ORIGINAL ARTICLE

Baseline Titre of Widal amongst Healthy Blood Donors in Raichur, Karnataka

Abdul Kaleem Bahadur[1], B. V. Peerapur[1]*
[1]Department of Microbiology, Raichur Institute of Medical Sciences, Raichur - 584101, (Karnataka) India

Abstract:

Background: Lack of proper knowledge of baseline titre of Widal test can lead to over diagnosis of typhoid fever leading to mismanagement of patients. Rapid semiquantitative slide Widal test has replaced conventional tube Widal test in many laboratories, but there is paucity of scientific data regarding how far the results of this rapid test correlate with that of conventional tube Widal test. *Aims and Objectives:* To evaluate baseline titre of widal using quantitative tube Widal test, to suggest the cut-off value for positive Widal test in this region, and to correlate the results obtained in quantitative tube Widal test with that of rapid semiquantitative slide Widal test. *Material and Methods:* Sera of 107 apparently healthy blood donors (AHDs) were subjected to quantitative tube and semiquantitative slide Widal test to know the titre. *Results:* Highest titre obtained by tube Widal test for TO was 1:160, for TH - 1:320, for AH- 1:20, and for BH- 1:80. Tube Widal titres of $\leq$ 1:80 for TO and TH were seen in 103(96.2%) and 97(90.6%), TO and TH titres of $\geq$ 1:160 were seen in 4(3.7%) and 10 (9.3%) respectively. TH titre of 1:320 was seen in 1(0.93%) and no such high titre was reported in relation to TO. Highest titre obtained by semiquantitative slide Widal for TO was 1:320, for TH- 1:320, for AH-1:40 and for BH- 1:160. *Conclusion:* We recommend TO and TH titre of $\geq$ 1:320 as diagnostic of typhoid fever and for AH and BH, titres of $\geq$1:40 and $\geq$1:160 should be considered diagnostic respectively in our region. Because of high expected false positivity rate of slide Widal test even at the higher cut-off titre of 1:320, single slide Widal test appears to have little value in the diagnosis of typhoid fever in this region.

Key Words: Typhoid Fever; Tube Widal; Slide Widal; Baseline Titre.

Introduction:

Typhoid fever has continued to pose considerable health problems in developing world. While bacteriological culture remains the gold standard for definitive diagnosis of typhoid fever, lack of immediate availability of its results during the acute febrile illness and wider use of rapid Widal test have led to underutilization of this test. Widal agglutination test is still the most commonly done laboratory test for diagnosis of suspected typhoid fever in developing countries [1]. But the test suffers from serious cross-reactivity with other infectious agents; it may produce false-positive results, leading to an over-diagnosis of typhoid fever [1]. This problem is made worse by misdiagnosis through the use of a single pretreatment Widal agglutination test [2]. Erroneous interpretation of this test results may lead to misdiagnosis and mismanagement of the patients, resulting in major morbidity and mortality.

Background:

In endemic country like India sera of proportion of healthy individuals contain antibodies capable of reacting to variable titre in Widal test [3]. The knowledge of local baseline titre in healthy individuals will help in correct interpretation of this commonly done test. Hence the baseline titre among Apparently Healthy Blood Donors (AHDs) in Raichur district, Karnataka was evaluated. To our knowledge no such evaluation was done till date in this region. In this region where the use of a single slide Widal test appears to be a norm, for routine screening of suspected typhoid cases, tube Widal test which is technically demanding is often replaced by a semiquantitative slide Widal test by many laboratories for estimation of titre for those who test positive in rapid slide screening test. Many studies in various endemic regions were conducted in the recent past to know the baseline titre of Widal test using tube agglutination test and few similar studies have used rapid slide agglutination test [4, 5]. But we have found dearth of literature, comparing the results of rapid semiquantitative slide Widal (which is more popular because of its simplicity to perform and quick results) with the tube Widal agglutination test which is standard recommended test for this purpose. Hence we took the task of comparing the titre of semiquantitative slide Widal test with that of tube Widal test.

Material and Methods:

One hundred and seven (107) Apparently Healthy Blood Donors (AHDs) attending RIMS teaching hospital blood bank were included in the study. Average age of the AHDs was 26.7 years ranging from 18-47 years. Amongst whom 103(96.2%) were males and 4(3.7%) were females. AHDs who gave the history of suffering from fever in last three months and those who have taken typhoid vaccine in past were excluded from the study. Five milliliters of blood sample was collected separately using sterile syringe with aseptic precautions (blood bags were not used to collect the samples). Serum was separated and whenever required was stored at 4^{o}C and tests were performed within 48hrs. Samples were subjected to semiquantitative slide Widal and quantitative tube Widal test on the same day to know the titre. Readings of both the tests were recorded by two different trained technicians independently. Span diagnostics test reagents were used for both slide and tube Widal test. Clearance from ethics committee of Raichur Institute of Medical Sciences was taken. An informed consent was taken from all the participants in their vernacular language.

Semi Quantitative Slide Widal Test:

Clean glass slides supplied in the kit were used for the test. 0.005ml (corresponding to titre of 1:320), 0.01ml (corresponding to titre of 1:160), 0.02ml (corresponding to titre of 1:80), 0.04ml (corresponding to titre of 1:40) and 0.08ml (corresponding to titre of 1:20) of undiluted serum were dispensed in respective circles using calibrated micropipette. One drop of appropriate antigen suspension was added to each circle and mixed using separate stick and rotated for one minute to take the readings (Procedure as recommended by the manufacturer). Highest dilution of the serum showing minimum of 50% agglutination was taken as titre.

Quantitative Tube Widal Test:

Standard procedure recommended by the manufacturer was followed and highest dilution of the serum showing minimum of 50% agglutination was taken as titre.

Results:

Table 1: Titers of Tube Widal Test amongst 107 Apparently Healthy Blood Donors

	<1:20	1:20	1:40	1:80	1:160	1:320
TO						
Numbers	33	31	28	11	4	0
(%)	(30.84%)	(28.97%)	(26.16%)	(10.28%)	(3.73%)	(0.00%)
TH						
Numbers	38	28	19	12	9	1
(%)	(35.51%)	(26.16%)	(17.75%)	(11.21%)	(8.41%)	(0.93%)
AH						
Numbers	105	2	0	0	0	0
(%)	(98.13%)	(1.86%)	(0.00%)	(0.00%)	(0.00%)	(0.00%)
BH						
Numbers	95	7	2	3	0	0
(%)	(88.78%)	(6.54%)	(1.86%)	(2.80%)	(0.00%)	(0.00%)

It can be observed from the table 1; that 30.8% (33 out of 107) and 35.5% (38 out of 107) of the AHDs showed no agglutination for TO and TH antibodies respectively by tube Widal test, 98.1% (105 out of 107) and 88.7% (95 out of 107) showed no agglutination for AH and BH antibodies respectively, 96.2% (103 out of 107) of AHDs showed TO titre of $\leq$1:80 whereas 90.6% (97 out of 107) were positive for TH less $\leq$1:80. TO and TH titre of $\geq$1:160 was observed in 3.7 %(4 out of 107) and 9.3 % (10 out of 107) respectively. TH titre of 1:320 was noted in 1(0.93%) case and no such high titre was observed in relation to TO. All the cases of AHD showed AH titre of $\leq$ 1:20 whereas BH titre of 1:40 and 1:80 was noted in 2(1.86%) and 3(2.8%) cases respectively.

Comparison of Titre of Tube Widal with Semiquantitative Slide Widal Test:

Larger number of AHDs showed higher titre when tested by semiquantitative slide Widal test. Titre of $\geq$1:80 for TO was seen in 14.0% (15 out of 107) cases of tube agglutination test in contrast to 37.3% (40 out of 107) cases in semiquantitative slide test. (Chi squire value =15.2, $p<0.001$). Titre of $\geq$ 1:160 for TO was seen in 3.7% (4 out of 107) cases in tube Widal test as compared to 11.2%(12 out of 107) cases in semiquantitative slide Widal test. (Chi squire value = 4.32, $p<0.05$). None of the cases showed titre of 1:320 in tube Widal test whereas 1.8% (2 out of 107) cases showed this

Table 2: Titre of Tube Widal and Semiquantitative Slide Widal Test Among 107 Apparently Healthy Blood Donors

	<1:20	1:20	1:40	1:80	1:160	1:320
TO						
Tube Test	33(30.84%)	31(28.97%)	28(26.16%)	11(10.28%)	4(3.73%)	0(0.00%)
Slide Test	33(30.84%)	6(5.60%)	28(26.16%)	28(26.16%)	10(9.34%)	2(1.86%)
TH						
Tube Test	38(35.51%)	28(26.16%)	19(17.75%)	12(11.21%)	9(8.41%)	1(0.93%)
Slide Test	42(39.25%)	6(5.60%)	27(25.23%)	21(19.62%)	9(8.41%)	2(1.86%)
AH						
Tube Test	105(98.13%)	2(1.86%)	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)
Slide Test	104(97.19%)	0(0.00%)	3(2.80%)	0(0.00%)	0(0.00%)	0(0.00%)
BH						
Tube Test	95(88.78%)	7(6.54%)	2(1.86)	3(2.80%)	0(0.00%)	0(0.00%)
Slide Test	96(89.71%)	2(1.86%)	7(6.54%)	1(0.93%)	1(0.93%)	0(0.00%)

titre in semi quantitative slide Widal test in relation to TO.

The titre of ≥1:80 for TH was observed in 20.5%(22 out of 107) cases by tube Widal test in contrast to 30% (32 out of 107) cases by semiquantitative slide Widal test. Titre of ≥ 1:160 for TH was observed in 9.3% (10 out of 107) cases by tube Widal test compare to 10.3% (11 out of 107) cases in semiquantitative slide Widal test. Titre of 1:320 for TH was seen in 0.93% (1 out of 107) and 1.8% (2 out of 107) cases of tube and semiquantitative slide Widal test respectively.

Titre of ≥1:40 for AH was not seen in any of the case in tube Widal test whereas semiquantitative slide Widal test reported 2.8% (3 out of 107) cases. Titre of ≥1:40 for BH was observed in 4.6% (5 out of 107) cases of tube Widal test compared to 8.4% (9 out of 107) cases reported by semiquantitative Widal test. Titre of 1:160 for BH was reported in 0.93% (1 out of 107) case by semiquantitative tube test whereas tube Widal test has reported none. In contrast to the higher titres which were found more commonly when tested with semiquantitative slide test the number of AHDs tested positive for lower titre of 1:20 were significantly lower when compared to the tube test. The number of AHDs showing titre of 1:20 for TO when tested by tube Widal test was 31(28.9%) compared to 6 (5.6%) by semiquantitative slide test. The difference being significant (Chi squire value = 20.42, $p<0.001$). Likewise the number of AHDs showing titre of 1:20 for TH when tested by tube widal test was 28(26.16%) compared to just 6(5.6%) by semi quantitative slide Widal test (Chi squire value = 16.92, $p<0.001$).

Discussion:

Baseline Titre by Tube Widal Test

The highest titre reported by quantitative tube Widal test was found to be 1:160 for the TO and 1:320 for the TH of *Salmonella enterica* serovar typhi whereas the highest titre for *Salmonella enterica* serovar paratyphi A (AH) and serovar B (BH) were found to be 1:20 and 1:80 respectively, 103 out of 107 (96%) and 97 out of 107 (90.6%) of AHDs have shown TO and TH titre of ≤1:80. Hence the baseline titre for TO and TH in our study was ≤1:80. Taking this into consideration if TO and TH titre of >1:80 (i.e. ≥1:160) is considered as diagnostic (i.e. titre above the baseline), 3.7% cases tested for TO and 9.3% cases tested for TH would give false positive Widal test at this presumptive titre in our area. To address this high false positive rates we recommend a titre of >1:160 (i.e. ≥1:320) for TO and TH as diagnostic of typhoid fever. This would reduce the false positive rate from 13% to 0.9%. Similarly we recommend AH and BH titre of >1:20 (i.e. ≥1:40) and >1:80 (i.e. ≥1:160) as diagnostic titre for paratyphoid A and B respectively in this region. In this study TO and TH titre of ≥1:160 has been observed in 3.7 %(4 out of 107) and 9.3 % (10 out of 107) respectively. Pang T et al (1983) have reported TO and TH titre of ≥1: 160 among 5% and 2% of 300 normal subjects from Malaysia [6]. Bharat MP et al (2009) have reported TO and TH titre of ≥1: 160 among 15% and 12% of 100 blood donors respectively from Nepal [7]. In contrast to these studies some studies from India, Prashant P (2012) [8] and Punia JN (2003) [3] have shown TO titre of ≥1: 160 in none of the 490 and 255 normal subjects tested and TH titre of ≥1: 160 only in 0.6% (3 out of 490) and 1.5% (4 out of 255) respectively. This wide variation in titre of antibodies in different endemic places signifies the importance of evaluating the local titre and interpreting the results of Widal test accordingly. This variation may be the result of difference in safe water supply and sanitary conditions and hence the endemicity of typhoid in these places. In this study ADHs have shown higher titre for BH antibodies compared to AH, this is in contrast to other recent studies from India [3, 8]. Although *Salmonella paratyphi* A is reported to be second most common cause of enteric fever and its incidence is increasing in endemic countries like India and Thailand [9]. There are occasional reports of *Salmonella paratyphi* B infection from our country [10]. Hence, possible endemicity of *Salmonella paratyphi* B organism has to be ruled out in our region by application of proper identification methods of enteric bacilli.

Baseline titre by semi quantitative slide Widal test

The highest titre for both TO and TH obtained using semiquantitative slide Widal test was 1:320. Taking TO and TH titre of >1:80 (i.e. ≥1:160) as presumptive diagnostic titre (i.e. titre above the baseline) as indicated by the tube Widal test above, 21.5% (23 out of 107 cases) would be falsely diagnosed as typhoid cases as compared to 13% (14 out of 107 cases) by tube Widal test. Four (3.7%) AHDs showed titre of 1:320 for TO and TH. Because of this higher titre and high expected false positivity rates even at higher cut off titre of 1:320, slide Widal test in area endemic for typhoid fever provides minimal if any, diagnostic assistance. If at all used, the cut off titre for tube Widal test can-

not be applied to the slide Widal for declaring positive test. Teddy C, et al. (2010) has reported 31(15.5%) blood donors with antibody titre of 320 and 9(4.5%) donors with the antibody titre of 640 against *S. typhi* (D) antigen in the study consisting of 200 blood donors [11]. Another study by Musa A (2011) have reported titre of 1:320 for TO and TH in 7(8.7%) and 11(13.7%) of 80 healthy individuals from endemic area of Iraq [12]. Further large scale studies using titre of more than 1:320 (i.e. 1: 640 and 1: 1280) may be required to address the issue of cut off titre in slide Widal test. Many studies which have used slide Widal test for evaluation of endemic titre have reported higher endemic titre compared to studies which have used the tube Widal test [4, 11, 13].

Conclusion:

A substantial number of apparently healthy blood donors in this region have shown titre of 1:160 for TO and TH by tube Widal test, hence we recommend titre of ≥ 1:320 as diagnostic of typhoid fever. For AH and BH, titre of ≥1:40 and ≥1:160 should be considered diagnostic respectively. Titre observed in semi quantitative slide Widal test were significantly higher than those seen in tube Widal test hence the cut off titre used for tube Widal test cannot be used for slide Widal test. Because of high expected false positivity of single slide Widal test even at the higher cut-off titre of 1:320, slide Widal test appears to have little value in diagnosis of typhoid fever in our region. Further studies are needed to evaluate Widal titre of more than 1:320 in healthy individuals and proven typhoid cases which will help to know the actual accuracy of Widal test in terms of sensitivity and specificity at different cut off titre in this region.

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***Author for Correspondence:** Dr. Abdul Kaleem Bahadur, Assistant Professor, Department of Microbiology Raichur Institute of Medical Sciences Raichur - 584101, Karnataka. India. Cell: 09900177058, E-Mail: kaleembahadur@gmail.com*