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Relying on Widal test alone could lead to over diagnosis of typhoid fever: Findings from a records review of febrile patients at Damphu Hospital, Bhutan, 2011-2012

Tej N. Nepal[1], Tshering Dorji[2], Tsheten[3], Karma Tenzin[4], Dorji Pelzom[5], Mongal S. Gurung[6], Namgay Rinchen[7], Sonam Wangchuk[8]

[1]*Gedu Hospital, Chukha, Bhutan*

[2,3,8]*Royal Centre for Disease Control, Ministry of Health, Thimphu, Bhutan*

[4]*Faculty of Postgraduate Medicine, Khesar Gyalpo University of Medical Sciences of Bhutan, Thimphu, Bhutan*

[5,6]*Policy and Planning Division, Ministry of Health, Thimphu, Bhutan*

[7]*Tsimalakha Hospital, Chukha, Bhutan*

ABSTRACT

Introduction: The Widal test is widely used in hospitals in Bhutan for diagnosis of typhoid fever. The right test with high sensitivity and specificity supplements clinical judgement and contributes to correct diagnosis of disease. This study focuses on the contribution of the Widal test in the diagnosis of typhoid fever. **Methods:** Data was collected from records of patients who presented to Damphu hospital from March 2011 to June 2012 with clinical suspicion of typhoid fever. Blood samples were collected from patients and tested at Damphu Hospital, Tsirang and the Royal Centre for Disease Control, Thimphu. Seventy records were used for the study. **Results:** There was no growth of *Salmonella typhi* on blood cultures from patients who had tested positive in the Widal test. There were 20 (28.57%) samples which tested positive for scrub typhus; among these Widal test was positive in 10 (50%) samples. Thirty four out of 36 (94.44%) patients had duration of illness less than seven days and among them 26 (74.47%) had positive Widal test results. **Conclusions:** None of the samples that tested positive by Widal test gave a definite diagnosis of typhoid fever with blood culture. Clinical judgement may be more challenging because patients with other febrile illnesses like Scrub typhus also have positive Widal test result. We conclude that it is best not to rely on the Widal test alone for the diagnosis of typhoid fever and this test should be replaced by more accurate ones.

Keywords: Salmonella typhi; Typhoid fever; Widal test.

INTRODUCTION

Typhoid fever is a febrile illness caused by *Salmonella typhi* bacteria and is especially prevalent in developing countries[1]. *Salmonella typhi* is transmitted to humans through the ingestion of food or drink contaminated by the faeces or urine of infected people[1]. Symptoms of typhoid fever are gradual onset of sustained fever, chills, and abdominal pain. Some patients experience rash, nausea, anorexia, diarrhea or constipation and headache. Common signs include bradycardia, hepato-splenomegaly and reduced level of consciousness[1,2]. The differential diagnosis for presentation of typhoid fever, especially in the first week of fever, include malaria, dengue, chikungunia, infectious mononucleosis, leptospirosis, rickettsial diseases and other causes of acute gastroenteritis[3]. A review of 22 eligible studies estimated that typhoid fever caused 21,650,974 illnesses and 216,510 deaths during 2000 and South-east Asia has a high incidence of typhoid fever with more than 100 cases per 100,000 population[4].

Corresponding author:
Tej N. Nepal
tejnnepal@gmail.com

The diagnosis of typhoid fever is done through isolation of *Salmonella typhi* either by bone marrow (gold standard) culture, blood culture, urine culture or stool culture, by Polymerase Chain Reaction (PCR) or by serological tests such as Widal, typhi dot, and typhidot M[3,5,6].

In Bhutan, the burden of typhoid fever is unknown but sporadic outbreaks have been reported[7,8]. Damphu Hospital records show high numbers of typhoid cases based on positive Widal test results for many years. The diagnosis of typhoid fever in most district hospitals in Bhutan, including Damphu Hospital, is based on the Widal test as there is no capacity for cultures or other tests. However, recent literature indicates that Widal test lacks sensitivity and specificity, with the positive predictive value ranging from 5.70% - 9.25% and negative predictive value ranging from 95.65% - 98.9%[5,9–11]. Therefore, there is the possibility of over diagnosis of typhoid fever in the district hospitals of Bhutan due to false positive results on the Widal test with subsequent unnecessary treatment and exposure of patients to drugs[8]. Moreover, studies in other hospitals in the region indicate that typhoid fever was not being correctly diagnosed because higher Widal titre values were observed from samples of healthy persons[12,13]. However, these studies did not explore whether there

could be other diseases with similar clinical presentation which also give positive Widal test results[12–14].

This study was thereby conducted to assess the diagnosis of typhoid fever by Widal test and to address gaps in clinical practice in management of patients presenting with fever.

METHODS

The study was a retrospective review of records of patients who visited Damphu Hospital, Tsirang district, Bhutan. Tsirang is one of the 20 districts in central Bhutan with a population of 21,816[15]. It has one district hospital (Damphu Hospital) and seven Basic Health Units providing services to its populace[16]. Modern sanitation coverage of the district is 98.8% and access to improved drinking water source is 82.4%[17]. People from four gewogs of the adjacent district (Dagana) also avail hospital level services from Damphu Hospital. The patients present to clinical officers or health assistants for consultations after registration at reception counter. These providers initiate clinical investigations and patients go to the laboratory for tests. Samples are collected in the laboratory, tests are conducted and patients report to the doctors with the reports.

During the period of March 2011 to June 2012, there were increased number of patients presenting with symptoms suspicious of typhoid fever at Damphu Hospital for whom Widal tests were performed. These patients comprised the study sample. Additional samples were collected from the same patients and transported to the Royal Centre for Disease Control (RCDC) for confirmation of *Salmonella typhi* infection by blood culture. Double recording of the same patient was avoided by the patients` citizenship identity card number. All the 70 samples were also tested at RCDC for Scrub typhus, Leptospirosis and other causes of gastroenteritis such as *Salmonella*, *Shigella*, *Campylobacter* and *Vibriocholera*.

Study variables included Widal test results (positive and negative by antigens O, H, AH and BH), blood culture results for *Salmonella typhi* (positive and negative), and scrub typhus serological results (positive or negative). A Widal titre of 1:80 and above dilutions to any of the antigens O, H, AH and BH was considered positive. Other variables were duration in days of symptoms such as fever, headache, joint pain, body ache, diarrhoea and malaise. Data was collected from medical records of patients and laboratory test results from RCDC. Demographic characteristics (age, gender, occupation, address) were inconsistent in the records and could not be used in the study.

Data was double entered and validated in EpiData version 3.1. Data analysis was done using STATA software IC/13.1. Widal test report, *Salmonella typhi* blood culture report, any other pathogens, and duration of illness were described using counts and proportions. The duration of illness was categorized as up to 7 days and 8 days or more.

Prior ethical approval was obtained from the Research Ethics Board of Health, Bhutan (REBH/Approval/2016/073) dated 21st November, 2016. The study did not collect any individually identifiable information of patients and the medical records obtained were kept confidential and secured by the investigator. The need for informed consent was waived by REBH since this is a record-based study.

RESULTS

Out of 70 blood samples cultured, none showed growth of *Salmonella* species (Table 1). However, 60 (85.71%) of these same samples had positive Widal test results. Of these, 51 (72.86%) were positive for O antigen and 32 (45.71%) were positive for H antigen. Twenty-five (35.71%) samples tested positive for more than one antigen. Only 2 (2.86%) were positive for paratyphi AH and BH antigens.

Twenty of these 70 samples (28.57%) tested positive for scrub typhus and of these 10 (50%) had positive results for both scrub typhus and Widal test. One sample (1.42%) tested positive for both Leptospirosis and Scrub typhus and also had Widal test positive titre of 1:160 for S. typhi O.

Data on the duration of illness was available for 36 (51.42%) participants and it was up to seven days in 34 (94.44 %) of them. Among them 26 (76.47%) had positive Widal test (Table 2).

DISCUSSION

Our study found little evidence for the contribution of Widal test in the diagnosis of typhoid fever in our patient population at a district hospital in Bhutan. Surprisingly, none of the blood cultures of patients with clinical features of typhoid fever yielded a growth of *Salmonella typhi*, despite there being positive Widal test result on these samples. In addition, one-fifth of the samples had a positive serological result for scrub typhus. Moreover, Widal test was positive for over three-fourths of the samples even when the duration of illness was less than seven days.

The results of the *Salmonella typhi* blood cultures are similar to a study conducted in four large hospitals in Bhutan where only two out of 109 Widal test positive samples (1.8%) had a growth of *Salmonella typhi* on blood culture[13]. Of note, the RCDC has been successfully evaluated as the national reference laboratory to carry out blood cultures. In support of our negative findings, other circumstantial evidences suggest that the cases may not have been typhoid fever - the district's sanitation conditions during that period had 98.8% coverage and 82.4% access to an improved drinking water source[17].

The study also found that some patients who had symptoms suspicious of typhoid fever tested positive for scrub typhus. Patients manifest with similar undifferentiated fever and no localizing signs in both scrub typhus and typhoid fever[18].

Table 1. Reports of Widal test and blood culture for Salmonella typhi of blood samples collected from patients with clinical presentation suspicious of typhoid fever at Damphu hospital, Bhutan, March 2011-June 2012. *n*=70

Widal test titre (dilution)	Antigens				Salmonella typhi (culture positive) n (%)
	O n (%)	H n (%)	AH n (%)	BH n (%)	
<1:80	19 (27.14)	38 (54.29)	68 (97.14)	69 (98.57)	0 (0.00)
1:80	15 (21.43)	11 (15.71)	0 (0.00)	0 (0.00)	0 (0.00)
1:160	24 (34.29)	9 (12.84)	1 (1.43)	0 (0.00)	0 (0.00)
1:320	12 (17.14)	12 (17.14)	1 (1.43)	1 (1.43)	0 (0.00)
Total	**70 (100)**	**70 (100)**	**70 (100)**	**70 (100)**	**0 (0.00)**

Table 2. Widal test results by the duration of illness in patients presenting with clinical presentation suspicious of typhoid fever at Damphu hospital, Bhutan, March 2011 to June 2012. *n*=70

Widal test result*	Duration of illness			
	≤7 days n (%)	> 8 days n (%)	Missing data n (%)	Total n (%)
Positive	26 (76.47)	1 (50.00)	33 (97.06)	60 (85.71)
Negative	8 (23.53)	1 (50.00)	1 (2.94)	10 (14.29)
Total	**34 (100)**	**2 (100)**	**34 (100)**	**70 (100)**

**Widal test result of 1:80 and above dilutions to all the antigens O, H, AH and BH was considered as positive.*

With regards to the relationship between Widal test and typhoid fever, available literature states that on average, it takes approximately 7 to 10 days after the illness of typhoid fever for antibodies to develop in body which the Widal test will detect for positive results[19]. Therefore, a negative Widal result does not exclude typhoid fever in the early stage of symptoms. Despite the duration of illness being less than seven days for almost all participants in the current study, over three-fourths had a positive Widal test. This suggests the positive Widal test was not related to early typhoid fever. Relying on Widal test for diagnosis of typhoid fever could lead to over-treatment with antibiotics in patients with undifferentiated febrile illness.

Further, a study from India reported that a cut-off titre for O and H antigens on the Widal test should be ≥1:80[20], as used in the present study. For AH and BH titre, the recommended baseline titre was ≥1:160 because *Salmonella* agglutinins are common even among apparently healthy people[20]. In the current study, the baseline titre for AH and BH was also kept at 80 but there were no positive reports with reference to this baseline. Therefore, as an alternative when there are no newer sensitive tests, the Widal test should be interpreted with caution[12].

Widal test titres may also increase due to other infections like malaria, dengue and scrub typhus, immunological disorders and chronic liver failure[21,22]. Relying on the Widal test may therefore result in under-diagnosis of these other illnesses emerging in Tsirang. Further, treatment based on clinical assessment and Widal test alone could lead to over treatment of febrile illness with antibiotics, possibly leading to antibiotic resistance. Therefore, due to the various factors that can influence the results of a Widal test and due to the lack of accuracy found in our study, it is best not to rely much on this test to diagnose and treat typhoid fever. These recommendations are consistent with WHO guidelines.

The following are the strengths of the study. First, this is one of the two studies conducted in Bhutan which sheds light into the validity of Widal test in diagnosis of typhoid fever. Secondly, we adhered to Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines in conducting and reporting the study[23].

The study is not without limitations. This was a record-based study with incomplete information and a small sample size. The socio demographic variables could not be described

due to incomplete data. Incomplete data on the duration of illness was also substantial. Lastly, the results cannot be necessarily generalized to the whole country. Therefore, a prospective study of the whole country with an adequate sample size may be needed to more definitively assess the role of Widal test for the diagnosis of typhoid fever in Bhutan.

Nonetheless, our study has relevant implications. Given that the results of a Widal test can be influenced by various factors, it is best not to rely too much on this test, as recommended by the World Health Organization. Widal-positive test results need differentiation from scrub typhus and other acute febrile illnesses, warranting the introduction of a more sensitive and specific test for the correct diagnosis of typhoid fever in the district hospitals of Bhutan.

CONCLUSIONS

The study found that none of the samples that tested positive by Widal test gave a definite diagnosis of typhoid fever with blood culture. It is consistent to an earlier study done in four large hospitals in Bhutan. Further, Widal testing for the diagnosis of typhoid fever can unnecessarily increase laboratory burden, cause over diagnosis of typhoid fever and inappropriate treatment. Until a more accurate test is introduced in district hospitals in Bhutan, diagnosis of typhoid fever lies uncertainly on clinical suspicion and interpretation of Widal test results. The clinical judgement may further be complicated by scrub typhus and other acute febrile illnesses. Therefore, it is best not to rely completely on Widal test for diagnosis of typhoid fever and this test should be replaced with more accurate ones.

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REFERENCES

1. WHO. Typhoid Fever. Published 2016. Accessed September 13, 2016. [Full Text]
2. Azmatullah A, Qamar FN, Thaver D, Zaidi AK, Bhutta ZA. Systematic review of the global epidemiology, clinical and laboratory profile of enteric fever. J Glob Health. 2015;5(2):20407. [Full Text | DOI]
3. Wattal C, Prasad K, Oberoi J, Goel N. Comparative evaluation of two rapid Salmonella-IgM tests and blood culture in the diagnosis of enteric fever. Indian J Med Microbiol. 2015;33(2):237. [Full Text | DOI]
4. John A, Crump, Stephen P, Luby EDM. The global burden of typhoid fever. Bull World Health Organ. 2004;82(5).
5. Bhutta Z. Current concepts in the diagnosis and treatment of typhoid fever. BMJ. 2006;333(7558):78-82. [Full Text | DOI]
6. Maulingkar SV, Prakash R, Salabha PH and B. Study of baseline Widal titres in a healthy adult population of Wayanad district, Kerala, India. Trop Doct. 2015;45(1):12-14. [Full Text | DOI]
7. Pem T. Typhoid fever outbreak in Bjemina. Bhutan Observer. Published August 10, 2010.[Full Text]
8. Wangmo T. Five epidemic break outs within four months. The Bhutanese. Published April 18, 2012. [Full Text]
9. Andualem G, Abebe T, Kebede N, Gebre-Selassie S, Mihret A, Alemayehu H. A comparative study of Widal test with blood culture in the diagnosis of typhoid fever in febrile patients. BMC Res Notes. 2014;7:653. [Full Text | DOI]
10. Lalremruata R, Chadha S, Bhalla P. Retrospective audit of the widal test for diagnosis of typhoid fever in pediatric patients in an endemic region. J Clin Diagnostic Res. 2014;8(5):22-25. [Full Text | DOI]
11. Wain J, Hosoglu S. The laboratory diagnosis of enteric fever. J Infect Dev Ctries. 2008;2(6):421-425. [Full Text | DOI]
12. Islam K, Sayeed MA, Hossen E, Khanam F, Charles RC, Andrews J, et al. Comparison of the Performance of the TPTest, Tubex, Typhidot and Widal Immunodiagnostic Assays and Blood Cultures in Detecting Patients with Typhoid Fever in Bangladesh, Including Using a Bayesian Latent Class Modeling Approach. PLoS Negl Trop Dis. 2016;10(4):1-10. [Full Text | DOI]
13. T Tshokey, N Tshering, K Wangchuk, R Sharma, A Mongar, T Dorji, et al. A descriptive analysis of clinico-demographic features and microbiological results of patients with suspected typhoid fever in four large hospitals of Bhutan. Sri Lankan J Infect Dis. 2016;Vol.6((2)):106-113. [DOI]
14. Adhikari A, Rauniyar R, Raut PP, Manandhar K Das, Gupta BP. Evaluation of sensitivity and specificity of ELISA against Widal test for typhoid diagnosis in endemic population of Kathmandu. BMC Infect Dis. 2015;15(1):523. [Full Text | DOI]
15. National Statistics Bureau B. Population. Thimphu; 2017. Accessed July 8, 2017. [Full Text]

16. Ministry of Health B. Annual Health Bulletin. Thimphu; 2017.
17. Ministry of Health B. Annual Health Bulletin. Thimphu; 2017.
18. Trowbridge P. Diagnosis of scrub typhus. Expert Rev Anti Infect Ther. 2015;12(December 2014):12. [DOI]
19. Willke A, Ergonul O, Bayar B. Widal Test in Diagnosis of Typhoid Fever in Turkey. Clin Vaccine Immunol. 2002;9(4):938-941. [Full Text | DOI]
20. Gunjal SP, Gunjal PN, Patil NK, Vanaparthi N, Nalawade AV, Banerjee S, et al. Determination of baseline widal titres amongst apparently healthy blood donors in ahmednagar, maharashtra, India. J Clin Diagn Res. 2013;7(12):2709-2711. [Full Text | DOI]
21. Banu A, Hassan MMN, Anand M, Srinivasa S. Baseline antibody titres against Salmonella typhi in apparently asymptomatic HIV positive individuals in a tertiary care hospital. Australas Med J. 2013;6(7):354-357. [PubMed | Full Text | DOI]
22. Mazumder RN, Pietroni MAC, Mosabbir N, Salam MA. Typhus fever: an overlooked diagnosis. J Health Popul Nutr. 2009;27(3):419-421. Accessed June 22, 2017. [Full Text]
23. Elm EV, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. Strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. BMJ. 2007;335(7624). Accessed July 12, 2017. [Full Text]

AUTHORS CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

TN: Concept, study design, analysis of data, manuscript drafting and critical reviews
TD: Concept, samples testing, samples shipment and critical reviews
T: Concept, samples testing, samples shipment and critical reviews
KT: Concept, study design, analysis of data, manuscript drafting and critical reviews
DP: Concept, study design and critical reviews
MS: Concept, study design, analysis of data, manuscript drafting and critical reviews
NR: Concept, study design, manuscript drafting and revision
SW: Concept, study design, manuscript drafting and critical reviews

Authors agree to be accountable for all respects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

None

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