and paratyphoid fevers. *Am J Public Health* 1936; 26:248-55.
31. Schroeder AS. Interpretation of serologic tests for typhoid fever. *JAMA* 1968; 206:839-40.
32. Reynolds DW, Carpenter RL, Simon WH. Diagnostic specificity of Widal's reaction for typhoid fever. *JAMA* 1970; 214:2192-3.
33. Hook EW. Salmonella species (including typhoid fever). In: Mandell GL, Douglas RG, Bennett JE, eds, Principle and practice of infectious diseases, 2nd edn. New York: *Wiley Medical Publications,* 1985; 1256-67.
34. Aquino RL, Lansong MAD, Quimpo VS, Sambrero LT, Saniel MC. Evaluation of a single Widal test in the diagnosis of enteric fever. *SE Asian J Trop Med Public Health* 1991; 22:375-9.
35. Levine MM, Grados O, Gilman RH, et al. Diagnostic value of Widal test in areas endemic for typhoid fever. *Am J Trop Med Hyg* 1978; 27:795-800.
36. Mohammed I, Chikwem JO, Gashau W. Determination by Widal agglutination of baseline titre for the diagnosis of typhoid fever in two Nigerian states. *Scand J Immunol* 1992; 11(suppl):153-6.
37. Rasaily R, Dutta P, Saha WR, et al. Value of a single Widal test in diagnosis of typhoid fever. *Indian J Med Res* 1993; 97:104-7.
38. Pang T, Puthucheary S. Significance of Widal test in the diagnosis of typhoid fever in endemic areas. *J Clin Pathol* 1983; 36:471-5.
39. Choo KE, Razif AR, Oppenheimer SJ, et al. Usefulness of Widal test in diagnosing childhood typhoid fever in endemic areas. *J Paediatr Child Health* 1993; 29:36-9.
40. Wicks ACB, Cruickshank JG, Museeve N. Observations on the diagnosis of typhoid fever in an endemic area. *SAfr Med J* 1974; 48:1368-70.
41. Kulkarni ML, Rego SJ. Value of single Widal test in the diagnosis of typhoid fever. *Ind. Pediatr* 1994; 31:1373-77.
42. Collard P, Sen R, Montefiore D. The Distribution of Salmonella Agglutinins in sera of Healthy Adults at Ibadan. *J. Hygiene* 1959; 57:427-33.
43. Shukla S, Patel B, Chitnis DS. 100 years of the Widal test and its reappraisal in an endemic area. *Indian J Med Res* 1997; 105: 53-57.
44. Punia JN, Joshi RM, Gupta V, Arora RK. Determination of the baseline Widal titre in Chandigarh. *Ind. Journal of Medical Microbiology* 2003; 21 (2):144.
45. Patil AM, Kulkarni ML, Kulkarni AM. The baseline Widal titre in healthy children. *Ind. J. Paed* 2007; 74:1081-83.
46. Pal S, Prakash R et al. The Baseline Widal Titre Among the. Healthy Individuals of the Hilly Areas in the. Garhwal Region of Uttarakhand, India. *JCDR* 2012; 4889: 2508.

*All correspondences to: Dr. Gufran Ahmed M. Bijapur, Associate Professor, Department of Microbiology, Kannur Medical College, Anjarakandy, Kannur District-670612, Kerala, India. E-mail: drgufran@rediffmail.com