

Validity of TUBEX Test versus Widal Test in Detection of Typhoid Fever in Zagazig, Egypt

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Background and study aim: Typhoid fever is considered an endemic disease in Egypt. Widal test has been used for many years for diagnosing typhoid fever in the developing countries but it has serious doubts regarding its validity. TUBEX test is a simple rapid serodiagnostic test which has the advantages of Widal test without its controversy and it specifically detects the antibodies against *S. typhi* O9 lipopolysaccharide antigen. The aim of this study is to estimate the validity of TUBEX test, its sensitivity and specificity, as compared to Widal test.

Patients and methods: The study included 123 patients who presented with fever (≥ 3 days duration and temperature $\geq 38^\circ\text{C}$). Stool culture, Widal test, and TUBEX test were performed in all patients. TUBEX and Widal tests were compared as regard sensitivity and specificity in

detection of typhoid fever using stool culture as a reference test.

Results: 40 out of 123 febrile patients had positive stool culture and considered to have typhoid fever. Among them, Widal test was positive in 34 patients, giving a sensitivity of 85%, specificity of 88%, positive predictive value of 77.3% and negative predictive value of 92.4% while TUBEX test was positive in 38 patients with sensitivity, specificity, positive predictive value and negative predictive value of 95%, 90.4%, 82.6% and 97.4% respectively. Both tests showed a high statistically significant agreement with stool culture.

Conclusion: TUBEX test shows higher sensitivity and specificity than Widal test. It is a simple test that gives rapid diagnosis for typhoid fever and can be useful in areas where facilities for culture are not available.

INTRODUCTION

Typhoid fever is a multisystemic illness caused mainly by *Salmonella enterica*, subspecies *enterica* serovar *typhi* (*S. typhi*) [1]. Ingestion of contaminated drinking water and food is the most common route of disease transmission [2,3]. Worldwide, typhoid fever continues to be a health problem in the developing countries whose sanitary conditions are poor. It is difficult to obtain sufficient data to estimate the burden of the disease in these areas, since many hospitals lack facilities for isolating the organism, and up to 90 % of patients with typhoid are treated as outpatients. Community based studies have consistently shown higher levels of typhoid fever than public health figures suggest [4,5].

Blood, bone marrow and stool culture remains the most reliable methods for diagnosis of typhoid fever. Isolation is

highest in the first week and becomes more difficult as time passes [6,7]. However, the methods of bacterial isolation are long and slow, and are not always successful as it requires laboratory equipments and technical training that are beyond the means of most primary health care facilities in the developing world. That is why; serologic analysis becomes more important [8,9]. In many countries, the Widal test is the most widely used test in typhoid fever diagnosis because it is relatively cheaper, easy to perform and requires minimal training and equipment [10]. The classic Widal agglutination test demonstrates the presence of antibodies in the serum of an infected patient against the O (somatic) and H (flagellar) antigens of *S. typhi*. A four-fold increase in the titer (from 1:40 to 1:160) in the course of the infection would be consistent with a typhoid infection. However, such a rise is not always demonstrable, even

in culture-confirmed cases It is a simple test to perform, but it lacks sensitivity and specificity [11,12].

Therefore, a fast, reliable, and affordable sero-diagnostic test that is both sensitive and specific is required for rapid diagnosis and management of typhoid cases. TUBEX test is a simple and rapid in vitro diagnostic test for detection of acute typhoid fever. It specifically detects the presence of antibodies to the *S. typhi* O9 lipopolysaccharide antigen [13]. This antigen is highly specific to *S. typhi* and other *Salmonella* serogroup D bacteria by its extremely rare sugar (α -D-tyvelose). Elevated levels of anti-O9 IgM antibodies together with typical clinical symptoms of typhoid fever probably indicate acute infection with *S. Typhi* [14,15,16]. The aim of this study was to estimate the validity of TUBEX test, a rapid serological test for detection of typhoid fever, its sensitivity and specificity as compared to Widal test.

PATIENTS AND METHODS

This comparative cross sectional study was carried out at Zagazig Fever Hospital, Sharkeia governorate, Egypt, within a period from April 2013 to April 2014. All admitted patients with fever (≥ 3 days duration and temperature $\geq 38^\circ\text{C}$) [17] were included in the study. Patients received antibiotic treatment for their symptoms or for any other reason, 2 weeks before admission to the hospital, were excluded from the study. This study was approved by the ethical committee of the Egyptian Ministry of Health, including the informed consents which were obtained from all patients.

Over a year, 123 febrile patients (91 males and 32 females), their ages ranged from 15 to 46 years old (30.1 ± 9.4) were included in this study. Febrile patients with positive stool culture (40 out of 123 patients) were considered to have typhoid fever, after complete history taking and thorough clinical examination. All patients were subjected to routine laboratory investigation including complete blood count, Liver and kidney profiles, complete urine and stool analysis and culture (during 1st week of fever). Also, chest X-ray, electrocardiogram and pelvi-abdominal ultrasound were done. TUBEX test was done and Widal test was performed twice, one week apart, to detect the rising titer.

Stool culture:

It was done for the detection of *S. typhi* in the stool specimen. One gram of each stool was inoculated into 10 ml of selenite F broth and incubating at 37°C for 12-18 hours. Selenite broth inhibits the motility of *E. coli* found in stools but does not kill this bacterium. A subculture of selenite broth on a selective agar which is (*Salmonella-Shigella*). The plate is incubated at 37°C for 24 hours; the isolated colonies were identified as *S. typhi* by biochemical reaction and agglutination with specific antisera [18].

TUBEX test (IDL Biotech, Sweden):

A 5-minutes procedure for the detection of anti-*Salmonella* O9 immunoglobulin M [IgM] in the patients serum by assessing their ability to inhibit the binding between 2 reagent particles: An indicator (colored) particle coated with a monoclonal antibody specific for the O9 antigen found in *S. Typhi* lipopolysaccharide (LPS) and a magnetic particle coated with *S. Typhi* LPS. Reaction of the indicator particle is revealed by magnetic separation of the particles and consequent color development [15].

Statistical analysis:

Data were checked, entered and analyzed using SPSS version 19 for data processing and statistic. Data were expressed as mean \pm SD for quantitative variable, number and percentage for qualitative one. Chi-squared (X^2) or student "t" test were used when appropriate. $P < 0.05$ was considered significant.

RESULTS

According to the inclusion criteria, 40 patients (32.5%) were considered typhoid fever while 83 patients (67.5%) were considered non-typhoid febrile patients. Both groups were matched as regard age and sex. Among the typhoid group, in addition to fever, the clinical presentation were headache in 22 patients (55.0%), abdominal pain in 26 patients (65.0%), diarrhea in 9 patients (22.5%), constipation in 2 patients (5%), relative bradycardia in 18 patients (45.0%) and splenomegally in 11 patients (27.5%). As regard laboratory parameters in this study, patients with typhoid fever showed significant reduction in WBCs (1.9 ± 5.5) and neutrophil counts (44.9 ± 11.2), while there was significant increase in lymphocytic count (48.1 ± 10.5).

Results of positive cases of typhoid fever by different diagnostic tests among all the studied patients are shown in table (1). There was a highly statistical significant difference among both studied groups as regard rising titer of Widal test (O-antigen) ($P < 0.001$) and TUBEX test ($P < 0.001$) as shown in table (2). Among all the 40 patients in the typhoid group, who were having positive stool culture, rising Widal test

was positive in 34 patients, giving a sensitivity of 85%, a specificity of 88%, and a positive predictive value of 77.3% while TUBEX test was positive in 38 patients with sensitivity, specificity and positive predictive value of 95%, 90.4%, and 82.6% respectively. Both tests showed a high statistically significant agreement with stool culture (Table 3 and 4).

Table (1): Positive cases of typhoid fever by different diagnostic tests among all studied patients.

Diagnostic Test	Total Number of Cases (123)	
	No	%
Stool Culture		
+ve	40	32.5 %
-ve	83	67.5 %
Rising titre of Widal test (O – antigen)		
+ve	44	33.9 %
-ve	79	66.1 %
TUBEX Test		
+ve	46	37.4 %
-ve	77	62.6 %

Table (2): Results of the studied serological tests among both typhoid and non typhoid groups.

	Typhoid group (No=40)		Non-typhoid group (N0=83)		X ²	P
	No	%	No	%		
Rising titre of Widal test (O antigen)						
+ve	34	85.0	10	12.0	62.52	<0.001*
-ve	6	15.0	73	88.0		
TUBEX test						
+ve	38	95.0	8	9.6	84.01	<0.001*
-ve	2	5.0	75	90.4		

Table (3): Diagnostic validity of rising titre of Widal test (O-antigen) in relation to stool culture in diagnosis of typhoid fever

Sensitivity	85%
Specificity	88%
Positive predictive value	77.3%
Negative predictive value	92.4%
Accuracy	86.99%

Table (4): Diagnostic validity of TUBEX test in relation to stool culture in diagnosis of typhoid fever.

Sensitivity	95%
Specificity	90.4%
Positive predictive value	82.6%
Negative predictive value	97.4%
Accuracy	91.86%

DISCUSSION

The diagnosis of typhoid fever on clinical grounds is difficult, as the presenting symptoms are diverse [19]. Culture of the causative organism remains the most effective diagnostic procedure in suspected typhoid fever. However, its utility in early diagnosis is limited to early phase of illness thereby making the isolation of the organism difficult [20,21]. Widal test has been used for many years for diagnosing typhoid fever in the developing countries but it has a low sensitivity, specificity and positive predictive value, which changes with the geographical areas making serious doubts regarding its validity. 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 typhoid fever [5,22]. TUBEX test is a rapid, simple, and reliable sero-diagnostic test which studied in many endemic areas worldwide. In this study, the aim was to estimate the validity of TUBEX test versus the Widal test regarding sensitivity and specificity for detection of typhoid fever. It was observed that, TUBEX has a sensitivity of 95% and specificity of 90.4%, which was higher than that of Widal test and comparable to the studies done elsewhere worldwide.

Figures of the present study showed that sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the TUBEX test were 95%, 90.4%, 82.6%, and 97.4%, respectively. These figures are lower than that obtained in a study by Lim et al. who found that TUBEX have a sensitivity of 100% and a specificity of 100% and was recommended its utility in the laboratory or by the bedside as a simple, rapid aid to the routine diagnosis of typhoid fever [13]. The 100% specificity of TUBEX is not surprising, since previous investigators found *S. typhi* LPS to be very specific [23,24]. The test uses polystyrene particle agglutination to detect IgM antibodies to the O9 LPS antigen. This antigen has been found highly specific, by its extremely rare sugar (α -D-tyvelose) [8,13]. IgM anti-O9 antibodies are normally not present in healthy individuals, thus TUBEX test is highly specific [25]. However, the O9 determinant is present not only in *S. typhi* but also in several other serotypes of *Salmonella* (serogroup D) such as *S. enteritidis* and *S. sendai*. However, many of these bacteria are not invasive and may not stimulate a systemic antibody response [13, 14]. In another study group of typhoid patients in

Pakistan, TUBEX test had to some extent a comparable sensitivity of 89.29%, specificity of 84.62%, PPV of 86.21% and NPV of 88% [26].

The effectiveness of TUBEX test for rapid diagnosis of typhoid fever patients was also studied in two different studies. First study was in India by Dutta et al. who evaluated the performance of TUBEX test in a community field site and compared it with Widal and Typhidot tests for diagnosis of typhoid fever. The sensitivity, specificity, PPV, and NPV of TUBEX were not better than that of Widal test. They stated that there is a need for more efficient rapid diagnostic test for typhoid fever especially during the acute stage of the disease. Until then, culture remains the method of choice [27]. The other study by Bakr et al. in Egypt also reported that TUBEX results are not superior to Widal test results. TUBEX test had sensitivity of 74.62%, specificity of 75%, PPV of 89.28 % and NPV of 58.06% [28].

CONCLUSION

Both TUBEX and Widal tests showed comparability with stool culture. TUBEX test has higher sensitivity and specificity than Widal test. Since it is a simple test and takes less time in producing results, it can be used in all labs for rapid diagnosis of typhoid fever and it may be useful in areas where facilities for culture are not available.

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