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Evaluation of different diagnostic tests in respect to Diazo test against typhoid in Indian population

Ranbeer Kumar Singh[1], Farhan Usmani[2], Deepak Kushwaha[3], *S.S. Haque[4], Mussarat Perween[5], Sweta Kumari[6], Md. Tanweeruddin[7], Tarique Aziz[8], B.K. Singh[9]

[1]Department of microbiology Narayan Medical College and Hospital, Jamuhar, Sasaram, Bihar
[2,5]Department of Biochemistry, Patna Medical College and Hospital, Patna, India
[3]Department of medicine NMCT&H Birgunj, Nepal
[4,6]Department of Biochemistry, [7]Department of Anaesthesiology and Critical care, ECR, Danapur
[4,.9] Department of Biochemistry, Indira Gandhi Institute of Medical Sciences, Patna-14
[8]Department of Biochemistry, Rajendra Institute of Medical Sciences, Ranchi
[6]Department of Biochemistry, Vardhman Institute of Medical Sciences, Pavapuri, Nalanda

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ABSTRACT

Typhoid fever is an important health issue in developing countries like India and its diagnosis is also difficult. Widal test is commonly used in developing countries for the diagnosis of typhoid. The main aim of this study was to compare the result of Widal test, blood culture and to evaluate efficacy of Diazo test in the diagnosis of typhoid fever. Blood samples were collected from 90 febrile patients with symptoms clinically similar to typhoid fever and Blood culture was used to isolate S.typhi and S.paratyphi. Slide agglutination test and tube agglutination tests were used for the determination of antibody titer. Total Ninety patients' participants 40 (44.4%) were females and 50 (55.5%) were males. The total number of patients who have indicative of recent infection by either of O and H antigens Widal test is 60 (66.6%). Out of 90 suspected enteric fever patients, 55(61.11%) patients were positive for Diazo test. As far as blood culture was concerned total 50 (55.5%) cases were reported positive. In our study, Diazo test had more sensitivity than blood culture but less specificity. So for the quick diagnosis of enteric fever cases, as a bedside screening method, it can be used as an adjuvant testing method to conventional blood culture and widal test for diagnosis of enteric fever.

Keywords: Widal test, Blood culture, Stool culture, Diazo test

INTRODUCTION

In developing countries like India, Typhoid fever remains an important disease caused by *Salmonella* Typhi, is most commonly isolated from blood during the first, second or third week of illness, during the first week of antimicrobial therapy and during clinical relapse [1]. Isolation of *Salmonella* Typhi from bone marrow is the current gold standard method for confirming a case of typhoid fever. However, this requires equipment, and skilled laboratory personnel seldom found in primary health-care facilities in the developing world [2,3]. Blood culture is a more practical in comparison to bone marrow culture. However, it is not always available and, when it is, it takes 2 to 3 days. As a result, diagnosis may be delayed or overlooked and patients without typhoid fever may receive unnecessary and inappropriate antimicrobial treatment. For this reason, in developing countries typhoid rapid antibody tests can facilitate diagnosis and disease. New commercially available typhoid rapid antibody tests have been evaluated in Asia, where typhoid fever is known to be highly endemic [4]. In Asian studies the tests have shown variable performance. Rapid typhoid tests have not been evaluated in sub-Saharan Africa, where the typhoid fever burden may be smaller than in Asia.[5-7] The World Health Organization (WHO) has issued no recommendations on the use of typhoid rapid antibody tests.[8] Accurate diagnostics for typhoid fever could provide valuable diagnostic information for patient management and make it possible to estimate the incidence of typhoid fever in low-resource settings. Widal test measures

Corresponding Author Address: Dr. S.S. Haque, Department of Biochemistry, Indira Gandhi Institute of Medical Sciences, Patna-14
Email: sshaq2002@yahoo.co.in

antibodies against O and H antigens of S. Typhi but lacks sensitivity and specificity in endemic areas. Widal test alone is prone to error due to false positive and false-negative results. So widal test is the choice for typhoid fever especially in rural area.[9] One of the major drawback of widal test is cross-reactivity due to which some other bacteria of same genus often produces false positive results, so the positive results must correlate clinically before prescribing medicine. Diazo test is a simple non-invasive, inexpensive bedside test, of urine sample; a chemical test for typhoid illness has elucidated many claims to the level that it is the most valuable single test in the diagnosis of typhoid fever. Some authors have stated that it is 30-90% positive in typhoid cases [10,11] described by Huckstep in 1962, is a diagnostic tool in the diagnosis of Typhoid fever [12-17]. It is known that the putrefaction of a protein in the intestine of patients with typhoid fever leads to a breakdown product being excreted in the urine as a phenol-ring compound which is detected by Diazo test.

The main aim of this study was to compare the result of Widal test, blood culture and to evaluate efficacy of Diazo test in the diagnosis of typhoid fever in endemic rural and even urban areas where laboratory facilities may be inadequate or absent and used blood culture as the standard for comparison.

MATERIALS AND METHODS

Patients who met the criteria were asked to give informed consent and answer a brief questionnaire about clinical signs and symptoms, antimicrobial treatment, and history of typhoid fever and vaccination. Participants gave 5 ml of blood (3 ml from children 3 to 5 years old) upon routine venipuncture for blood culture. Only patients with a laboratory-confirmed etiology of their fever were included in the analysis.

Blood samples were centrifuged, and the serum was divided into aliquots and stored at _20°C. In order to minimize the degradation of the antibodies in the serum, the specimens were frozen immediately and remained frozen until the time of testing.

Diazo test was performed on early morning urine samples of all cases which were included in the study. Diazo reagent is prepared from two stock solutions 'A' and 'B'. Their compositions are as below:

a) Stock solution A: Sulphanilic acid- 0.5 gm, Concentrated HCL- 5 mL, Distilled water- 100 mL.
b) Stock solution B: Sodium nitrite- 0.5 gm, Distilled water- 100 mL.

RESULTS

Ninety patients were enrolled in which 50 (55.56%) were males and 40(44.44%) were females Participants had a median age of 26 years (range: < 1 to 76) (Table 1). Out of 90 cases 60 (66.6%) were Widal test positive on first instance and 40 (44.4%) blood culture were negative 50 (55.5%) were positive cases showed rise in titre in paired sera taken at interval of 7 to 10 days. In stool culture 40 (44.4%) were positive and 50 (55.5%) were negative (Table 1). 55 patients in the study group showed a positive diazo test. In the control group, Diazo test was positive in only four patients.

Table 1 Characteristics of patients

Sr.No	Parameter	Cases
1.	Total no. of patients	90
2.	Median age (range)	26 years
3.	No. (%) of female patients	40 (44.4%)
4.	Median days (range) between fever onset and enrollment	13(5-55)

Table 2: Results from diagnostic tests of different parameter

Sr.No	Diagnostic Test	Positive Number	%	Negative Number	%
1.	**Widal test**	60	66.6%	30	33.3%
2.	**Stool Culture**	40	44.4%	50	55.5%
3.	**Blood Culture**	50	55.5%	40	44.4%
4.	**Typhi dot test**	63	70%	27	30%
5.	**Diazo test**	55	61.11%	35	38.88%

DISCUSSION

Typhoid one of the major causes of mortality and morbidity in developing countries like India. Several factors contribute to the uncertainty of exactly identifying typhoid fever. The treatment of typhoid fever early is an essential factor in preventing the emergence of antibiotic resistance which is becoming increasingly common due to incorrect diagnostic test results and their interpretation. The advent of a rapid test for diagnosis of typhoid fever has come as a boon for clinicians and patients, but the reliability of these tests in the Indian perspective and among children is still questionable. The yield of blood culture was 55.55%, which is comparable with culture positivity reported in other studies, which varies from 14.3% to 67.8%. It can be negative in up to 30% of culture proven cases of typhoid fever. Diazo test is positive in 61.11% compare to stool and blood culture. Diazo test is a simple urine sample based test shows almost parallel results to other serum based test in north Indian population. In our study, Diazo test showed a promising results when validated against Widal and blood culture.

Conclusion

Diazo test is simple and quick test is a useful diagnostic tool in the early diagnosis of typhoid fever especially in areas with limited resources compare to other tests for typhoid.

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