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Research Article

COMPARISON OF WIDAL AND TYPHOID IMMUNOGLOBULIN M AND IMMUNOGLOBULIN G IN RAPID AND EARLY DIAGNOSIS OF ENTERIC FEVER

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ABSTRACT

Objective: Typhoid fever is one of the major public health problems in developing countries including India. A simple, reliable, rapid, and early diagnostic test has been one of the important needs of the clinicians. The present study was carried out to compare the Widal test and typhoid immunoglobulin M (IgM) and immunoglobulin G (IgG) rapid test in diagnosing of *Salmonella typhi* infection.

Methods: A total of 100 cases having clinical suspicion of typhoid fever and 40 controls (20 healthy persons and 20 non-typhoidal febrile patients) were studied. Participants were investigated by blood culture, clot culture, Widal test, and typhoid IgM and IgG rapid test, and the results were compared.

Results: Typhoid IgM and IgG test was positive for IgM in 70 cases and IgG for 6 cases of typhoid fever compared to Widal test which showed only 58 positive cases. The sensitivity, specificity, positive, and negative predictive value of typhoid IgM was found as 70%, 90%, 94.59%, and 54.55%, respectively. On the other hand, corresponding values for Widal test were 58%, 85%, 90.63%, and 44.74%, respectively.

Conclusion: In the present study, the typhoid IgM and IgG yielded remarkable high sensitivity and specificity to diagnose typhoid fever in the first week of illness, so it is recommended to use the test in small and less equipped laboratories as a complementary test to Widal.

Keywords: Widal, Typhoid immunoglobulin M/immunoglobulin G, Typhoid fever, Blood culture.

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INTRODUCTION

Enteric fever is a global health problem. Its real impact is difficult to estimate because the clinical picture is confused with other febrile infectious diseases. The incidence of typhoid fever has been estimated approximately 2.17 million cases with at least 217,000 deaths occurring worldwide annually [1]. The disease is endemic in the Indian subcontinent [2]. A delay in diagnosis and management may significantly increase the risk of adverse outcome and mortality [3]. Hence, an accurate diagnosis of enteric fever at an early stage is important not only for etiological diagnosis but also to identify the potential carriers and may be responsible for acute enteric fever outbreaks [4]. Although blood culture is the gold standard for the diagnosis of enteric fever, it is not routinely requested by physicians because it is expensive and there is delay in result of at least 2-3 days [5]. The Widal test has been used for almost 100 years and widely available in developing countries and is still regarded useful in endemic areas [6]. However, it becomes positive only in the second week of illness [7], and paired sera are required for confirmation of the diagnosis [8]. Hence, the limitation of the above traditional methods has prompted other novel tests to be developed. The typhoid immunoglobulin M (IgM)/immunoglobulin G (IgG) rapid test is also a new serologic test based on the lateral flow immunochromatographic principle. It detects the presence of specific IgM and IgG antibodies to *Salmonella typhi* lipopolysaccharide. The test becomes positive as early as in the first week of the fever; the results can be interpreted visually and available within 15 minutes. Hence, it provides an alternative for rapid and early diagnosis of typhoid fever.

METHODS

The study was designed as cross-sectional comparative study for 1 year. Relevant history, clinical findings, and laboratory records of every case were collected and recorded, and the data were analyzed using a computer program SPSS Version 20. The study population consisted of 100 clinically suspected typhoid fever cases and 40 controls comprising 20 healthy and 20 non-typhoidal febrile illness cases. 100 clinically suspected typhoid fever cases (irrespective of age and sex) were selected on the basis of following criteria of fever for >3 days, with no obvious focus of infection, abdominal discomfort, constipation or loose motion, coated tongue, toxic look, hepatomegaly, splenomegaly, relative bradycardia, rose spot, etc. [9].

Blood culture was done by conventional or traditional method using trypticase soy broth (TSB) with sodium polyanethol sulfonate. 5 ml of collected blood for adult and 3 ml of collected for pediatric patients were inoculated immediately into 50 ml and 30 ml TSB broth (which was brought to room temperature 30 minutes before inoculation), respectively. The inoculated bottle was inverted 3-5 times to mix blood with broth. Inoculated culture bottle was incubated at 37°C aerobically. Bottles were examined visually daily for growth. It was indicated by hemolysis of red blood cells, gas bubbles in the medium or turbidity in the broth. When macroscopic evidence of growth was apparent, a Gram-stained smear was done. In addition to daily visual examination, blind subculture from conventional bottle after the first 24 hrs of incubation was performed aseptically removing a few drops of the well-mixed medium and spreading this inoculum onto MacConkey and Blood agar plates. The plates were incubated at 37°C aerobically for 24-48 hrs. Culture negative bottles were then reincubated for 5-7 days [10]. Suspected colonies were identified as *S. typhi* by Grams stain, motility test and by following biochemical tests including - oxidase test, triple sugar iron agar (TSI), citrate, indole and urease tests, and slide agglutination test with high titer sera.

For blood clot culture, a modification of method described by Escamilla *et al.* [11] was used. The clot was broken up with a sterile glass rod and added to bottle of 50 ml bile broth. Streptokinase (100 units/ml) was added into the broth to facilitate lysis of the clot. Culture was incubated

at 37°C for 24 hrs. At interval of 1, 3, and 7 days, subculture was done on blood and MacConkey agar non-lactose fermenting colonies from the MacConkey agar plates were identified by Gram's staining, oxidase test, conventional biochemical tests, and slide agglutination test using high titer antisera.

The Widal test was done by tube agglutination using various dilutions and significant titer was taken as 1:80 and above for TO and 1:160 and above for TH [12].

The rapid test for detection of IgM and IgG antibody in patient's serum was done in all groups of cases and controls. The test is based on lateral flow immune-chromatographic principle. The device contains two test strips: One for IgM detection and another for IgG detection. The IgM strip consisting of a dried conjugate pad containing antihuman IgM conjugated with colloidal gold, a nitrocellulose membrane immobilized with *S. typhi* antigen lipopolysaccharide (LPS) at test line region "T" and a control line protein at control line "C." The IgG strip consisting of a dried conjugate pad containing protein A conjugated with colloidal gold, on a nitrocellulose membrane immobilized with *S. typhi* antigen LPS at test line region "T" and control line protein at control line region "C." When test specimen is applied into sample well(S) of the test device, the specimen migrate by the capillary action across the nitrocellulose membrane. If antibody to *S. typhi* is present in the specimen, it will react to the colloidal gold conjugate and makes an immune complex. The immune complex moves on the membrane and reacts with immobilized antigen of *S. typhi* resulting information of pink/purple line at "T" region. The test contains an internal inbuilt control which should exhibit a pink or purple line at "C" region. The result is invalid if pink/purple line at "C" is invisible.

About 5 µl of specimen was added using micropipette into the S+B well or fill the provided disposable plastic dropper with the specimen up to the indicated mark on dropper and add into S+B well. Add 2 drops of buffer into S+B and wait for appearance of pink/purple lines in result window. Results were read within 15 minutes.

Positive result was observed as appearance of pink/purple lines at "T" and "C" region which indicates specimen has antibody to *S. typhi*. Negative result was observed as appearance of only one pink/purple line at "C" region which indicates that specimen has no antibody to *S. typhi*.

RESULTS

The present study was conducted among 140 participants. 100 were clinically suspected case of typhoid fever and 40 were age- and sex-matched healthy and sick controls. Out of 100 cases, 16 were culture positive for *S. typhi*, 58 were Widal positive, and rest of the 32 were clinically diagnosed typhoid fever, but blood culture and Widal test were negative. 70(70%) were typhoid IgM/IgG positive (Table 1). The rapid test was found to be highly significant than Widal test among clinically diagnosed typhoid fever ($p<0.001$).

The highest rate of blood culture positivity for *S. typhi* was found among 1-5 years of age group (16.66%). Among the 32 cases, that were both blood culture and Widal test negative 6 cases were rapid test positive. Typhoid IgM/IgG was positive in 10 (62.5%) out of 16 culture positive typhoid cases. The rapid test was also positive in 4 (20%) out of 20 febrile controls. None of the healthy controls were positive by rapid test (Fig. 1). The Widal test was positive in 6 (37.50%) out of 16 blood culture positive cases and in 52 (61.90%) out of 84 culture negative typhoid cases. Out of 16 blood culture positive typhoid cases, only 6 (37.50%) was Widal positive.

Out of 84 culture negative typhoid cases, 60 (71.40%) were rapid test positive and 52 (61.90%) positive for Widal. Among the non-typhoid febrile illness cases, 4 (20%) were rapid test positive (2 were *Salmonella paratyphoid* A cases and the other 2 had respiratory and urinary tract infection).

Of the healthy controls, none were positive for typhoid IgM/IgG, but 2(10%) were positive for Widal test. The rapid test was more significant in both culture positive and culture negative cases than Widal test. Sensitivity and specificity of typhoid IgM/IgG among typhoid cases are shown in (Table 2). Accordingly, sensitivity and specificity were calculated as 70.00% and 90.00%, respectively, for rapid test. The Widal test was positive in 58 (58.00%) out of 100 typhoid cases, and among 40 controls, 6 (15%) were positive and 34 (85.00%) were negative. Accordingly, sensitivity and specificity were calculated as 58.00% and 85.00%, respectively. The sensitivity of rapid test was found to be higher than Widal test (70.00% and 58.00%) in the early serodiagnosis of typhoid fever, whereas the specificity was found to be (90% and 85%), respectively (Table 2).

DISCUSSION

Isolation of the causative agent by culture has remained the gold standard for diagnosis of typhoid fever. Blood culture has got a limited utility due to its low sensitivity. Although the Widal test has been used for more than a century in many developing countries, it is non-specific, poorly standardized, often confusing, and difficult to interpret [13]. Moreover, sharing of O and H antigens by other *Salmonella* serotypes and other members of Enterobacteriaceae makes the role of Widal test even more controversial in diagnosing enteric fever [14]. On the other hand, typhoid IgG/IgM is a new, inexpensive, and reliable serodiagnostic test recently available commercially that enables direct observation of antibody binding to antigen profiles. Keeping this in mind, this study was carried out in search of an appropriate replacement for Widal test.

In our study, 100 febrile patients clinically suggestive of typhoid fever were screened for isolation of *S. typhi* by blood culture and clot culture. Among them, 16 (16%) were positive for *S. typhi*. This constituted

Table 1: Comparison of various diagnostic methods among clinically suspected typhoid cases

Test	Positive (%)	Negative (%)
Blood culture (n=100)	16 (16)	84 (84)
Clot culture (n=100)	16 (16)	84 (84)
Widal (n=100)	58 (58)	42 (42)
Typhoid IgM/IgG (n=100)	70 (70)	30 (30)

IgG: Immunoglobulin G, IgM: Immunoglobulin M

Table 2: Comparison of results among Widal and rapid test

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Thyphoid IgG/IgM	70	90	93.93	85.71
Widal	58	85	90.62	77.27

IgG: Immunoglobulin G, IgM: Immunoglobulin M

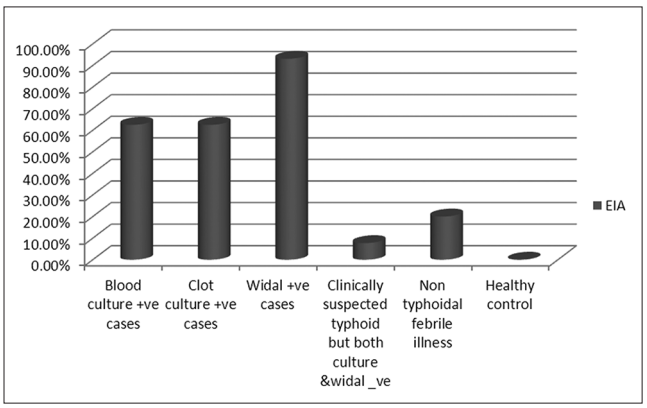

Fig. 1: Category-wise results of rapid test

the bacteriologically proven enteric fever. The clot culture yielded a sensitivity of 100% when compared to blood culture in our study. A similar study by Simanjuntak *et al.* [15], Indonesia compared the sensitivities of whole blood and clot culture in 155 patients who had typhoid or paratyphoid fever, *S. typhi*, and *S. paratyphi A* was isolated from 98.7% blood culture and 94.8% clot culture positives with nearly equal sensitivity. The results of the above study correlated well with ours. Mantur *et al.* [16] did a similar study where the clot culture was found to be much more sensitive for *Salmonella* than whole blood culture. Bacterial growth was significantly faster in culture of blood clots compared to whole blood.

The remaining 84 patients were culture negative but clinically suggestive of typhoid fever. In our study, the sensitivity of Widal was 58% and specificity was 85% which was almost same when compared with the study of Mathew and Jobin [12], from Chennai where the sensitivity was 47.5% and specificity was 67.2%. Widal test done on early phase of illness has shown limited diagnostic value because of its low sensitivity. Paired sera testing would be more advantageous than a single Widal test [12]. Another study also illustrated that a single Widal test is not diagnostic and four-fold rise in titer after an interval 7-10 days is more diagnostic for enteric fever [17]. In our study, among clinically suspected 100 enteric fever cases, 32 cases were both blood culture and Widal test negative. However, out of these 32 cases, 6 cases were rapid test positive.

In the present study, out of clinically diagnosed typhoid fever, 16% showed positive blood culture for *S. typhi*. Similar finding was also reported by Saha *et al.* [18] from Kolkata. They reported an isolation rate of 21.1% and Hossain [19] from Bangladesh reported an isolation rate of 16.67%. In contrast, Saha *et al.* [18] from Bangladesh and Jessudasen and Sivakumar [20] from India reported an isolation rate of only 8.40% and 6.97%, respectively. The relative low sensitivity of the blood culture in diagnosis of typhoid fever attributes to the widespread and irrational use of antibiotics and the difficulties in obtaining large volume of blood for cultures from children [7,21].

The most widely used serological test in typhoid fever is to detect antibody against O and H antigen of *S. typhi* by Widal test. In the present study, Widal test was carried out in a group of patients and controls. The cutoff value of Widal test was considered as 1:80 for TO and 1:160 for TH [12]. Although Widal test usually becomes positive from the second week, in this study out of 16 culture positive typhoid patients, 6 (37.77%) had an initial TO titer >80 and TH >160 in the first week of illness.

A study done by Shukala *et al.*, 1997 [22] found that 44.2% had TO titer of >160 in single sample collected in the early phase of illness from patients suspected to have typhoid in an endemic area of central India. These findings were most probably attributable to a hyperimmune or immunologically sensitized population which is continually exposed to *S. typhi* and other Salmonellae [23]. This observation is also of practical importance as second specimens are often not sent to laboratory. The results obtained are also of relevance to the concept that specimens which are taken in the first week of illness are of little use in the serodiagnosis of typhoid. The incidence of false-negative the Widal test among the bacteriologically proven cases of this study was 10 (62.50%). This finding was higher than other studies which showed false negatives in Bangladesh as 11.3% [24] in Iran as 24% [25]and 6.9% in Malaysian population [26]. Possible hypothesis put forward to explain this phenomenon was, prior use of antibiotics, the existence of less immunogenic strains of *S. typhi* and reduced immunity from severe nutritional hypoproteinemia [25].

Out of 20 non-typhoidal febrile cases, 4 (20%) showed high titer in the Widal test. These findings were closely similar to the findings of Duthie and French [27]. They found 23% false-positive results in the Widal test. Handojo *et al.* [28] in Indonesia also found 7% non-typhoid fever cases showing a false positive for Widal test. In contrast, Pang and Puthucheary [29] found that 3% of non-typhoidal fever gave a significant Widal reaction. This raised Widal titer in non-typhoidal febrile patients was perhaps due to the fact that these persons had been infected by *S. typhi* in the past as Salmonella agglutinating antibodies may show a non-specific raise as a result of non-typhoidal fever [29].

Out of 20 healthy controls, 2(10%) case was positive for TH (titer>160) our findings were almost similar to those of Saha *et al.* [25]. They reported 4.3% false positive out of 300 healthy Bangladeshi children who had a TH titers of >160. This raised TH titer among our healthy controls was probably due to previous exposure to *S. typhi* as typhoid is endemic in our region [25]. Antibodies to *S. typhi* specific LPS were detected in 70 sera by the rapid test typhoid IgG/IgM.

In our present study, it was found that typhoid IgG/IgM was positive in 70% out of 100 clinically suspected typhoid cases, whereas the Widal test was positive in only 58%. These findings were almost similar with Bhutta and Mansurali [8] and Sherwal *et al.* [30]. They reported a similar rapid test positive 70% and 79%, Widal positive 54% and 57%, respectively, among clinically suspected typhoid fever.

In our study, it was found that typhoid IgG/IgM has sensitivity of 70%. Our results were slightly lower than the findings of Sherwal *et al.* [30] from India, who reported a sensitivity 92% to a rapid test and Choo *et al.* [31], who found sensitivity of 90.3%. Several studies have reported much higher sensitivity for rapid tests in diagnosing typhoid fever cases [20]. Jesudasson *et al.* [20] and Choo *et al.* [31] reported 100% and 98% sensitivity to rapid tests, respectively, in detecting enteric fever. This difference was probably due to the fact that in those studies unlike us, they have included all typhoid patients irrespective of duration of fever and result of the repeat tests.

In our study, typhoid IgG/IgM was found to have high specificity 90%. In agreement with our finding, Bhutta and Mansurali [8] found 89% specificity, Sherwal *et al.* [30] found 87.5%, and Gopalakrishnan *et al.* [32] found 100% specificity of rapid tests in the diagnosis of enteric fever. In contrast, a study from Pakistan [8] reported a much lower specificity (77%) of rapid test. This was due to a high rate (23%) of positivity among non-typhoidal febrile patients. In our study, we got a positive predictive value of 94.59% and a negative predictive value 54.55%.

CONCLUSION

Thus, this study shows that typhoid IgM/IgG procedure is more sensitive than the established Widal agglutination assay for providing evidence of infection with *S. typhi.* It can be performed in a single working day, in contrast to the Widal agglutination assay, which takes 2 days. We suggest that typhoid IgM/IgG should be considered as a viable alternative to the long established Widal agglutination assay. After analyzing the findings of the present study, it is suggested that though blood culture is gold standard for diagnosis of typhoid fever along with rising titer of Widal test, typhoid IgM/IgG with its higher sensitivity and specificity might actually be a practical alternative test for diagnosis of enteric fever. Further multicentric intensive research on typhoid IgM/IgG test is recommended.

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