Original Article

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Comparative Evaluation of Tubex TF (Inhibition Magnetic Binding Immunoassay) for Typhoid Fever in Endemic Area

Microbiology Section

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ABSTRACT

Background: Typhoid fever remains a significant health problem in endemic countries like India. Various serological tests for the diagnosis of typhoid fever are available commercially. We assessed the usefulness of rapid test based on magnetic particle separation to detect Immunoglobulin against *Salmonella typhi* O9 lipopolysaccharide.

Aim: Aim of this study was to compare the sensitivity and specificity of widal test, typhidot and tubex TF test for the diagnosis of typhoid fever in an endemic country like India.

Materials and Methods: Serum samples collected from 50 patients of typhoid fever, 50 patients of non typhoid fever and 100 normal healthy individuals residing in Amritsar were subjected to widal test, typhidot test and tubex TF test as per manufacturer's instructions. Data collected was assessed to find sensitivity and specificity of these tests in an endemic area.

Results: Significant widal test results were found positive in 68% of patients of typhoid fever and only 4% of non typhoid fever patients. Typhidot (IgM or IgG) was positive in 72% of typhoid fever patients and 10% and 6% in non typhoid fever and normal healthy individuals respectively. Tubex TF showed higher sensitivity of 76% and specificity of 96-99% which was higher than typhidot and comparable to widal test.

Conclusion: This was the first evaluation of rapid tubex TF test in northern India. In countries which can afford high cost of test, tubex TF should be recommended for the diagnosis in acute stage of the disease in clinical setting. However, there is urgent need for a highly specific and sensitive test for the diagnosis of typhoid fever in clinical settings in endemic areas.

Keywords: *Salmonella,* Widal test

INTRODUCTION

Typhoid fever remains an important cause of disease in developing countries like India. Isolation of *Salmonella typhi* is the current gold standard method for confirming a diagnosis of typhoid fever. For the isolation of *Salmonella typhi* now-a-days either costly automated blood culture machines are required or trained staff is needed for manual methods. These are not found in primary health care settings in a developing country like India. As the symptoms of typhoid fever are diverse and non specific, sometimes the patients having similar symptoms due to other diseases are put on unnecessary antimicrobial treatment or the treatment is delayed in some cases [1,2].

However, *Salmonella typhi* can be isolated from blood, urine, stool and bone marrow but these tests takes 2-3 days so the diagnosis is delayed [3]. Serological tests based on antibody detection have been used as an alternative for blood culture in the diagnosis of typhoid fever. Most widely used serological test is widal test that detects agglutinating antibodies to TO and TH antigen of *salmonella typhi*. Some researchers however found false positive and false negative results with this test and processing time of upto 18 hours limits its usefulness [4,5]. Many commercial tests that detect the presence of *Salmonella enterica serovar typhi* antigen/antibody have been developed for early diagnosis. These commercial tests are rapid and user friendly and do not require specially trained staff. IMBI (Inhibition Magnetic Binding Immunoassay) is a semi quantitative test based on visual interpretation of the test results. It detects infection specific *Salmonella typhi* anti O9 IgM antibodies in patient's serum. These antibodies inhibit the reaction between the antigen coated on magnetic particles and antibody coated on coloured latex particles. The colour is proportional to the concentration of inspection specific antibodies in the patient's serum. Results are calculated by visual interpretation of the colour developed. The scores are 0-10 on the colour scale (zero corresponds to absence of infection specific antibodies in the patients serum). Another new generation of rapid test is Typhidot (dot enzyme immunosorbent assay) which detects both IgM and IgG antibodies against typhoid antigen [6,7]. While tubex test was the most sensitive and specific in the Phillipines whereas neither tubex nor typhidot was both sensitive and specific in an evaluation in Vietnam [8,9]. Performance was also poor in trails in Bangladesh and Egypt [10,11].

We undertook this study, firstly to determine a area specific cut off point for the widal test and secondly to compare the performance of widal test, typhidot and tubex test in the diagnosis of typhoid fever.

MATERIALS AND METHODS

This cross-sectional study was done in the Department of Microbiology, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, during the six month period from March 2014 to August 2014. Blood samples were collected from three groups of individuals observing strict aseptic precautions after taking the informed consent.

Group I comprised of 100 normal healthy individuals residing in Amritsar (Punjab).

Group II comprised of 50 patients with a definite diagnosis of typhoid fever as indicated by isolation of *Salmonella typhi* from blood. The blood was collected in the acute phase preferably in the second week of illness.

Group III comprised of 50 patients with non enteric fever who had not been previously immunized with TAB vaccine. These included patients with urinary tract infection, respiratory tract infection, other infections like wound infection, burns and malaria. The diagnosis

was made on the basis of laboratory tests such as demonstration of malarial parasite in peripheral blood film, sputum smear, positive urine culture, pus and blood culture.

Blood samples from patients in all the three groups were collected under aseptic conditions. A 5ml of the blood collected was put in the bile broth for blood culture and 2 ml was put in a vial for separation of serum to be used for widal test.

A co-incidental salmonella infection was excluded by blood cultures in group I and III also.

The blood was dispensed in blood culture bottles containing 50 ml bile broth. After incubation of 48 hours at 37ºC sub culturing was done on blood and McConkeys agar (In case there was no growth then blood culture bottles were reincubated further for seven days. These bottles were observed for signs of growth daily and were subcultured before being discarded). Suspecting non lactose fermenting colonies on the above media were screened by biochemical tests and agglutination with specific antisera (CRI Kasauli) for final confirmation. The serum was separated with sterile precautions from 2 ml of the clotted blood taken from the patient earlier along with blood collected for culture. Widal test was carried out by tube dilution technique with the antigens obtained from CRI Kasauli [12].

The tubex test (IDL Sweden) was conducted on all the three group of samples. To the reaction well strip provided with the kit added 45 microlitre of Tubex TF brown reagent. Then added 45 microlitre of the patient's serum sample and incubated for two minutes. After incubation 90 microlitre of blue reagent was added and the well strip was sealed with a tape. After shaking the well for two minutes, the well was placed on tubex colour scale. The reading was taken after five minutes by comparing the colour with tubex colour scale (colour ranging from clear pink (negative) to intense blue (Positive) are given scores of 0 and 10 respectively. Scores of ≥ 4 were taken as positive [7].

Typhidot was carried out as per the manufacturer's instructions. (Typhidot: Malaysian Biodiagnostic Research, Kuala Lampur, Malaysia). A 250 micro liters of diluent was added to the test strip and then 2.5 micro liters of the patients serum was added to it. This mixture was incubated at 37ºC for 20 minutes. After washing the strip three times added 250 micro litres of Antihuman IgM conjugate and incubated for 15 minutes. Again after washing the strip thrice added 250 micro litres of colour development solution and incubated for 15 minutes. After that the strip was washed and result read. If the dots were dark or darker than the positive control, the result was reported as positive. Absence or faint dots than the control were reported as negative [6].

Taking the blood culture results as gold standard, the sensitivity and specificity of the kits were assessed. Sensitivity of the test kit was the percentage of culture positive patients which were correctly identified with these kits. Whereas specificity of the test kit was the percentage of the culture negative patients which were correctly identified with these kits.

RESULTS

The titre of H & O agglutinins against *Salmonella typhi* among all the three group of individuals were assessed by widal tube agglutination test. Based on the titre of agglutinins in normal individuals, a titre of ≥ 1:160 for O and ≥1:320 for H were taken as significant and indicative of typhoid fever. Using the above criteria the significant widal test results in typhoid and non typhoid fever group were calculated as shown in [Table/Fig-1]. Sixty eight percent patients in the typhoid fever group showed significant widal test result and among these significant O titre only were seen in four percent and significant H titre only were seen in 10 percent. In the present study, of the 17 cases in the typhoid fever group who had taken prior antibiotics significant widal test results were obtained in 8 and of the 33 cases who had not taken prior antibiotics 26 showed significant widal test result ($p<.05$).

Comparison of the widal test result in typhoid fever group with the control groups (both normal and non typhoid fever group) was statistically highly significant ($p<.05$).

The performance of Tubex TF was examined and score of four was taken as cut off for negative reaction (red colour of solution) according to manufacturer's instruction. In the typhoid fever group the tubex test showed a sensitivity of 76% (38 out of 50 showed positive result i.e. score >4). The specificity of tubex test ranged from 96-99%. Whereas the typhidot test showed a sensitivity of 72% but the specificity was low as compared to widal test and tubex TF test [Table/Fig-2].

Sr No	Group	Total no of cases	Significant result for O and H agglutinins		Significant widal test results
			O> or =160	H>or =320	
I	Typhoid	50	29(58%)	31(62%)	34(68%)
II	Non typhoidv	50	1(2%)	1(2%)	2(4%)
III	Normal healthy individual	100	NIL	1	1(1%)

[Table/Fig-1]: Showing comparison of widal test results in 50 patients of typhoid fever, 50 patient of non typhoid fever and 100 normal healthy individuals

Assay	Typhoid n= 50	Non Typhoid Fever n=50	Normal healthy individuals n=100	Senstivity (%)	Specificity (%)
Significant Tubex (>4)	38(76%)	2(4%)	1(1%)	76	96-99
Significant IgG/IgM	36(72%)	5(10%)	6(6%)	72	90-94
Significant Widal Test	34(68%)	2(4%)	1 (1%)	68	96-99

[Table/Fig-2]: Showing significant results with the three tests and their Senstivity and Specificity

DISCUSSION

In an endemic area, normal healthy individuals may contain antibodies against *Salmonella typhi* due to previous subclinical infection. Moreover, immunization with typhoid vaccine may lead to elevated H agglutinins. Therefore, widal test in endemic areas should be assessed by keeping the local cut off endemic titre in mind [4].

In the present study, agglutinins against O antigen of *Salmonella typhi* were obtained in 36 percent of individuals and against H antigen of *salmonella typhi* in 41 percent of normal healthy individuals. Agglutinin against O antigen were present in the present study in titres of 1:80 in 4 percent of healthy individuals and H agglutinins against *salmonella typhi* were present in titres of 1:160 in two percent of healthy individuals residing in Amritsar. High titres in normal individuals obtained in the present study were in accordance to the high titres obtained in other endemic areas [4,5]. As interpretation of widal test must be made against this baseline information, agglutination titres considered as significant for evaluating the result of widal test in the present study were > or = 1:160 for O antigen and >or =1:320 for H antigen of *Salmonella typhi*. Similar diagnostic criteria have been used by other workers in their studies [4,5].

The result of the widal test performed on 50 patients of bacteriologically proven typhoid fever showed that agglutinins against O antigen of *salmonella typhi* were present in 92 percent cases and against H antigen of *salmonella typhi* in 98 percent of cases [Table/Fig-2] Significant widal test results (O titres> or =1:160 and H titres> or =1: 320) were obtained in 68 percent of cases of typhoid fever. The highest titre of O agglutinins was > 1:1280 in

two percent cases and H agglutinin was > 1:1280 in four percent cases [Table/Fig-2]. Similar results were obtained by other workers however higher percentage of significant widal test results were seen by some authors [4,13].

While majority of patients (96 percent) of non typhoid fever in our study showed non significant widal test result, a diagnostic widal test result was obtained in only 4 percent of cases of non typhoid fever [Table/Fig-1]. Pang and Puthucheary found significant widal test result in 3 percent cases of non typhoid fever [4]. It has been reported in literature that previous inoculation with TAB vaccine could make the interpretation of widal test result difficult as H agglutinins may persist for years after TAB inoculation [14]. This was not a problem in our study since almost all the patients of typhoid fever, non typhoid fever and normal healthy individuals gave no history of TAB inoculation.

With regards to the possibility of false positive reactions, the present study has shown that the large majority of sera from proven cases of other febrile illnesses common in the region e.g. urinary tract infection, malaria, tuberculosis, wound infection and septicemia did not give a significant widal test results. Only one patient (four percent) showing false positive reaction in non typhoid fever group in our study was suffering from falciparum malaria. Similar false positive widal reactions in malaria have been seen in other studies also. However, these findings do not discount the possibility of false positive reaction seen in infections with other salmonellae, immunological disorders and chronic active hepatitis as seen in various studies [4,5,15].

Inhibition magnetic binding immunoassay allow for detection of infection specific antibodies in patients serum. These antibodies inhibit the reaction between the antigen coated on magnetic particles and antibody coated on coloured latex particles. The reaction is separated using magnetic force and colour is proportional to the concentration of infection specific IgM anti O9 antibodies against *Salmonella typhi*. All the three tests performed poorly compared to blood culture. In endemic country like India, cost and ease are also important considerations. The tube widal test and typhidot test were cheaper than tubex TF test. Among the three tests, widal test had the lowest sensitivity of 68% whereas the tubex test had the highest sensitivity of 76%. Similar study in phillipines found the Tubex TF test to be highly sensitive (94.7%) but with a lower cut off of >2 for a positive test [8]. Widal test performed with paired sera might have given better results as with other studies, but in our setting getting the paired sera after about two weeks was unlikely [16]. Tubex test had greater specificity than typhidot when compared in non typhoid group and normal healthy individuals. The sensitivity of blood culture is low in our setting and is further reduced by the antimicrobial use.

In a similar study in India, the performance of tubex test was evaluated and compared with typhidot and widal test in 2006. The study found that during the acute stage of fever the result of tubex and typhidot were not better than the widal test [17]. Tubex TF was found to have low sensitivity in a study in China. [18] Similar variations of sensitivity and specificity of Tubex TF were seen from different regions of the world [Table/Fig-3]. Other similar studies comparing the three serological tests reported typhidot to be most sensitive (79%) followed by tubex (78%) and widal test (61-69%) for acute typhoid fever [9]. One review study showed that Tubex TF had an average sensitivity of 69% and specificity of 88% and

Author	Year	Senstivity (%)	Specificity (%)
Kawano et al., [8]	2007	94.7	80.4
Naheed et al., [10]	2008	60	64
Dong et al., [18]	2007	69	95
Dutta et al., [17]	2006	56	88

[Table/Fig-3]: Various studies showing the Performance of Tubex TF using typhoid culture positive as true positive (gold standard) and two different control groups as true negatives. (non typhoid fever group and normal healthy individuals)

recommended that Tubex TF should not be used exclusively for the diagnosis of typhoid fever [19]. A study in Papua New Guinea suggested that PCR should be used in conjugation with blood culture as gold standard for the diagnostic evaluation of various kits for typhoid fever. The advantage of using PCR was its ability to detect non viable organisms in patients taking antibiotic treatment but this was not possible in a developing country like ours due to its limited resources [20]. Moreover a study in Zimbabwe showed the usefulness of both the Typhidot and Tubex TF for rapid diagnosis of typhoid fever in cases of typhoid outbreaks [21].

The widal test is an easy inexpensive test that can be of diagnostic value in unvaccinated individuals in endemic areas in situations where blood culture cannot be obtained as in a developing country like India. Both the agglutinins, Somatic and Flagellar, are equally important for that purpose. But the result of the widal test must be interpreted cautiously with the foreknowledge of the tests shortcomings. Tubex test has severe shortcomings regarding the scoring of its result, as clear cut scores were obtained only in two extremes. The score were even subjected to variation with different operators. The scoring system should be standardized and operator bias should be removed by taking the reading with the help of machine only. The other limitations of the study was that widal test were performed in acute stage and not convalescent sera samples, but for the study our intention was to compare techniques which can be performed in the acute stage of the disease for diagnosis as in the clinical settings.

The advantage of Tubex TF test over the blood culture was that of less time required and moreover there was no need of establishing a local cut off titre as with widal test. In countries which can afford relatively high cost of tubex TF test and require instant diagnostic kits for the support of clinical diagnosis of typhoid fever, Tubex TF should be recommended. For screening and surveillance purposes and in low cost settings the widal test should be preferred.

CONCLUSION

Typhoid fever is a major public health problem in a developing country like India. The study showed better results with the Tubex TF test as compared with widal test/typhidot test. These results should be further confirmed by large cross sectional studies in other endemic areas. However, there is urgent need for a highly sensitive/specific test kit for the diagnosis of typhoid fever in clinical settings, so that early effective treatment could be given to the patient.

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