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Research Article

Enteric Fever

Determining significant cut off titre of single widal test for identifying presence of active typhoid/paratyphoid infection by studying baseline endemic widal titres from Meerut (UP), India & adjoining sub-urban regions

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Introduction: Globally enteric fever is one of the most infectious disease and endemic in all parts of India. Widal test is considered positive with a fourfold rise in titre in paired sera collected at intervals of one to two weeks or if the antibody titres are higher than the cut -off value in a single test. In endemic areas due to previous exposure and cross reacting antigens, the healthy population may contain antibodies capable of reacting at variable titres in widal test which is called as baseline antibody titre. Widal titres among healthy population of different areas differ substantially and depends upon the endemicity of typhoid in each area which has been changing over time. **Aim:** To establish the baseline endemic titre inorder to determine significant cutoff values for the single Widal test in Meerut and adjoining suburban areas. **Material and Methods:** 580 sera were tested by the Widal tube agglutination test from January 2012 to June 2013. **Results:** Baseline endemic titre for enteric fever in our region for TO and TH was found to be 1:80 and that for AH and BH was noted to be 1:40 and 1:20 respectively . **Conclusion:** For single Widal test, significant cutoff titre for TO and TH was ≥1:160 and that for AH and BH was ≥1:80 and ≥1:40 respectively. Single Widal test can be widely used as the diagnostic tool for enteric fever in endemic areas if we know the baseline endemic titres and this should be updated periodically with time.

Keywords: Baseline endemic Titre, Enteric Fever, Salmonella, Significant Cut-off titre, Widal Test

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Introduction

The term, 'enteric fever' includes typhoid fever which is caused by Salmonella typhi and paratyphoid fever which is caused by Salmonella paratyphi A, B and C [1]. Typhoid fever, a food and waterborne disease caused by Salmonella enteric serotype typhi (S. typhi), is a serious public health problem in developing countries that claims 600000 lives every year [2]. Enteric fever is endemic in India and it continues to be one of the major health problems here [3]. The isolation of Salmonella from blood, stool, bone marrow, bile, or other body fluid is considered the gold standard for the diagnosis of enteric fever [4]. However the isolation rate of Salmonella from blood culture of enteric fever patients is relatively low and is often jeopardized by lack of bacterial culture facilities particularly in developing countries endemic with enteric fever. Culturing bone marrow aspirates has a much higher sensitivity, but for large population-based studies it is neither appropriate nor feasible to perform this in all fever cases. Large studies have to rely on blood cultures which have a sensitivity between 30% and 70% [2, 3]. Reason for reduced Salmonella etection rates in blood cultures include pre-medication with antibiotics prior to culture and low bacterial load in the eripheral circulation. Thus culture positive cases are very less, time consuming and expensive. For these reasons, laboratory diagnosis of enteric fever relies heavily on serological tests such as the Widal test [5]. The Widal test is the most simple, over utilized, specific diagnostic investigation tool available in the local laboratories of developing countries. It's relied upon because of its convenience. The Widal test was developed by Georges Fernand Isadora Widal in 1896. The Widal agglutination test is the diagnostic test which is mostly commonly used for the diagnosis of enteric fever, ever since its introduction 100 years back [2]. Since its introduction as a serologic means of etecting the presence of typhoid fever, the Widal test continues to be plagued with controversies involving the quality of the antigens used and interpretation of the results, particularly in endemic areas [6]. This test detects the antibodies against the O and H antigens of Salmonella typhi and against the H antigens of Salmonella paratyphi A and B. A diagnostic Widal agglutination titre of 'H' and 'O 'agglutinin is considered useful in the diagnosis of typhoid fever in our environment [7]. There are various difficulties associated

With an evaluation of the Widal test in an area where malaria, bacterial septicemia and now due to other salmonellae with cross reacting antigen [8, 9]. In endemic areas due to previous exposure and cross reacting antigens, the healthy population may contain antibodies capable of reacting to variable titres in widal test and this is called as baseline antibody titre. The Widal test is considered positive if in a single test the antibody titres are higher than the significant cut -off value considering the baseline endemic titre at a particular time period for a particular area or a rising titre in paired sera collected at intervals of one to two weeks. The test becomes reliable if at least two properly staged tests show about a four-fold rise in antibody levels. Local baseline endemic titre should be known before interpreting the results of a single test. Though it is generally stated that in India where studies have not been done, titres of 1:100 or more for O agglutinins and 1:200 or more for H agglutinins are significant, the results in a single test by no means prove the presence of enteric fever nor negative results its absence.

In India, most patient's presents late to the hospital and require an immediate diagnosis and specific treatment and often a single sample has to be relied upon, instead of paired serum sample [10]. Therefore baseline titre of antiO and anti-H for salmonella typhi and anti-AH and antiBH for salmonella paratyphi A and B should be known in the area in which one is practicing medicine and thus further a single cutoff value can be decided and widely used [11]. Widal test can be used as a diagnostic tool in endemic areas, if we know the baseline titre in a population. Interpretation of a single Widal test result needs to be based on the baseline titre which is seen among healthy individuals in a particular geographical area. The Widal titres among the healthy populations of different areas differ substantially and this depends upon the endemicity of typhoid in each area, which has been changing over time. Regular updating of the baseline Widal titre is a must for the proper interpretation of the Widal test being performed at a particular time period [12, 13, 14]. With treating physicians having an updated knowledge of the community titre, it is possible for him/her to attach importance to the value of a single acute phase Widal test for the diagnosis of typhoid fever at a particular time period. Recently there has been effort on the part of many developing

Countries to determine the community level of the antibodies against the Salmonella in their endemic zones [8, 10, 15,16,17,18 and 19].Hence, the following study was undertaken with an objective to determine and update the baseline endemic Widal titre (titre of the antibodies to the O and H antigens of S. typhi and to the H antigens of S. paratyphi A and B) amongst apparently healthy individuals in Meerut District and its adjoining suburban areas of western Uttar Pradesh in India. It was also aimed to define and update the significant cut off titre for the Widal agglutination test in a single serum test for the diagnosis of active enteric fever requiring treatment, in community around district of Meerut and adjoining suburban regions.

Material and Methods

The present observational and prospective study was conducted in the Blood Bank and Microbiology department at a Tertiary care Hospital, situated in, Meerut. Approval of the institutional ethical committee was obtained for carrying out this study.

Participants were explained the study protocol and objectives. After obtaining their informed consent verbally, randomly selected, non-repetitive 580 blood samples (2ml each) were collected from healthy blood donors who were in the age group of 18 to 60 years, of both the genders, who attended our blood bank from January 2012 to June 2013. Participants who were apparently healthy were only included as study subjects All the donors who did not have any obvious signs and symptoms of infectious diseases were included in the study. Those with obvious signs and symptoms were excluded. All the donors who were found to be positive for the following screening tests like those for Malaria, Microfilaria, HBsAg and antibodies to HIV, HCV and Treponema pallidum, those who were vaccinated for enteric fever in the preceding three years, those whose blood samples which were submitted for the Widal test or individuals with the history of fever of unknown origin were excluded from the study. Serum was separated immediately and labelled. All the serum samples were processed according to standard tube dilution method. The Widal test was performed with standardized S. Typhi O and H antigen, Para typhi A & Para typhi B (H Antigen). Commercially available coloured antigens which contained the "O" and "H" antigens of S. typhi and "H" antigens of S. paratyphi A and B were used, which were procured from Span Diagnostics Ltd. Master Dilution was prepared by diluting all the serum samples in 1:20 ratio with isotonic normal saline (8.5 g/liter) in such a way that final volume contained a total of 1 ml. The test used two fold serially diluted sera of patients, i.e. doubling dilution. For each sample further 4 dilutions of 1:40, 1:80, 1:160 and 1:320 were made in four test tubes. Then one drop (0.03 ml) each of the antigen suspensions was added to corresponding tubes. All tubes were mixed well, treated in water bath at 37°C for four hours and further read after overnight refrigeration at 4°C. Appropriate positive (positive polyspecific control) and negative (physiological saline) controls were put up for each test. Each tube was checked visually for the agglutination. When antigen suspensions were mixed and incubated, anti-Salmonella antibodies which if present in the serum reacted with the corresponding antigens to give agglutination. The 'O' antigen being a somatic antigen, brought about a granular agglutination at the bottom of the tube resembling "Carpet formation", whereas 'H' antigen being a flagellar antigen, brought about a loose, cottonwool clumps, agglutination. Negative test was represented as compact deposit "Button formation" at bottom for both'O'and 'H' antigen. The antibody titre was reported as the highest dilution of serum which showed distinct visible agglutination.

Results

In the present study 580 blood samples of adults more than 18 years were taken for detection of antibodies against S.typhi-O, S typhi-H, S. paratyphi-AH and S.paratyphi-BH. Of the total 580 samples, there were 202(34.83%) males and 378 (65.17%) females.

Table I- Number and (%) of Sera with End Titre

Salmonellae(n=580)	<1:20	1:20	1:40	1:80	1:160	1:320
S.typhi O	532(91.72)	25(4.31)	21(3.62)	2(0.34)	0	0
S.typhi H	503(86.72)	45(7.75)	26(4.48)	6(1.03)	0	0
S.paratyphi AH	573(98.79)	4(0.69)	3(0.52)	0	0	0
S.paratyphi BH	579(99.83)	1(0.17)	0	0	0	0

The level of O and H agglutinin in 580 (100%) sera is presented in Table I. For S.typhi, it was seen that the majority of sera tested 532(91.72%) had an O agglutinin titre of <1:20 and only 2(0.34%) cases had a titre of 1:80. For S.typhi- H agglutinin,

Titer of <1:20 was seen in 503(86.72%) cases and only 6(1.03%) cases were having a titre of 1:80. H agglutinin titre for paratyphi A & B was <1:20 in 98.79% and 99.83% cases respectively. None of the sera tested for S. paratyphi AH, had a titre of 1:80 and above. For S.paratyphi BH, none of the sera had a titre of 1:40.

The analysis of the data suggested that titre of ≥1:160 is significant cut off titre for S. typhi 0 & H. whereas, for S.paratyphi AH, titre of ≥1:80 is significant cut off titre and for S.paratyphi BH, titre of ≥1:40 is significant cut off titre.

Table II- Number and (%) of Sera with End Titre in Adult Males

Salmonellae(n=202)	<1:20	1:20	1:40	1:80	1:160	1:320
S.typhi O	185(91.58)	10(4.95)	5(2.98)	2(0.99)	0	0
S.typhi H	155(76.73)	25(12.27)	21(10.39)	1(0.49)	0	0
S.paratyphi AH	198(98.01)	2(0.99)	2(0.99)	0	0	0
S.paratyphi BH	202(100)	0	0	0	0	0

Table II shows the distribution of Salmonella agglutinin titres in 202 male. All the males had S. typhi-O, S.typhi-H, S paratyphi A-H and S. paratyphi B-H agglutinin titre present in the sera. Only 0.99% & 0.49% had titre of 1:80 for S.typhi-O and S.typhi-H respectively. Salmonella paratyphi-A agglutinin titre of 1:80 was seen in none of the sample. Only 0.99% males had a titre of 1:40 in their sera. Salmonella paratyphi B agglutinin titre was less than 1:20 in all adult males.

Table III- Number and (%) of Sera with End Titre in Adult Females

Salmonellae(n=378)	<1:20	1:20	1:40	1:80	1:160	1:320
S.typhi O	347(91.79)	15(3.96)	5(1.32)	0	0	0
S.typhi H	348(92.06)	20(5.29)	5(1.32)	5(1.32)	0	0
S.paratyphi AH	375(99.20)	2(0.52)	1(0.26)	0	0	0
S.paratyphi BH	377(99.74)	1(0.26)	0	0	0	0

Table III shows the distribution of Salmonella agglutinin titres in 378 female. All the females had S. typhi-O, S.typhi-H, S paratyphi A-H and S. paratyphi B-H agglutinin titre present in the sera. 347 (91.79%) females had a titre of < 1:20 whereas highest positive titre reported was 1:40 in 5(1.32%) samples. Only 5(1.32%) cases had titre of 1:80 for S.typhi-H whereas 348(92.06%) sera had titre < 1:20 for S.typhi-O among 378 females. Salmonella paratyphi-AH agglutinin and Salmonella paratyphi-BH agglutinin titre of 1:40 Was seen in none of the sample except one (0.26%) case where Salmonella paratyphi-An agglutinin titre was 1:40. More than 99% of the sera from 378 females had Salmonella paratyphi-AH & BH agglutinin < 1:20.

Table IV- Baseline endemic titre of O and H agglutinins in different regions of India

Authors	Study Region	Year	Baseline endemic titre			
			TO	TH	AH	BH
Shukla S et al., [24]	Central India	1997	1:80	1:80	0	0
Punia JM et al.,[15]	Chandigarh	2003	1:80	1:160	1:20	1:20
Patil Anand M et al., [23]	Karnataka (In children)	2007	1:80	1:80	1:40	1:40
Prashant Peshattiwar[22]	Amlapuram(AP)	2011	1:80	1:80	1:40	1:20
A.J Sneha [25]	Pondicherry	2011	1:80	1:80	1:40	1:40
Shekhar Pal et al., [21]	Srinagar Garhwal (UK)	2011	1:40	1:80	1:20	1:20
Vijay K Kataria et al., [27]	Dehradun(UK)	2013	1:160	1:160	1:80	1:160
Shraddha Prasad Gunjal et al.,	Ahmednagar(MS)	2013	1:40	1:40	1:80	1:80
K.Sreenath et al., [26]	Kollam, Kerala	2013	1:160	1:160	1:40	1:20
Garima Mittal et al., [28]	Dehradun(UK)	2013	1:40	1:80	1:20	1:20
Present study	Meerut(UP)	2013	1:80	1:80	1:40	1:20

Discussion

Typhoid fever continues to be a major problem in the developing world. The present study, probably the first one in our area, with a large sample size (n=580), reflects base line community titre in urban and sub-urban population of Meerut (North India). It is an established fact that the titre of agglutinin detectable in non-infected population of different area varies considerably [7]. A high frequency of significant titre for S.typhi and S. Paratyphi was also found in our study.

In our study S.typhi-O agglutinin was present in 1:80 dilution in 2(0.34%) cases and S. typhi-H agglutinin was present in 1:80 dilution in 6(1.03%) cases. J.Punia etal. (2003) [15] from Chandigarh (a highly developed planned city of North India) did not find any case having S.typhi O agglutinin in 1:80 dilution. However, S.typhi- H agglutinin was found in 1:160 dilution in 6(1.03%) cases in their study which is much higher than we found in our study. Study done in Srilanka, another endemic area by Senewiratne K. [9] also found agglutinin titres of up to 1:80 in the normal

Population. T Pang and SD Puthucheary [17] in their study clearly showed that in an endemic area such as Malaysia, S typhi agglutinins against both H and O antigens may be present in the normal population at titres of up to 1:160.

The diagnostic significance of agglutinin O or H has been a matter of controversy. It is widely held that raised O agglutinin is of greater significance [7,9] but few studies have suggested that raised levels up to 1:80 of both S.typhi-O and S.typhi-H can be found in a very small percentage of normal population.

S.paratyphi AH agglutinin was found to be positive in 1:40 dilution in only two cases in our study. On the other hand, no case of S.paratyphi BH agglutinin having titre of 1:40 was seen. There was only the one patient having S.paratyphi BH agglutinin positive in 1:20 dilution, similar to our observations, prevalence of much lower titre of S.paratyphi BH compared to of S.paratyphi AH has been reported in many recent studies e.g. G.O. Oyeyinka etal.[19] observed that mean titre for S.paratyphi BH agglutinin in Ibadan, Nigeria. Bharat M Pokhrel et.al. [10] also, in their study on 100 healthy adults reported S.paratyphi AH agglutinin titre of 1:20 or more in patients (8 had titre of 1:40 and one having 1:80) compared to only 3 cases who had positive titre of 1:20 or more against S.paratyphi BH.

The present study showed noteworthy titre of 1:80 for S.typhi O & S.typhi H in only 0.99% & 0.49% respectively in males (n=202), whereas it was 0% and 1.32% respectively in females (n=378).Very few studies have highlighted variation of the titre based on the sex. Studies on healthy population in Awka (South Eastern Nigeria) showed S.typhi O titre positivity in dilution of 1:80 in 2.4% males and 1.8% female. The reason for S.typhi O titre in males is not clear. It may be due to socio-cultural practices in developing countries where male tends to be more often out of homes where they eat or drink water. However, Ibekwe A.C et al [20] in their study they found S.typhi H titre to be marginally higher in males than females unlike our study where it was marginally higher in females.

S.paratyphi–AH of 1:40 dilutions was seen in 2 males (0.99%) compared to only one female (0.26%) in our study but the minor difference was not found to be statically significant.

Ibekwe A.C. et al. study also found higher positivity of S.paratyphi AH titre in males compared to females. S.paratyphi BH titre was found to be almost similar in both the sexes, as has been reported in few other studies eg. Ibekwe A.C [20].

Summary and Conclusion

We conclude that in an endemic area like Meerut & adjoining Sub-urban Regions (U.P), India, the baseline endemic Widal titre in the community is < 1:80 for S.typhi-O agglutinin in more than 99% population,< 1:80 for S.typhi-H agglutinin in more than 99% population,< 1:20 for S.paratyphi-AH agglutinin in more than 99% population, < 1:20 for S.paratyphi-BH agglutinin in more than 99% population. Community titre for S.typhi is less in females than males. Community titre for S.paratyphiAH & S.paratyphi-BH is, 1/20 in 99% of population irrespective of sex. ased on the above results, baseline endemic titre for enteric fever in our region for TO and TH was found to be 1:80 and that for AH and BH was found to be 1:40 and 1:20 respectively . Positive blood culture is the gold standard for diagnosis of Typhoid fever. In its absence, a fourfold rise of Widal titre both for O & H agglutinin in paired sera at interval of 4 to 7 days is considered diagnostic. Where bacterial culture many a times is not possible in a developing country like ours, in such a situation, single reading of Widal test higher than the community titre in the early stage of the disease may be considered as diagnostic. This will reduce morbidity and mortality from the disease.

We thus conclude that Widal test is an accessible, cheap and simple test which has diagnostic significance in endemic areas provided judicious interpretation of the test is made based on the agglutinin levels prevalent in the normal population of the region at a particular time period. Based on results of our study, for the population of Meerut & adjoining Sub-urban regions, we recommend significant cut off levels for single antibody titre against S.typhi-O and S.typhi-H as ≥1:160, S.paratyphi A as ≥1:80 and S.paratyphi BH as ≥1:40. It is clear that *Salmonella* agglutinins are common among apparently healthy people and as endemicity of typhoid in an area may change over time, more studies should be carried out periodically to determine *Salmonella* agglutinin titre in apparently healthy populations, so

That a better judgment which is based on the prevailing agglutinin titres can be made at a particular time period. Periodic evaluation of baseline titers of Salmonella serotypes agglutinins particularly in endemic areas is necessary to avoid false positive results.

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