Typhidot M and Diazo test *vis-à-vis* blood culture and Widal test in the early diagnosis of typhoid fever in children in a resource poor setting

ABSTRACT

Objective: Typhoid fever is a major public health problem. A test which is simple, reliable and can be carried out in small laboratories is the need of the hour. We prospectively evaluated typhidot M and Diazo tests *vis-à-vis* blood culture and Widal test in children. **Methods:** Patients aged 6 months to 12 years, having fever of more than four days duration with clinical suspicion of typhoid fever were enrolled. Patients in whom other diagnosis was made served as control. The tests under scrutiny were validated against blood culture and then all the four tests were evaluated among patients who presented in the first week of illness. **Results:** Blood culture was positive in only 27.3% of the cases. Among these culture positive cases, typhidot M test had the highest sensitivity, specificity, PPV and NPV of 90% (95% CI = 74.4-96.5), 100% (95% CI = 90.1-100), 100% (95% CI = 87.5-100), and 92.1% (95% CI = 79.2-97.3) respectively. Diazo test ranked next with sensitivity, specificity, PPV and NPV of 86.7% (95% CI = 70.3-94.7), 85.7% (95% CI = 70.6-93.7), 83.9% (95% CI = 67.4-92.9), 88.2% (95% CI = 73.4-95.3) respectively. Among clinically suspected typhoid cases, the overall sensitivity, of blood culture, Widal, typhidot M, Diazo was 27.3% (95% CI = 19.8- 36.3), 64.6% (95% CI = 55.3-72.9), 89.1% (95% CI = 81.9-93.7), 80.9% (95% CI = 72.6-87.2) respectively. In the first week of illness, typhidot M showed the best sensitivity [86.2% (95% CI = 69.4-94.5)] followed by Diazo [79% (95% CI = 61.6-90.2)], Widal [41.4% (95% CI = 25.5-59.3)] and blood culture [31% (95% CI = 17.3-49.2)]. **Conclusion:** Both Typhidot M and Diazo are good screening tests for the diagnosis of typhoid fever. Typhidot M is superior to Diazo but the latter is more suitable to resource poor settings being economic and easy to perform.

Keywords: Typhidot test; Diazo test; Widal test; Typhoid fever.

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INTRODUCTION

Typhoid fever is a global health problem. According to the best global estimates approximately 600,000 deaths[1] occur annually due to typhoid fever, majority of which occur in developing countries. The annual incidence rate of 980 per 100,000 has been reported in India.[2] Most serotypes of typhoid infections are diagnosed purely on clinical grounds and treated presumptively leading to delayed diagnosis, emergence of drug resistance and missing other clinical diagnosis which could be mistaken as typhoid fever. Isolation of bacteria from blood remains the gold-standard for diagnosing typhoid fever. However this requires laboratory equipments and technical support which is lacking or limited in the peripheral health facilities in developing countries and the time taken for culture reports is rather long, usually taking about seven days. Hence, it does not contribute towards an early diagnosis. Moreover, widespread use of antibiotics in the community makes it difficult to isolate the bacteria from the blood even in true typhoid fever. The commonly used Widal test for diagnosis of typhoid fever needs to be interpreted with caution in endemic areas where anti-O and anti-H antibodies are already present in the population as a result of past subclinical infections with *salmonella* species, enterobacteriaceae, malaria, etc.[3] Widal test is now regarded as inaccurate, non-specific, poorly standardized and of limited diagnostic value.[4-11] To overcome such limitations, several assays and serological tests have been developed but none is found optimal.[12-14] A rapid serological/biochemical test to diagnose typhoid

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fever accurately at an early stage is thus currently needed. Recent advances in immunology have led to the discovery of more sensitive and specific markers of typhoid fever and hence newer serological tests like typhidot M has come up. A simple bedside test like Diazo which has served in epidemic situations in the past, should also be reevaluated for those working in remote health settings. This prospective study was, therefore, carried out to evaluate the role of preexisting tests and newer diagnostic tests in the early diagnosis of typhoid fever.

METHOD

The study was conducted in the department of pediatrics Jawahar Lal NehruMedical College Hospital, a tertiary care hospital in the northern part of India. It was a hospital based prospective study which included 145 clinically suspected enteric fever cases. All children between 6 months and 12 years of age with fever of more than four days having a clinical suspicion of typhoid fever were enrolled and admitted to the hospital. The criteria for clinical suspicion were those already used by previous workers.[15-17] Detailed clinical evaluation was done and findings were recorded on a standardized format. Complete blood count, smear for malarial parasite, urine and stool routine microscopy and urine culture were done in all cases. Other appropriate investigations like liver function test, lumber puncture, electrocardiogram, abdomen ultrasound were also done where indicated. The four tests Diazo, Widal, typhidot M and blood culture were done in all enrolled cases. Diazo test was performed by mixing 5 mL of urine with equal amount of freshly prepared Diazo reagent and then adding five drops of 30% ammonium hydroxide. The mixture was shaken and the color of the froth was noted. Pink or red was taken as positive and all other as negative. The Widal test was performed by double dilution technique using *salmonella* antigens (Span Diagnostic Limited, Surat, India). The antibody titer was considered positive at H and O titer of > = 200 and > = 100, respectively. For typhidot M the kit manufactured by Malaysian Bio-Diagnostics was used. Blood culture was done by inoculating 5 mL of blood immediately in a culture bottle containing brain heart infusion with 0.025% sodium polyanethol sulphonate. The culture bottles were then examined at different stages, subcultures done and positive colonies identified. Children in whom an alternative final diagnosis was made served as control. The remaining were labeled as "clinical typhoid" cases which was further divided into culture positive and culture negative groups. The study protocol was reviewed and approved by the Institutional Ethical committee. The statistical analysis was done using SPSS version 10.0. Sensitivity, specificity, positive predictive value and negative predictive values were calculated for each test.

RESULTS

One hundred and forty-five cases were enrolled in the study. The 35 cases in which an alternative diagnosis was made served as control. The remaining 110 were labelled as clinical typhoid cases of which 30 were blood culture positive and 80 were negative. In the control group ten were diagnosed as malaria, four as tubercular meningoencephalitis, five as lobar pneumonia, two as chronic liver disease, five as pharyngotonsillitis, one as pyogenic meningitis and eight had blood cultures positive for organisms other than typhoid.

Among the 30 culture positive cases, the typhidot M test was positive in 27 cases giving sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of 90%, 100%, 100%, 92.1% respectively (Table 1). The Diazo test was found to have sensitivity, specificity and PPV of 86.7%, 85.7% and 83.9% respectively and Widal test was positive in 12 cases giving sensitivity, specificity and PPV of 40.0%, 91.4%, 80% respectively. On comparative evaluation of all the tests for the entire cohort of 110 clinical typhoid

Table 1. Diagnostic parameters of various tests among culture positive cases (n = 30)

Diagnostic tests	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV (95% CI)	NPV (95% CI)
Widal test*	40.0 (24.6-57.7)	91.4 (77.6-97.0)	80.0 (54.8-92.9)	64.0 (50.1-75.9)
Typhidot M test**	90.0 (74.4-96.5)	100 (90.1-100)	100 (87.5-100)	92.1 (79.2-97.3)
Diazo test***	86.7 (70.3-94.7)	85.7 (70.6-93.7)	83.9 (67.4-92.9)	88.2 (73.4-95.3)

* positive Widal in cases = 12, positive Widal in controls = 3
** positive typhidot M in cases = 27, positive typhidot M in controls = 0
*** positive Diazo in cases = 26, positive Diazo in controls = 5

fever cases (Table 2), the typhidot M was positive in 98 of them giving the sensitivity, specificity and negative predictive values of 89.1%, 100% and 74.5%, respectively, while that of blood culture was 27.3%, 100%, and 30.4% respectively. The Diazo test was positive in 89 of 110 cases and hence had sensitivity and specificity of 80.9% and 85.7%, respectively. Among the culture negative typhoid cases (Table 3), typhidot M test was the most sensitive and specific test (88.8% and 100%) while Diazo and Widal test had a sensitivity of 80% and 71.3% and specificity of 85.9% and 91.4%, respectively. The results of all diagnostic tests in patients presenting in the first week of illness were compared and it was found that the sensitivity of typhidot M, Diazo test, blood culture, Widal test was 86.2%, 79%, 31%, 41.4%, respectively (Table 4).

Table 2. Diagnostic parameters of various tests for the entire cohort of clinically suspected typhoid fever cases (n = 110)

Diagnostic tests	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Blood culture[#]	27.3 (19.8-36.3)	100 (90.1-100)	100 (88.7-100)	30.4 (22.8-39.4)
Widal test[*]	64.6 (55.3-72.9)	91.4 (77.6-97.0)	95.9 (88.8-98.6)	45.1 (34.1-56.6)
Typhidot M test[**]	89.1 (81.9-93.7)	100 (90.1-100)	100 (96.2-100)	74.5 (60.5-84.8)
Diazo test[***]	80.9 (72.6-87.2)	85.7 (70.6-93.7)	94.7 (88.2-97.7)	58.8 (45.2-71.3)

[#] positive blood culture in cases = 30, positive blood culture in controls = 0
[*] positive Widal in cases = 7 1, positive Widal in controls = 3
[**] positive typhidot M in cases = 98, positive typhidot M in controls = 0
[***] positive Diazo in cases = 89, positive Diazo in controls = 5

Table 3. Diagnostic parameters of various tests among culture negative cases

Diagnostic tests	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Widal test[*]	71.3 (60.5-80.0)	91.4 (77.6-97.0)	95.0 (86.3-98.3)	58.2 (45.0-70.3)
Typhidot M test[**]	88.8 (79.9-93.9)	100 (90.1-100)	100 (94.9-100)	79.6 (65.5-88.9)
Diazo test[***]	80 (69.9-87.3)	85.7 (70.6-93.4)	92.8 (84.1-96.9)	65.2 (50.8-77.3)

[*] positive Widal in cases = 57, positive Widal in controls = 3
[**] positive typhidot M in cases = 71, positive typhidot M in controls = 0
[***] positive Diazo in cases = 64, positive Diazo in controls = 5

Table 4. Comparative evaluation of various tests in the first week of illness

Diagnostic tests	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Blood culture[#]	31 (17.3-49.23)	100 (91.1-100)	100 (70.1-100)	63.6 (50.4-75.1)
Widal test[*]	41.4 (25.5-59.26)	91.4 (77.6-97.0)	80 (54.8-92.9)	65.3 (51.3-77.1)
Typhidot M test[**]	86.2 (69.4-94.5)	100 (90.1-100)	100 (86.7-100)	89.7 (76.4-95.9)
Diazo test[***]	79 (61.6-90.2)	85.7 (70.6-93.7)	82.1 (64.4-92.1)	83.3 (68.1-92.1)

[#] positive blood culture in cases = 9, positive blood culture in controls = 0
[*] positive Widal in cases = 12, positive Widal in controls = 3
[**] positive typhidot M in cases = 25, positive typhidot M in controls = 0
[***] positive Diazo in cases = 23, positive Diazo in controls = 5

DISCUSSION

In our study culture positivity among clinically suspected typhoid cases was 27.3% and 31% in those who came for care in the first week. Culture positivity reported in other studies varies from 14.3% to 67.8%.[18-21] While the lower values in our study could be due to the rampant use of antibiotics by private practitioners, the fact remains that in the majority of the studies the culture yield was around 40%.[22-25] This value is too low to satisfy the criterion of a diagnostic test, irrespective of the reasons for its low yield. Nonetheless, blood culture is the foolproof method for the diagnosis of typhoid fever and hence a substitute has to be validated against it. Furthermore, the feasibility of a test has to be taken into account. The idea of the study was to find the utility of various available tests as applied to various levels of health care, especially the resource poor settings. Typhidot M test is based on IgM antibodies which appear in detectable titers as early as the second day of illness. It showed sensitivity of 90% and specificity of 100% in blood culture proved cases. In simple words, we could pick up 9 out of 10 cases of true typhoid fever *vis-à-vis* blood culture which could pick up only 2.7 out of 10 cases. Even in the first week of illness when blood culture is supposed to have maximum positivity, the ratio proportion was only marginally different, i.e. blood culture could pick up 3.1 out of 10 cases as against typhidot M which picked up 8.6 out of 10 cases of true typhoid. So typhidot M is a reasonable substitute for blood culture having good correlation. However, it has limitations due to high cost and laboratory technique involved.

The Diazo test had a sensitivity of 86.7% which was comparable to previous studies,[26,27] but false positivity of 14.3% is rather high. However, the test is much simpler than blood culture, typhidot M and Widal test. Thus, it can be used in resource poor settings and primary health centers. If we consider substituting Diazo for Widal, we find that Diazo has better sensitivity, specificity, PPV, NPV of 86.7%, 85.7%, 83.9%, 88.2%, respectively, among culture positive cases. In simple terms, the Diazo test was able to pick up about 8 out of 10 cases of typhoid fever in the first week of illness as compared to Widal which could pick up only 4 out of 10 cases. The specificity of Widal test seen in such an early period was high (91.4%) probably because we had excluded the common causes of fever like, tuberculosis, malaria, sepsis, pneumonia, UTI.

CONCLUSION

Both typhidot M and Diazo tests are good screening tests for the diagnosis of typhoid fever. Typhidot M is superior to Diazo, but the latter is more suitable to resource poor settings as it is economic and easy to perform.

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