Baseline antibody titres against Salmonella *typhi* in apparently asymptomatic HIV positive individuals in a tertiary care hospital

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RESEARCH

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Abstract

Background

Enteric fever is common in tropical regions and is caused by Salmonella enterica serotype typhi (*S typhi*). For diagnosis of enteric fever, the Widal test is the most widely used test after blood culture. In HIV infected individuals false positive and false negative Widal reactions are common. The result is variable titres and baseline titres that are unusual in this patient population.

Aims

This study was done to determine the baseline antibody titres for *S typhi* among HIV infected individuals.

Method

Average baseline antibody titres against O and H antigens of *S typhi* were measured by standard Widal test in 200 HIV positive asymptomatic individuals, as well as 200 age and sex-matched controls. The results were compiled and statistically analysed.

Results

A total of 84 (42%) of the cases had an H antibody titre of >1:20 and 105 (52.5%) had a titre of >1:20 against O antigen. This implies that positive titre of H and O antigen is significantly associated with HIV positive cases with P<0.001. Correlation of CD4 count with antibody titres shows that there is no significant association between CD4 counts and antibody titres against either H (P=0.634) or O antigen (P=0.765)

Conclusion

This study shows that HIV infected individuals had increased titres of antibodies against *S typhi* from the baseline. This indicates a need for evaluation of current cut-off values of diagnostic titres for this group. We also suggest that it is best to perform baseline titres against S *t*yphi for each patient at the time of diagnosis of HIV status, and to use this for future reference.

Key Words

Salmonella typhi, Widal test, HIV/AIDS

What this study adds:

1. Enteric fever is common in developing countries and Widal test is most widely used in diagnosis. In HIV positive cases, false positive and false negative Widal reactions are common and baseline titres are not known.
2. In HIV positive patients the titres of antibodies against *S typhi* considered as baseline are higher than in the general population
3. Evaluation of baseline titres against *S typhi* among the HIV positive patient is recommended at the time of diagnosis of HIV for future reference.

Background

"The brunt of the human immunodeficiency virus (HIV) pandemic has been borne disproportionately by resource-poor regions of the world, where tropical infectious diseases continue to hold greatest sway".[1] Enteric fever is a common systemic infection in tropical regions caused by *Salmonella enterica* serotype typhi. In 2000, more than 2.16 million episodes of typhoid occurred worldwide, resulting in 216,000 deaths. More than 90% of these cases with associated morbidity and mortality occurred in Asia. According to a study by the WHO, the prevalence of typhoid per 1000 febrile episodes in India was 28.[2] Although the number of people living with HIV/AIDS (PLHA) in India is estimated at 23.9 lakh (19.3 – 30.4 lakh) in 2009,[3] there is still no available data on typhoid-HIV co-infection at this point in time.

In HIV infected cases with febrile illness, clinical diagnosis alone is unreliable, and it is imperative to have a laboratory diagnosis in each case. For diagnosing a case of enteric fever, Widal test is the most widely used test after blood culture. Widal test using antigen suspensions appropriate to the diagnosis of the prevalent enteric fever agents has been used either to compare paired sera or to test a single serum sample taken on admission to see significant antibody levels.[4] In endemic countries like India, sera of a proportion of healthy individuals contain antibodies capable of reacting to a variable titre in Widal test. This is due to previous stimuli, and is known as the baseline titre.

In HIV infected cases, a false positive Widal reaction can occur due to cross-reacting antibodies, and false negative Widal reaction occurs due to immunosuppression. Therefore, in this group especially, the significance of results should be assessed against baseline antibody titres for enteric fever organisms. Since there is no available reference of baseline titres for HIV positive cases in India, this study was conducted to determine the baseline antibody titres of enteric fever-causing Salmonellae amongst HIV infected individuals. These were then compared with the baseline antibody titres of enteric fever organisms in normal healthy individuals.

Method

This study was undertaken in the microbiology department of a tertiary care referral hospital attached to a medical college. Institutional ethical clearance was obtained for this study and informed consent was taken from each subject prior to inclusion in the study. Under aseptic precautions, 2ml of blood was collected from each of 200 HIV positive individuals with and without antiretroviral treatment (ART) presenting to our department, and from 200 healthy blood donors between 18-65 years of age who acted as controls. The serum samples were processed according to the standard tube Widal method. Suspensions of Salmonella enterica serotype Typhi O and H antigens and Salmonella Paratyphi A and B H antigens were used. These are stabilised suspensions of smooth, non-fimbriate, killed bacilli, which were standardised to produce appropriate reactivity. The O antigen being a somatic antigen brings about a coarse, compact, granular agglutination, whereas the H antigen being a flagellar antigen brings about larger, loose, fluffy agglutination. The IgM somatic O antibody appears first and represents the initial serologic response in acute typhoid fever, while the IgG flagellar H antibody usually develops more slowly but persists longer. After overnight incubation at 37°C (16–20 hours) the results were quantitatively defined as titres ranging from 1:20 to 1:320. The results were then tabulated and statistically analysed.

Results

A total of 200 HIV positive cases that presented to the department of microbiology were screened for the presence of Salmonella antibody titres. A majority of cases were between the age group of 21-50 years i.e. 164 (82%) while 109 (54.5%) were males and 91 (45.5%) were females. Of the 200 HIV patients studied, only 5 (2.5%) gave a known history of enteric fever.

Table 1: Comparison of positivity of Salmonella typhi H in cases and controls

H antigen	Cases (n=200)	Controls (n=100)
<1:20	116 (58.0%)	91 (91.0%)
>1:20	84 (42.0%)	9 (9.0%)
Inference	Positive titre of H is significantly associated with cases (42.0%) when compared to controls (9.0%) with P<0.001**	

Table 1 shows the titre of antibodies against H antigen in cases and controls. 84 (42%) of the cases had a titre of >1:20 while only 9 (9%) of the controls had a titre of >1:20. This titre of 1:20 is used as the baseline titre of normal control population in Bangalore, India, and is used as standard for comparison. For diagnosis, the titre considered positive is H titre >1:80 or 1:160 and 0 antibody >1:80. The inference of this analysis is that positive titre of H is significantly associated with HIV infected cases (42.0%) when compared to controls (9.0%) with P<0.001.

Table 2: Comparison of positivity of O in cases and controls

O antigen	Cases (n=200)	Controls (n=100)
<1:20	95 (47.5%)	91 (91.0%)
>1:20	105 (52.5%)	9 (9.0%)
Inference	Positive titre of O antigen is significantly associated with cases (52.5%) when compared to controls (9.0%) with P<0.001**	

Similarly, Table 2 shows the titre of antibodies against O antigen in cases and controls; 105 (52.5%) of the cases had a titre of >1:20 while only 9% of the controls had a titre of >1:20. The inference of this analysis is that positive titre of O antigen is significantly associated with HIV positive cases (52.5%) when compared to controls (9.0%) with P<0.001. Table 3 shows the distribution of antibody titre against H and O antigens in the cases which showed a titre of >1:20.

Table 3: Antibody titres against H and O antigens of Salmonella Typhi

Titre	Titre against H antigen	Titre against O antigen
1:40	43 (21.82%)	65 (32.99%)
1:80	6 (3.04%)	33 (16.75%)
1:160	0 (0%)	6 (3.04%)
1:320	0 (0%)	0 (0%)

Table 4: Correlation of Antigen H with CD4 counts

CD4 count	Number of patients (n=200)	Antigen H	
		<1:20 (n=110)	>1:20 (n=84)
<100	6 (3%)	4 (3.6%)	2 (2.4%)
101-200	13 (6.5%)	6 (5.5%)	7 (8.3%)
201-300	24 (12%)	16 (14.5%)	8 (9.5%)
301-500	56 (28%)	34 (30.9%)	22 (26.2%)
501-1000	85 (42.5%)	53 (48.2%)	32 (38.1%)
>10000	8 (4%)	3 (2.7%)	5 (6%)
Inference	Levels of CD4 count is not statistically associated with antigen H titre with P=0.634		

Table 4 shows the correlation of CD4 count with antibody titres against H antigen in which the levels of CD4 count are not statistically associated with antigen H titre (P=0.634). Table 5 shows the correlation of CD4 count with antibody titres against O antigen in which the levels of CD4 count are not statistically associated with antigen O titre (P=0.765). The titres for Salmonella paratyphi A and B were <1:20, in all the cases and controls and therefore considered normal.

Discussion

The gold standard for diagnosis of enteric fever is blood culture with isolation of *Salmonella enterica.* However, most health care facilities in developing countries do not have ready access to this diagnostic method.[5] In addition, conventional tests are time consuming, often requiring specialised equipment and expertise. Widal agglutination tests are widely used in many developing countries, including India, as an alternative laboratory procedure for diagnosis of enteric fever. Baseline titre of antibodies is normally elevated in healthy individuals in endemic areas. This factor must be considered before interpretation of Widal titres. In India, a titre of <1:20 is considered as the baseline of antibody titres against *S typhi* in normal healthy individuals.[4]

Since our hospital is a referral centre for HIV infected patients, interpreting Widal titres on these patients presenting with fever is challenging. There is no data currently available for comparison regarding baseline titres in asymptomatic HIV positive individuals. Hence this study was undertaken to compile the baseline titres for this study group.

This study shows that a significant proportion of HIV infected individuals had increased titres of antibodies against *S typhi* compared to those considered as baseline, indicating a need for evaluation of current cut-off values of diagnostic titres in this group.

In our study we found that a titre of > 1:20 against the H antigen of *S. typhi* was present in 42% of the HIV positive cases of which, 43 (21.8%) had a titre of 1:40 and 6 (3.04%) had a titre of 1:80. Since the H antibody titres of S. Paratyphi A and B were less than 1:20, it was considered normal. Similarly, a titre of >1:20 against the O antigen was present in 105 (52.5%) of the cases of which, 65 (32.99%) had a titre of 1:40, 33 (16.75%) had a titre of 1:80 and 6 (3.04%) had a titre of 1:160. When compared to the normal healthy age and sex matched controls who had a titre of <1:20 the standard baseline titre used in India, these titres were significantly high.

Only 2.5% of the HIV positive cases gave a history of typhoid fever in the past. Also, there was no correlation between the number of years the patient was on antiretroviral therapy and the antibody titres against *S typhi*. When the correlation of titres with CD4 counts was done, it was found that levels of CD4 count were not statistically associated with antibody titres to H antigen (P=0.634). Similarly, levels of CD4 count were not statistically associated with antibody titres to O antigen (P=0.765).

The Widal test results may vary depending on the level of antibodies to cross-reacting antigens. Elevated levels of *S. typhi* anti H and anti O antibodies have been reported in patients with a variety of other infections, including those caused by other Salmonella spp like S.paratyphi A and B, *E coli*, *Klebsiella* spp, *Staphylococcus aureus* and in other conditions including malaria, dengue, immunological disorders and chronic liver failure. This could be the reason for high titres in our patients. Frequent infections are common in HIV positive individuals, with decreased CD4 counts.

Conclusion

To the best of our knowledge, this is the first study done to determine the baseline antibody titres against *Salmonella*

typhi in HIV positive individuals. Proper hygiene and sanitation is the main cause of low prevalence of enteric fever in developed countries resulting in low baseline antibody titres. Studies support re-evaluation of Widal baseline titres, which should be done at regular intervals. Since high baselines tires are seen in HIV infected individuals and there is no particular pattern of titres and no significant correlations with other parameters, it is best to perform the baseline titres for each patient at the time of diagnosis of HIV status and to use this as the baseline for future reference, thereby facilitating the appropriate interpretation and diagnosis.

References

1. Karp CL, Auwaerter PG. Coinfection with HIV and tropical infectious diseases. Clin Infect Dis. 2007 Nov 1;45(9):1208-13.

2. Ochiai RL, Acosta CJ, Danovaro-Holliday MC, Baiqing D, Bhattacharya SK, Agtini MD, Bhutta ZA, Canh do G, Ali M, Shin S, Wain J, Page AL, Albert MJ, Farrar J, Abu-Elyazeed R, Pang T, Galindo CM, von Seidlein L, Clemens JD; Domi Typhoid Study Group. A study of typhoid fever in five Asian countries: disease burden and implications for controls. Bulletin of the World Health Organization. Bull World Health Organ. 2008 Apr;86(4):260-8.

3. NACO Annual Report 2011. [Online] National AIDS Control Organization. Department of AIDS Control. Available at http://www.nacoonline.org/upload/Publication/Annual%20Report/NACO_AR_Eng%202011-12.pdf.

4. Punia JN, Joshi RM, Gupta V, Arora RK. Determination of baseline Widal titres from Chandigarh. Indian J Med Microbiol. 2003 Apr-Jun;21(2):144.

5. Pokhrel BM, Karmacharya R, Mishra SK, Koirala J. Distribution of antibody titre against Salmonella enterica among healthy individuals in Nepal. Ann Clin Microbiol Antimicrob. 2009 Jan 7;8:1.

PEER REVIEW

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests. We also declare that all the authors have approved the final version of this manuscript.