A Comparative Study of Diazo Test and Blood Culture in Children With Clinically Compatible Typhoid Fever

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ABSTRACT

Introduction: Typhoid fever causes a clinically indistinguishable disease with a wide range of clinical severity. This study was done to compare the Diazo test with blood culture.

Methods: A cross-sectional comparative study was done for one year with a sample size of 100 children up to the age of 15 years in the Department of Paediatrics, Sree Balaji Medical College and Hospital, Tamilnadu, India. Diazo test and blood culture were done in enrolled children who had clinical symptoms and signs suggestive of typhoid fever.

Results: Blood culture-positive cases were 26% and diazo-positive cases were 34%. Out of 26 blood culture-positive cases, 19 cases showed positive results and seven had a negative result by Diazo test and this was statistically significant. Diazo test had sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, positive predictive value, negative predictive value of 73.08%, 79.73%, 3.61, 0.34, 55.8%, 89.4% respectively. The measure of agreement Kappa value was found to be 0.480 which is considered to be a significant moderate agreement between the Diazo test and blood culture.

Conclusions: Diazo test is a simple bedside test with a comparable degree of sensitivity and specificity and can be utilized for the diagnosis of typhoid fever in children in areas of scarce resources and thereby reducing the complications.

Key words: Diazo test; Sensitivity; Specificity

INTRODUCTION

Typhoid fever is one of the most common infections which continue to cause a significant disease burden in developing countries like India. Global burden of disease study in 2015 estimated 17 million cases of typhoid and paratyphoid fever globally, with the higher incidence and larger burden in Southeast Asia. Though Asia and Africa contribute to the major proportion of the disease incidence worldwide, regional differences were present both within and between these countries. As the age advanced the incidence of typhoid fever showed a decreasing trend.[1]

Certain infections like malaria, typhoid, leptospirosis and scrub typhus share similar clinical features with typhoid fever and hence it is very difficult to recognize this disease with the clinical features alone. It is not feasible to send blood culture to all suspected cases. The increasingly common practice of taking antibiotics before the confirmation of diagnosis has brought down the sensitivity of blood culture to approximately 60% and till now no other ideal diagnostic test is available for diagnosis of enteric fever.[2]

An inexpensive simple test like Diazo test which was used in the past epidemic situations should be re-evaluated especially in resource-limited health settings. Diazo test might take five days to detect typhoid fever and becomes negative when the clinical symptoms subside.[3] This study was done to compare the sensitivity and specificity of the Diazo test with blood culture in clinically suspected typhoid cases in our tertiary health care center in Chennai, India.

METHODS

This cross-sectional comparative study was done in the Department of Paediatrics, Sree Balaji Medical College and Hospital, Chennai, Tamilnadu for a period of one year (April 2018 to April 2019). The sample size was calculated considering the specificity of the Diazo test with the blood culture of 93.3%, with 95% confidence interval and 5% of absolute precision.[4] Hundred children up to the age of 15 years with a history of fever more than three days with clinical symptoms and signs suggestive of typhoid fever were included in this study. Participants who did not consent to participate and children with alternative diagnoses were excluded from this study. The study was started after obtaining ethical clearance from the ethical committee of the institution. Parents or guardians were informed about the study and consent was taken. The details of clinical symptoms and positive signs were recorded in the proforma.

Complete blood count, Diazo test, and blood culture were done in all enrolled patients on the day of admission. Diazo reagent is prepared from two stock solutions 'A' and 'B', the constituents of which were as follows: (i) Stock solution 'A': Sulphanilic acid - 0.5 g, Cone HC1 - 5 ml, Distilled water - 100 ml; and (ii) Stock solution 'B': Sodium nitrite - 0.5 g, Distilled water - 100 ml. Forty parts of the stock solution 'A' is mixed with one part of the stock solution 'B'. Two ml of this mixture and two ml of early morning urine sample were mixed. To those, five drops of 30% ammonium hydroxide was added. The sample was shaken well. A positive reaction was indicated by a red or pinkish coloration of the froth. Statistical analysis was performed using SPSS statistical software (Version 24). Chi-square test was used to observe statistical differences among the observed value and experimental value of variables. The differences were considered statistically significant at a p value < 0.05. Sensitivity, specificity, positive predictive value, negative predictive value, positive and negative likelihood ratios were calculated for the Diazo test by comparing the results with the gold standard blood culture.

RESULTS

Out of 100 children in this study, 26 children were culture positive and 34% were found positive by the Diazo test. Blood culture positive patients were taken as confirmed cases of typhoid fever as the blood culture method is the gold standard for diagnosing typhoid fever. Table 1 shows the percentage of blood culture and Diazo test positive cases.

Of 26 blood culture-positive cases, 19 cases showed positive results and seven had a negative result by Diazo test. The remaining 15 cases which were found to be positive using the Diazo test were

Table 1. Results of blood culture and Diazo test

Results	Blood culture		Diazo test	
	No.	%	No.	%
Positive	26	26%	34	34%
Negative	74	74%	66	66%
Total	100	100%	100	100%

found negative with blood culture. The p - value for the below tabulation is 0.001 which shows that it is statistically significant. Table 2 shows the comparison of the Diazo test and blood culture.

It has been observed from the study that for Diazo test, sensitivity was 73.08 % (CI = 52.21% - 88.43%), specificity was 79.73% (CI = 68.78% - 88.19%), positive likelihood ratio was 3.61 (CI = 2.17 - 5.99), negative likelihood R = ratio was 0.34 (CI = 0.18 - 0.64), positive predictive value was 55.8% (CI = 43.2% - 67.8%) and negative predictive value was 89.4% (CI = 81.6% - 94.1%). Table 3 represents the values of the Diazo test.

The measure of agreement Kappa value was found to be 0.480 which is considered to be a significant moderate agreement between the Diazo test and blood culture which is shown in table 4 and the test was found to be statistically significant.

Table 3. Values of Diazo test

	Estimate	95% Confidence interval
Sensitivity	73.08%	52.21% - 88.43%
Specificity	79.73%	68.78% - 88.19%
Positive likelihood ratio	3.61	2.17 - 5.99
Negative likelihood ratio	0.34	0.18 - 0.64
Positive predictive value	55.8%	43.2% - 67.8%
Negative predictive value	89.4%	81.6% - 94.1%

Table 2. Comparison of Diazo test and blood culture

		Blood culture		Total	Pearson Chi-Square test
		Positive	Negative		
Diazo test	Positive	19	15	34	$x2 = 23.9$
	Negative	7	59	66	p = 0.001*
Total		26	74	100	

**statistically significant*

DISCUSSION

The clinical diagnosis of typhoid fever is difficult because of its common presentation with many other febrile illnesses, especially in the endemic regions. Easy availability of antimicrobials along with its usage without prescription in countries like India is another major issue hindering accurate diagnosis.[5] Along with this, the high burden of the disease in especially younger children[6] poses a greater risk in terms of drug resistance and interference with confirmatory diagnosis. This tendency leads to mitigation of more severe symptoms of typhoid fever and therefore the real burden of disease goes unrecognized. In endemic areas, the diagnosis of typhoid fever with Widal test should be done with caution as antibodies are present in the population because of past subclinical infections with salmonella species.[7] Though immunodiagnostic tests are simple, easy to perform giving early diagnosis with high sensitivity but they are limited by their suboptimal specificity, cost and availability.[8]

In our study, 26% of the cases were positive by blood culture which is more or less comparable to the observations made by Retnosari S et al.[9] who have reported 24% positivity for blood culture. Other studies have reported variable blood culture positivity from 7.96% to 27.3%.[10-12] Although blood culture is considered as the gold standard test for diagnosis of enteric fever, there are several factors like volume of blood cultured because of difficulty in drawing the required amount of blood for culture in children, the ratio of blood volume to culture broth volume, and inclusion of anti

Table 4. Measure of agreement of Diazo test

Symmetric measures					
	Value	**Asymptomatic standard error**	**Approximate T**	**Approximate significance**	
Measure of Agreement	Kappa	0.480	0.094	4.890	0.001
Number of valid Cases	100				
a. Not assuming the null hypothesis.					
b. Using the asymptotic standard error assuming the null hypothesis.					

complementary substances such as bile in the medium affecting the recovery of an organism from culture leads to the lower percentage of blood culture positivity.[13] In developing countries, the feasibility of taking blood culture before starting antibiotics, along with its poor sensitivity[14] and also delay in getting the culture report makes it not an ideal choice for early diagnosis of typhoid fever.

Of 26 blood culture-positive cases, 19 cases showed positive results and seven had negative result by Diazo test. The remaining 15 cases which were found to be positive using the Diazo test were found negative with blood culture. Diazo test is not affected by prior antibiotic intake and it is a simple bedside test. This explains the reason for those 15 cases who were found to be positive using the Diazo test but were found negative with blood culture and also blood culture results are affected by varied reasons mentioned above.

We had seven cases that were positive by blood culture but were detected negative by Diazo test. We probed the literature on Diazo test to find out the reasons for variability in the results, but we couldn't get any further references. On average Diazo test usually becomes positive on day five of fever but in our study children with fever more than three days but clinically compatible with typhoid fever were also included. This could be the possible reason for this discrepancy. Our study showed a statistically significant p-value for this comparison.

In the present study, the sensitivity of 73.08% is due to the fact that out of 26 blood culture positive typhoid cases, 19 were detected positive by the Diazo test. The specificity of 79.73% is because the Diazo test showed positive in only 15 out of 74 blood culture-negative cases. These results prove the utility of this test. Other parameters like positive likelihood ratio (3.61), negative likelihood ratio was (0.34), positive predictive value (55.8%) and negative predictive value was (89.4%) justifies the diagnostic use of this test. In low middle-income countries, the real burden of typhoid fever goes underestimated in infants and young children, as blood culture is not done for the majority of cases, especially those treated in nonhospital settings.[2]

The major limitation of this study is that children who had taken prior antibiotics were also included which might have lowered the blood culture sensitivity. We had to include those children as it was not possible to exclude them as most of the children would have already taken antibiotics before admission. This highlights the indiscriminate usage of antimicrobials prevailing in the current scenario. Diazo test can also have false positive[15] result of about 5% which can be seen in tuberculosis, measles, typhus and therefore it is very essential to rule out other alternative diagnoses. Diazo test showed a high degree of sensitivity and specificity in the present study. This simple, inexpensive, rapid diagnostic test can be used for supporting the early diagnosis of typhoid fever in places with simple laboratory facilities. As antibiotics administered before the test do not interfere with the results[15] it can be used as a good screening test in resource-limited areas.

CONCLUSIONS

Though blood culture is the ideal test in the diagnosis of typhoid fever, it is underutilized because of many limitations and also not routinely available everywhere. A simple cost-effective bedside test like Diazo test can be used as a diagnostic tool for typhoid fever especially in

resource-poor settings and primary health centers and henceforth reducing morbidity and mortality.

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