

# Baseline Titer and Diagnostic cut off value for Widal test: A Comparative Study in Healthy Blood Donors and Clinically Suspected of Enteric Fever

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## ABSTRACT

Widal test is one of the easily available tools in many areas of developing countries for the diagnosis of enteric fever. The diagnostic titer value should correspond to the baseline titer of the population. In this retrospective study, antibody level against O and H antigens of *S. enterica* serovar Typhi and H antigen of *S. enterica* serovar Paratyphi A and B was determined by single slide Widal test for 400 normal blood donors and 200 clinically suspected patients of enteric fever aiming to establish both the baseline and cut off titer for enteric fever. In blood donors, 5.50% sera were found to have significant agglutinin titer ( $\geq 1:80$ ), with a seroprevalence rate of 5.59% in male and 5.30% in female. However, suspected patients of enteric fever exhibited relatively higher positivity (27%) for at least one of the *Salmonella* antigens with 22.93% of males and 31.86% of females. Among the Widal positive cases, sera positive against *Salmonella* ser. Typhi antigens were 77.27% in blood donors and 70.37% in clinically suspected patients of enteric fever while 18.18% and 14.81% cases were infected with *S. enterica* serovar Paratyphi in respective study groups. Majority of positive cases (>55%) for both O and H antigens of *S. Typhi* showed agglutinin titre of 1:160 in both study groups. In conclusion, the diagnostic value for enteric fever should be established on the basis of local baseline titer and in endemic areas, only the higher value from single slide Widal test can be of diagnostic utility.

**Key words:** Enteric fever; Antibody titer; Widal test; Seroprevalence, *Salmonella* antigens

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## INTRODUCTION

Enteric fever which includes typhoid and paratyphoid fever is a systemic febrile illness caused by the bacterium *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar A, B or C respectively and *S. enterica* ser. Typhi is found to be associated with more than 90% of cases of enteric fever. Paratyphoid fever is clinically similar but milder than typhoid fever.<sup>1</sup> Many middle and low income countries are endemic regions of enteric fever due to an inappropriate level of sanitation. While in industrialized countries; it is mainly manifested after the travel to endemic countries.<sup>2</sup>

There was a global estimation of more than 21.6 million of cases of typhoid fever in 2000 and 5412,744 illness were due to paratyphoid fever.<sup>3</sup> These fevers are considered as a major cause of morbidity and mortality in developing countries with more than 90% of cases found in Asia only.<sup>4</sup>

Nepal is a considered as an endemic region to enteric fever and Kathmandu, the capital city of Nepal is also taken as the capital of enteric fever.<sup>5</sup> In Nepal, 11,337 patients were admitted to the hospital due to typhoid and paratyphoid fever which was the fifth major cause of hospitalisation and 3.49% of the total cases of hospitalisation.<sup>6</sup>

The standard method for the diagnosis of enteric fever is the

isolation of organism from specimens especially blood, bone marrow and other various anatomical lesions.<sup>7</sup> In addition, ELISA, and immunofluorescence and Widal test are also often used. However, the only universally practiced diagnostic procedure is the Widal test because other methods are either invasive, expensive and have poor utility.<sup>8</sup> Moreover, the isolation of causative agent from the culture requires a good microbiological laboratory facility which makes the Widal test a better alternative method of diagnosis of typhoid and paratyphoid fever mainly in many developing countries like Nepal.<sup>9</sup>

*Salmonella* exhibits various antigenic structures such as somatic, flagellar, vi antigen, M antigen, fimbrial antigen etc.<sup>7</sup> Widal test demonstrates the presence of agglutinating antibodies against somatic, O antigen and flagellar, H antigen of *S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi.<sup>10</sup> However, in endemic area, people are often exposed to these bacteria and remain healthy as subclinical reservoir and cross reactivity with other bacterial infection and protozoan infection like Malaria, which eventually makes the test doubtful.<sup>11,12</sup>

In Widal test, bacterial suspensions of *S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi A and B are employed to detect corresponding antibodies in the serum

of the patients suspected to enteric fever.<sup>13</sup> The interpretation of Widal test is another arduous task. Depending upon O antigens, *Salmonella* are divided into five serological groups, A to E. Since the entire group D organisms including non-typhoidal organisms, possess O antigen 9 and most of group D organisms and all groups A and B organisms also exhibit O antigen 12, cross reaction may occur with any of the organisms of these groups.<sup>14,15</sup> In addition to that, in endemic regions, the previous immunization against typhoid fever or other infectious agents causing malaria should also be taken into account.<sup>10</sup> Since the majority of the normal healthy individuals also carry detectable antibodies due to the repeated prior exposure with low inoculums of typhoid bacilli, the knowledge of baseline titer is important for using the Widal test as diagnostic tool for enteric fever in endemic area.<sup>16</sup>

Therefore, this study was aimed to determine the antibody titre against *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Paratyphi in normal population in Kathmandu Nepal. We further aimed to establish the cut off titer value for the diagnosis of enteric fever by using comparative analysis of results from normal and clinically suspected population. The outcome of this study would provide a good platform to test the diagnostic utility or significance of the single slide Widal test.

**MATERIAL AND METHODS**

The comparative study was done for the detection of serum antibody levels in normal blood donors and suspected patients of enteric fever against different bacteria causing enteric fever. Blood serum of 400 blood donors (268 males, 132 females) were collected from Nepal Red Cross Society, Central Blood Transfusion Services, Exhibition road, Kathmandu and 200 serum samples (109 males and 91 females) were collected from the suspicious patients of enteric fever visiting Kathmandu Model Hospital, Exhibition road, Kathmandu, Nepal.

Blood donors were from the age of 18-60 years without having any current or previous history of febrile or other diseases as indicated by Nepal Red Cross Society. Single acute sera from patients visiting Kathmandu Model Hospital with febrile illness and suspected of enteric fever were tested.

Blood sera were first screened for the agglutination with the *Salmonella* antigens. The sera with positive agglutination were semi quantitatively analyzed for the determination of antibody titre. The Widal test kits containing O, H, AH, BH antigens and positive and negative controls were supplied from RFCL company, India. The serum samples showing positive agglutination with *Salmonella* antigens were analyzed by semi quantitative Widal test to determine the antibody titer against different antigens.

Chi-Square ( $\chi^2$ ) test was used to test the association of different variables with each other using the data obtained from the Widal test for different test categories. Results were considered significant if p values were less than 0.05, i.e. at 5% level of significance.

**RESULTS**

Among the total healthy blood donors and suspected febrile patients sample, relatively higher, 5.44% (22/400), sera taken from healthy blood donors were found to have agglutination titre of  $\geq 1:80$ . There was no significant difference in serum antibody level between male and female. Antibody titre  $\geq 1:80$  is generally considered as indication of infection with *Salmonella*. While in case of febrile patients suspected of enteric fever, seroprevalence was found to be 27% (54/200).

**Age wise distribution of cases**

Since most of the blood donors were from age group 20-30 yrs, 50% of the positive cases (11/22) were also from that age group. However, the highest prevalence 8.69% was found in age group of 31-40 yrs (Table 1). In case of patients suspected of enteric fever, highest prevalence 42.85% (15/35) was found in age group of 31- 40 years (Table 1). Very few numbers of positive cases were seen in both blood donors and patients.

**Table 1: Age wise distribution of single slide agglutination Widal test results for healthy blood donors (p=0.85) and suspected patients (p=0.1913)**

Age group (in yrs)	Widal positive	Widal negative	Total	Widal positive	Widal negative	Total
> 20	2(9.09%)	21(5.55%)	23(5.75%)	19(35.18%)	59(40.41%)	78(39%)
20-30	11(50%)	219(57.93%)	230(57.5%)	14(25.92%)	42(28.76%)	56(28%)
31-40	6(27.27%)	83(21.95%)	89(22.25%)	15(27.77%)	20(13.69%)	35(17.5%)
41-50	3(13.63%)	48 (12.69%)	51(12.75%)	4(7.40%)	13(8.90%)	17(8.5%)
> 50	0(0%)	7(1.85%)	7(1.75%)	2(3.70%)	12(8.21%)	14(7%)
Total	22(100%)	378(100%)	400 (100%)	54(100%)	146(100%)	200(100%)

**Distribution of antibodies against different *Salmonella* antigens**

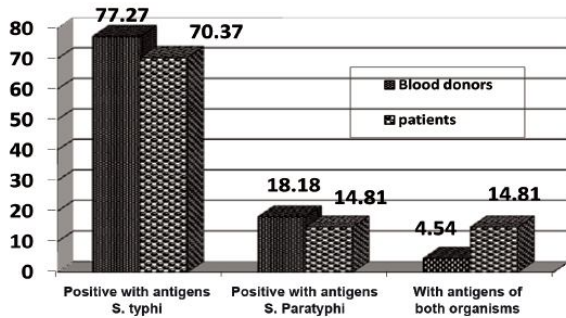
Among Widal positive cases in blood donors, 31.81% (7/22) of cases contained agglutinin against lipopolysaccharide O antigen of *Salmonella enterica* serovar Typhi followed by 27.2% (6/22) against flagellar (H) antigen of the same organism. Positive agglutination against both O and H antigens was recorded to be 18.18%. In contrast, very high 46.29% of cases showed positive results with both O and H antigens in clinically suspected patients of enteric fever (Table 2).

**Table 2: Distribution of antibodies against *Salmonella* antigens (p< 0.001)**

Agglutinin against	In blood donors		In suspected patients	
	reactive cases	%	reactive cases	%
O Ag	7	31.81	7	12.96
H Ag	6	27.27	6	11.11
AH Ag	4	18.18	6	11.11
BH Ag	0	0	2	3.70
Both O and H Ag	4	18.18	25	46.29
Both O and AH Ag	0	0	5	9.25
Both O and BH Ag	0	0	0	0
Both H and AH Ag	0	0	3	5.55
Both H and BH Ag	1	4.54	0	0
Both AH and BH Ag	0	0	0	0
Total	22	100	54	100

**Disease in terms of etiology**

*Salmonella enterica* serovar Typhi was found to be dominant infecting agent in both blood donors and clinically suspected patients of enteric fever. *S. typhi* was involved in 77.27% and 70.37% of cases in blood donors and suspected patients respectively. 18.18% and 14.81% cases were infected with *S. paratyphi* in respective categories of study. Combined infection with both *S. typhi* and *S. paratyphi* were found more (14.81%) in patients than 4.54% in blood donors (Figure 1).



**Figure 1: Comparison of results in reference of antibody in blood donors and clinically suspected patients (in %)( P value: 0.046)**

**High antibody titre value and Risk Comparison in blood donors**

Among the 22 blood sera showing positive agglutination, 4 (18.18%) had the anti-O titre value of 1:160, 3 (13.63%) had anti-H titer of 1:160, 3 (13.63%) had both anti-O and anti-H titre of 1:160. Similarly, 2 (9.09%) each of anti-O and anti-H sera had titer of 1:320, 3 (9.09%) had anti-O titre of 1:320. When we compared the titer value between blood donors and the clinically suspected patients as a whole, a similar ratio of antibody level against O antigen and H antigen was found in both study groups. When comparing the data obtained from healthy blood donors and patients suspected of enteric fever, positivity of the test was increased to 27% for patients in reference to the 5.5% of the healthy blood donors. The risk ratio was found to be 0.203(1/RR=4.9) and odds ratio was 0.157 (1/OR=6.35) (Table 3).

**Table 3: Risk comparison between healthy blood donors and clinically suspected patients in single slide agglutination Widal test results.**

Test category	Positives	Negatives	Total	% of positives	Risk ratio (RR)	Odds ratio (OR)	P Value
Blood donors	22	378	400	5.59	0.203 (1/RR=4.9)	0.157 (1/OR=6.35)	P<0.001
Suspected patients	54	146	200	27			
Total	76	524	600				

**DISCUSSION**

Widal test, widely used diagnostic test for enteric fever in developing countries has been an exclusive choice either due to the non availability of other standard diagnostic tools or other reasons such as cost, technical demands and time consumption. We used single slide agglutination Widal test

to find out the baseline antibody titer in normal population in Kathmandu and diagnostic titer value in clinically suspected patients of enteric fever. By detecting the antibody level in healthy blood donors, we tried to find out the proportion of population showing higher titre ( $\geq 1:80$ ) value which is considered due to infection with either *Salmonella enterica* serotypes, Typhi or Paratyphi (A, B or C). On the other hand, we also assessed the titer value in clinically suspected patients of enteric fever in same region against the antigens of same infecting agents. The comparison of the titre value and the trend in both categories, not only helps to determine the baseline antibody titre of the population but also to establish the cut off titer value significant for the diagnosis of enteric fever along with the utility of Widal test in endemic area.

In this study, out of 400 serum samples from healthy blood donors, 5.44% of sera were found to have significantly higher agglutinin titer of  $\geq 1:80$ . Since Nepal and its capital Kathmandu are known as the endemic regions for enteric fever,<sup>12,17</sup> a proportion of healthy people also contain antibodies against these organisms capable of reacting to a variable titer in Widal test due to previous stimuli.<sup>11</sup> However the higher seroprevalence may also be associated with different epidemiological factors such as persistence of infection in the community and the longevity of the antibodies in the serum, cross reactivity of *Salmonella* serotype Typhi and Paratyphi with other *Salmonella* species.<sup>18</sup>

Our findings are less than that of the results obtained by a study by Pokhrel *et al.*, in 2008.<sup>9</sup> However the sample size (100) was very low in comparison to our research (400) and we only noted the titre value  $\geq 1:80$  but they also noted the lower titre value. Moreover the duration of the research was also different. The peak incidence of enteric fever was noted in months of April and May in a study carried out by Rai *et al.*, 2005.<sup>12</sup> The interpretation of Widal test is an arduous task and should be carried out carefully because of the cross reactivity of *Salmonella* antigens with antibodies against other *Salmonella* as well as non *Salmonella* species.<sup>9</sup> Different serologic groups of *Salmonella* (A to E) on the basis of O antigen, share the same antigens, for example, all group D organisms, such as *S. enterica* serovar Typhi possess O antigen 9, and about 60 of the 78 group D serotypes including also have *S. enterica* serovar Typhi O antigen 12.<sup>10</sup> Thus, the antibodies produced by any of Group D *Salmonella* may react with the O antigen.<sup>19</sup> Similarly, cross reactions with O antibody of group D serotypes may also occur with group A and B serotypes of O antigens because they possess O antigen 12 which can be enough to lessen the diagnostic significance of the test.<sup>14,15</sup> Other *Salmonellae* share the H (flagellar) antigens with *S. enterica* serotype Typhi exhibiting cross-reactions producing a false positive anti-O or anti H titer in the Widal test.<sup>20</sup> Other possible reasons for false positive results are infection with other organisms causing malaria, cryptococcal meningitis, immunological disorders and chronic liver failure patients.<sup>21</sup>

So the baseline antibody titer against O antigen and H antigen of *S. enterica* ser. Typhi and against H antigen of Paratyphi

A and B should be evaluated and reported accordingly.<sup>11</sup> In our country, the standard diagnostic titre value of Widal test has not been set yet. So the diagnostic titer suggested in the manufacturer's reference was followed throughout the study which was  $\geq 1:80$  for all antigens used.

Out of 200 patients suspected of enteric fever, 54 (27%) had antibody titre  $\geq 1:80$ . Another similar study in the Southern part of Nepal, Terai, in 2005, determined 29.8% positivity in patients with febrile illness and suspected of enteric fever.<sup>12</sup> In suspected patients, the seropositive cases were found higher, 31.86% in female as compared to 22.93% of male. From this, though the suspected male patients outnumbered the female patients, the incidence of the disease was higher in female than in male. Similar findings were also reported by some other studies.<sup>22,23</sup> However, no significant association at 95% confidence limit was obtained between the prevalence and sex with both the cases in blood donors and suspected patients of enteric fever. According to our study, most of the blood donors were from the age group 20-30 which shows that youths are actively involved in social welfare programs. The age group with high prevalence of disease was found to be different for the two study groups.

From the qualitative Widal test of serum from healthy blood donors, 31.81% positive cases showed agglutination against O antigen of *S. enterica* serovar Typhi, and was followed by 27.27% against H antigen of the same organism. While 18.18% (4/22) each had the agglutinin against AH antigen of *S. enterica* serovar Paratyphi A and against the both antigens of *S. enterica* serovar Typhi. Interestingly, 4.54% of positive cases exhibited mixed infection showing antibody against H antigen of *S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi B. Moreover the qualitative Widal test of serum from patients of enteric fever shows relatively very high proportion of samples showed agglutination with both H and O antigens of *S. enterica* serovar Typhi. 46.29 % contained agglutinin against both O and H antigen of *Salmonella enterica* serovar Typhi in comparison to 18.18 % that with blood donors.

While agglutination against O antigen of *S. enterica* serovar Typhi was found in 12.96% which was followed by 11.11% against H antigen of *S. enterica* serovar Typhi and against AH antigen of *S. enterica* serovar Paratyphi A each. In contrast to the result of blood donors, 3.70% cases with agglutinin against BH antigen of *S. enterica* serovar Paratyphi B were found.

Thus, in healthy blood donors, 77.27% (17/22) of people had the agglutinin against *S. enterica* serovar Typhi, 18.18% (4/22) donors had antibody against *S. enterica* serovar Paratyphi. Similarly, in patients suspected with enteric fever, 70.37% (38/54) of people had the agglutinin against *S. enterica* serovar Typhi, 14.81% (8/54) patients had antibody against *S. enterica* serovar Paratyphi and 14.81% (8/54) contained antibody against both organisms in their serum. From these

data it is evident that typhoid fever is predominant over paratyphoid fever. Similar prevalence was found in the study carried in study carried out in Kathmandu Model Hospital and in Kathmandu Medical College Teaching Hospital, Kathmandu in 2003.<sup>24</sup>

It has been reported from various studies that the ratio of diseases, typhoid fever and paratyphoid in most countries is found to be 10:1.<sup>25</sup> But the incidence of paratyphoid fever is now increasing.<sup>26,27</sup> While the 1997 Global Survey of *Salmonella* serotyping estimated an incidence of one case of paratyphoid fever for every four cases of typhoid fever, studies from India and Nepal suggest that in some settings, *S. enterica* serovar Paratyphi A can contribute up to half of all cases of enteric fever.<sup>28,29,30</sup> In our study the ratio of typhoid fever and paratyphoid fever was nearly 4:1. A possible explanation for the increasing prevalence of paratyphoid fever may be due to the extra household route of transmission of paratyphoid fever, which mainly includes consuming food from street vendors. Urban population is becoming even more dependent on inexpensive food obtained from the street vendors.<sup>31</sup>

Relatively very high percentage of positive samples in both blood donors as well as in suspected patients had shown antibody titre of 1:160 against both O and H antigen (more than 55% for each). Nearly 30% of positive samples in both group showed more elevated titer value of 1:320 and only nearly 10% of samples were found to have antibody titre of 1:80 against both O and H antigen in both study groups. 75% among the positive samples for *S. enterica* serovar Paratyphi A had the titer 1:320 and 25% with 1:80 in case of blood donors. This high antibody level may be shown due to improper selection criteria for blood donor, its implementation.

In our study, the comparative risk of enteric fever between two different groups of people i.e. healthy blood donors and clinically suspected patients of enteric fever was assessed. The risk ratio (RR) between the two groups was found to be 0.203 (1/RR =4.9). Here, the risk ratio between two groups is less than one. So, the risk of enteric fever was found to be less in blood donors than in clinically suspected people. The risk ratio gives the percentage difference between group one and group 2.<sup>32</sup> Thus, from our experiment, risk ratio was 0.203, 79.7% fewer people in blood donors suffer from enteric fever than in patients.

Similarly we also have estimated the odds ratio (OR) between these two groups and was found to be 0.157 (1/OR =6.35, CI at 95%: 0.093-0.268). Like risk ratio, odds ratio was also less than one. So, this is evident that the chance of occurring Widal positive cases is less in blood donors in clinically suspected people. The inverse of odds ratio is 6.35, which means that patients with clinical symptoms have 6.35 times higher chances of being infected with enteric fever than blood donors.

## CONCLUSION

Since the Widal test is easy, inexpensive, and relatively non-invasive, it can be of diagnostic value when blood cultures are not available or unpractical. However, the results of Widal test should be analyzed on the basis of endemicity and cross reaction of antigen with other *Salmonella* and non *Salmonella* species. As the level of antibody in Widal positive cases of healthy blood donors was higher and was in a proportionate level with clinically suspected patients of enteric fever and a considerable number of healthy individuals had antibody titre higher than 1:80. It is therefore concluded that O, H, AH and BH agglutinin titre of  $\geq 1:80$  from Widal agglutination test cannot be used as diagnostic value in the region of endemicity such as Nepal. On those areas, only the elevated levels of agglutinating O, H, AH and BH antibodies as measured in single slide Widal test can be helpful in making a presumptive diagnosis of typhoid fever if interpreted with care.

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