

A Study of Widal test in typhoid fever and in healthy population

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ABSTRACT

INTRODUCTION: Patients often receive antibiotics prior to blood culture sampling. So widal test is most widely used method for diagnosis of typhoid fever. Its popularity seems from the fact that it is simple to use and inexpensive. As the clinical features of typhoid fever are not always diagnostic and blood cultures for typhoid are falsely negative in many cases, widal test still has a role in the diagnosis. The cutoff level for widal test positivity has still not been established in Nepal This study will help in adding knowledge regarding this. The purpose of the study is to test the predictive values of positive serology for the population and to identify the specificity of the Widal test at various dilutions.

METHODS: This is a prospective study conducted at three different hospitals of Kathmandu, Nepal (Bir, Sukraraj Tropical and Infectious Disease and Birendra Army Hospital) from March 2005 to December 2005. Widal test was done on 8th to 12th day of onset fever. Widal test was reported as positive or negative agglutination at 1:80, 1:160 and 1:320 dilution for both O and H antibodies.

RESULTS: A total of 104 Widal tests were performed. The study group had 53 patients and control group had 51 individuals. When both 'O' and 'H' antigen titres are considered together, the sensitivity and specificity of the test increased. At 1:80 titre, the sensitivity is quite high (83.0%) and the specificity is also moderately high (76.5%). At a titre a 1:160, the sensitivity is still high (62.3%) and the specificity is quite high (86.3%). At titre of 1:320, the specificity is 100% but the sensitivity is very low (20.8%).

CONCLUSION: The cutoff point for positivity of Widal test is best taken as 1:80 dilution for both 'O' and 'H' antibodies. Although higher dilutions of Widal test give higher specificity, the sensitivity decreased greatly.

KEY WORDS: Widal test, Typhoid Fever, 'O' & 'H' antibodies

INTRODUCTION

Typhoid fever is an important cause of morbidity in many regions of the world, with an estimate of 12 to 33 million cases occurring annually and an estimated 13 million cases occurring annually in Asia alone.¹ According to the best global estimates, there are at least 16 million new cases of typhoid fever each year, with 600000 deaths.^{2,3} The average attack rate of enteric fever is 23/100000 population in Nepal. Sixty

percent of cases are males and 40% are females. Sixty one percentage of cases are seen in the age group of 15 to 44 years.⁴

The diagnosis of typhoid fever on clinical grounds is difficult, as the presenting features are diverse and similar to those observed in other common febrile illnesses. The laboratory diagnosis of typhoid fever is dependent upon either the isolation of *Salmonella* enteric serotype typhi from a body sample or detection of raised titres of agglutinating serum antibodies against the lipopolysaccharide 'O' or flagellum 'H' antigens of serotype typhi. The Widal test is used to demonstrate rising titres of antibodies.

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The cutoff value for positivity chosen in a particular community depends on the background level of typhoid fever and the level of typhoid vaccination, which may vary with time.⁵ However in our country since patients often receive antibiotics prior to medical diagnosis and culture facilities may not be available, serologic analysis becomes more important. The purpose of study was to identify the predictive values of positive serology for the population and to identify the specificity and sensitivity of the Widal test. The purpose of the study is to test the predictive values of positive serology for the population and to identify the specificity of the Widal test at various dilutions.

METHOD

This is a prospective study conducted at three different hospitals of Kathmandu, Nepal (Bir Hospital, Sukraraj Tropical and Infectious Disease Hospital and Shree Birendra Army Hospital). This study was conducted from March 2005 to December 2005. Patients aged more than 15 years with typhoid fever having positive blood culture for *Salmonella typhi* were included. Patients with past history of typhoid fever, history of vaccination for typhoid fever, Widal test performed before 8 days of fever onset and after 12 days of fever onset were excluded. For the control group, the required numbers of matched individuals were selected from healthy volunteer blood donors.

Verbal and informed written consent was obtained from all the participants of the study. Data collection was done from the OPD departments, emergency department and indoor medical wards of these hospitals. Blood sample for Widal test was taken between day 8 to day 12 of onset of fever. Blood culture was carried out according to standard procedures in the laboratories of the hospitals. All laboratories carried out Widal test by plate method and reported positive or negative agglutination at 1:80, 1:160 and 1:320 dilution for both 'O' and 'H' antigens.

Statistical analyses were performed using SPSS10 program. Sensitivity, specificity, positive predictive value and negative predictive value were calculated. The result was analyzed with the help of p value.

RESULTS

A total of 104 patients were included in this study. There were 53 patients in the study group and 51 individuals in the control group. In the study group, 48 (91%) were males and 5(9%) were females. Similarly in the control group, 42(82%) were males and 9(18%) were females.

When the titre of 1:80 for 'O' antigen is taken as the cut off value, the sensitivity and specificity of the widal test are 64.2% and 78.4% respectively. The positive predictive value (PPV) and negative predictive values (NPV) are 75.6% and 67.8% respectively. The p value was 0.00001 which was highly significant. (Table1 and 2)

At the cut off value of 1:160, although the specificity is very high (90.2%), the sensitivity decreases to a low level of 47.2%. Similarly at a cut off value of 1:320, the specificity reaches 100% but the sensitivity is very low (11.3%). At a titer of 1:80 for 'H' antigen, the Widal test has a moderately high sensitivity and specificity of 69.8% and 83.3% respectively. At a titer of 1:160, the sensitivity is less (58.5) but the specificity becomes very high (90.2%). At a titre of 1:320, although the specificity becomes 100%, the sensitivity becomes very low (18.9%). When both 'O' and 'H' antigen titres are considered together, the sensitivity and specificity increases. At 1:80 titre, the sensitivity is quite high (83.0%) and the specificity is also moderately high (76.5%). At a titre a 1:160, the sensitivity is still high (62.3%) and the specificity is quite high (86.3%). At titre of 1:320, the specificity is 100% but the sensitivity is very low (20.8%).

Table 1: Comparative chart

	O			H			T (either O or H)		
	80	160	320	80	160	320	80	160	320
Frequency	34	25	6	37	31	10	44	33	11
Sensitivity	64.2	47.2	11.3	69.8	58.5	18.9	83	62.3	20.8
Specificity	78.4	90.2	100	83.3	90.2	100	76.5	86.3	100
Positive predictive value	75.6	83.3	100	82.2	86.1	100	78.6	82.5	100
Negative predictive value	67.8	62.2	52	72.9	67.6	54.3	81.3	68.8	54.8
p value	0.00001	0.00003	0.013	0.000001	0.0001	0.001	0.00001	0.00001	0.00005

Table 2: Comparative chart

	O			H			T (either O or H)		
	80	160	320	80	160	320	80	160	320
Frequency (study)	34	25	6	37	31	10	44	33	11
Sensitivity	64.2	47.2	11.3	69.8	58.5	18.9	83	62.3	20.8
Frequency (control)	11	5	0	8	5	0	12	7	0
Sensitivity	21.5	9.8	0	15.6	9.8	0	23.5	13.7	0
p- value	0.0001	0.0001	0.013	0.0001	0.0001	0.0011	0.0001	0.0001	0.0006

DISCUSSION

Although the Widal test is widely used in Nepal to diagnose typhoid fever, only a few studies have been done in the past in Nepal to evaluate its usefulness.

The widal test is not a highly diagnostic test by itself. In a study conducted by Bhutta and Masrali, the sensitivity, specificity, PPV and NPV with the help of cutoff value of 1:100 were 63%, 81%, 85% and 55%⁶. Similarly, the sensitivity, specificity, PPV and NPV were 92%, 57%, 70% and 87% respectively in a study from Vietnam¹ where they used cutoff value of 1:100. In our study, the sensitivity, specificity, PPV and NPV were 64.2%, 78.4%, 75.6% and 67.8% respectively at 1:80 titre. Although the study from Vietnam showed the sensitivity of the test (when only 'O' agglutination was considered) to be 92%, it was only 64% in our study. Many cases of typhoid fever could be missed by relying on the widal test alone and therefore the clinical scenario should also be considered and treatment for typhoid fever should be initiated early in suspected cases even if the widal test is negative as the complications of typhoid fever are life- threatening if not treated appropriately in time.

In many studies the 'O' antigen has higher sensitivity than the 'H' antigen⁷. But some studies have shown that rise in 'H' agglutinin is more significant as compared to 'O' agglutinins⁸. In a study by T Pang, they have commented that a small proportion of patients with proven typhoid fever showed a rise in 'H' agglutinins only. In our study, the 'H' antigen had slightly increased sensitivity. The sensitivity for 'O' agglutinin, was 64.2% and for 'H' agglutinin it was 69.8% at 1:80 titre. We feel that for treatment purposes, both 'O' and 'H' antibody titre should be considered.

When 'TO' and 'TH' are considered together, the test can be more sensitive and specific. We found the sensitivity, specificity, PPV, and NPV to be 83%, 76.5%, 78.6% and 81.3% respectively when 'TO' and

'TH' were taken together at 1:80 titre. Similar findings have been reported in other studies^{7, 9}. So at titre of 1:80 the overall sensitivity and specificity of the test is reasonably high.

At higher titres, the widal test is more specific and the positive predictive value tends towards 100% but at the higher titres, many cases of typhoid fever will be missed. For example at 1:320 titre, only 11 (20.8%) of culture positive cases were having widal test positive in our study. So a large number of typhoid cases would be missed. But a study from Dhulikhel Hospital has reported that the titre of 1:320 can be useful in blood culture negative but clinically suspected enteric fever cases where they found that 63% of such cases tested positive at 1:320¹⁰.

In the present study, 11 (21.5%) controls tested positive for 'O' antigen and 8(15.6%) controls tested positive for 'H' antigen. This reflects the high background level of 'O' and 'H' titres in the general population in Nepal which is an endemic country for typhoid fever. This factor has to be considered when interpreting the test results in endemic areas.

CONCLUSION

The cutoff point for Widal positivity varies from place to place and has to be determined for each region. At 1:80 dilution, the sensitivity and specificity are quite high especially if both 'O' and 'H' titres are taken together. At higher dilutions, although the specificity tends towards 100%, the sensitivity decreases greatly and so many patients will be missed at these higher dilution level.

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