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Evaluation of Current Diagnostic Tests for Typhoid Fever in Al-Kindy Teaching Hospital

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ABSTRACT

Background: *Typhoid fever is a potentially fatal multisystemic illness caused primarily by Salmonella enterica (S enterica) subspecies enterica serovar typhi and, to a lesser extent, related serovars paratyphi A, B, and C and others.* ***Objectives:*** *Is to assess different methods in diagnosing typhoid fever in the patients attending Al-Kindy Teaching Hospital and to know which one of them is the best in the diagnosis.* ***Type of the study:*** *A retrospective cross-sectional study.* ***Patients and methods:*** *Total 100 patients consulted Al-Kindy Teaching Hospital, laboratory department in Baghdad, Iraq for diagnosis of typhoid fever from December 2017 to March 2018. About 50 of them were tested for Widal test and the rest were blood culture done for them.* ***Results:*** *Widal test showed positive results in 14% of the patients and the rest were negative. There was a significant difference between two groups (p=0.002), while results of blood culture showed a negative result in 86% of cases and the rest 14% were positive with other bacteria like E. coli, Pseudomonas spp. and Staphylococcus spp. There were no significant differences between the two groups (p=1).* ***Conclusion:*** *Widal test is not useful in diagnosing the disease and blood culture was negative due to the inappropriate time of blood collections.*

Keywords: Typhoid, Fever, *Salmonella*

INTRODUCTION

Typhoid fever is a multisystemic illness caused primarily by *Salmonella enterica* (*S. enterica*) subspecies *enterica* serovar typhi and, to a lesser extent, related serovars paratyphi A, B, and C and others. It causes fever, malaise, diffuse abdominal pain, and constipation. Untreated typhoid leads to delirium, intestinal hemorrhage, bowel perforation. Survivors may be left with long-term carriers [1]. Human beings are the only reservoir host for this disease [2]. The diagnosis of this disease can be done by Widal test which is an important test in the diagnosis for many years by measuring agglutinating antibodies against H (flagella) and O (somatic) antigens of *Salmonella typhi*. However, the major drawback of the Widal test is its cross-reactivity with some other bacteria of the same genus or with other pathogens [3,4]. Another diagnostic test like blood culture, stool culture due to shedding bacteria through the gallbladder, urine culture and bone marrow culture is also used [5]. Widal test is done since the 1950s [6]. Nowadays molecular methods are used for detecting specific DNA sequence in clinical specimens from patients. The food industry has used PCR technology for several decades and guidelines are published for quantitative detection of *Salmonella* in food by PCR [7].

The aim of this study was to assess different methods in diagnosing typhoid fever in the patients attending Al-Kindy Teaching Hospital and to know which one of them is the best in the diagnosis.

PATIENTS AND METHODS

This is a retrospective cross-sectional study of 100 patients consulted at Al-Kindy Teaching Hospital, laboratory department in Baghdad-Iraq for diagnosis typhoid fever from December 2017 to March 2018. The consent of medicinal morals board was obtained from them. The revision was accepted by the Al-Kindy College of Medicine and Al-Kindy Teaching Hospital. Knowledgeable permission was obtained from patients. Data was collected which

included demographic information such as age, sex, marital status, occupation, residential status if available from hospital records.

The inclusion criteria were febrile patients with leucopenia while the exclusion criteria were other bacterial and viral diseases that cause fever. About 50 of them were tested for Widal test and the rest were blood culture done for them.

Blood Culture

The skin was disinfected with 2% tincture of iodine, 10% polyvidone iodine, 70% alcohol, or 0.5% chlorhexidine in 70% alcohol. The disinfectant should be allowed to evaporate on the skin surface before blood is withdrawn. About 10 ml of the blood were aspirated per venipuncture and then added to blood culture bottles of device BacT/ALERT (which is an automated rapid microbial detection system) (Figure 1). Ideally, there were 2 bottles: the BacT/ALERT FA (green label) which is intended for the culture of aerobes and the BacT/ALERT FN (orange label) which is intended for the culture of anaerobes should be used for each set of cultures drawn. After inoculating the bottles with the sample, it is labeled with the patient's name and the date then should be loaded immediately to the auto-analyzer and incubated for up to 7 days for *Salmonella*. Each BacT/ALERT® bottle contains a sterile culture medium and is imbedded with a colorimetric sensor that changes from grey to yellow in the presence of CO_2 produced by growing microorganisms (Figure 2). Once bottles are loaded, the colorimetric sensors are scanned after every 10 minutes. Once growth is detected, the system alarms both audibly and visually and the sample data is recorded. Once a positive growth is detected subculture was done using a sterile syringe, in which some blood from the positive bottle is dropped on the agar culture media (Chocolate, Blood, *Salmonella Shigella* and MacConkey) and spread on the agar using a sterile loop. After 24 hours we examined the plate for *Salmonella* on MacConkey Agar: Non-lactose fermenting smooth colonies was observed and on blood agar non-hemolytic smooth white colonies were observed.

Figure 1 BacT/ALERT device

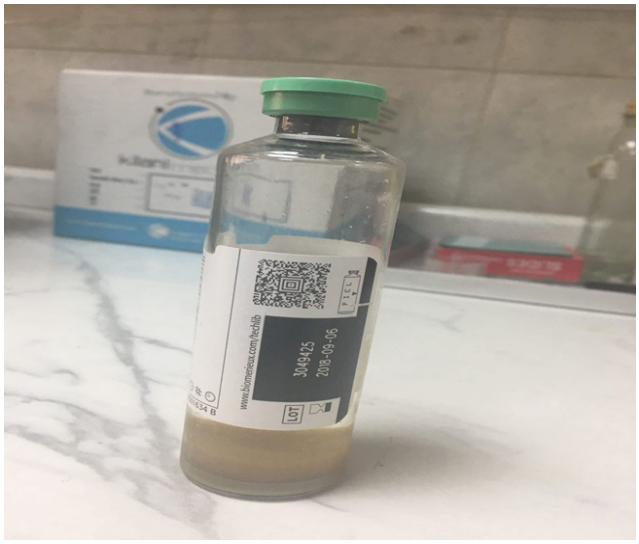

Figure 2 Blood culture bottle (aerobic)

Widal Test

The reagent and samples were brought to room temperature. About 50 µl of the serum sample should be tested and one drop of each control into separate circles on the slide test. Mix the antigen vial vigorously or on a vortex mixer before using. Add 1 drop of antigen to each circle next to the sample to be tested. Mix with a disposable stirrer and spread over the entire area enclosed by the circle. Rotate the slide gently for 1 minute and observe for agglutination.

Slide Agglutination Method (Titration)

This is performed for the samples which showed positive agglutination during the qualitative test. Using a micropipette, deliver 80 µl, 40 µl, 20 µl, 10 µl and 5 µl of undiluted serum into separate circles of the slide test. Place 1 drop (50 µl) of antigen to each circle next to the sample to be tested. Mix with a disposable stirrer and spread over the entire area enclosed by the circle. Rock the slide, gently back and forth and observe for agglutination macroscopically within 1 minute.

Reading and Interpretation

Examine macroscopically the presence or absence of clumps within 1 minute after removing the slide from the rotator comparing test results with control serum. The reactions obtained in the slide titration method, are roughly equivalent to those which would occur in a test tube with serum dilutions of 1/20, 1/40, 1/80, 1/160 and 1/320 respectively. If a reaction is found it is advisable to confirm the reaction and establish the titer by a tube test.

Statistical Analysis

Data were analyzed statistically using:

- Descriptive statistics: Percentages are calculated
- Inferential statistics: Chi-square tests and fisher exact test

All of these were done using Minitab statistical software program version 13.20. A p-value ≤ 0.05 was considered to be statistically significant.

RESULTS

A total of 100 patients were estimated for the diagnosis of typhoid fever. They were complaining of splenomegaly and complete blood picture showed leucopenia. They were sent for the Widal test and blood culture to assess their benefits in diagnosis. Total 50 of them were tested using Widal test. About 50 (50%) of them were males and the rest were females (50%). The other 50 patients were examined by blood Culture, 48% of them were males and the rest 52% were females (Figures 3 and 4).

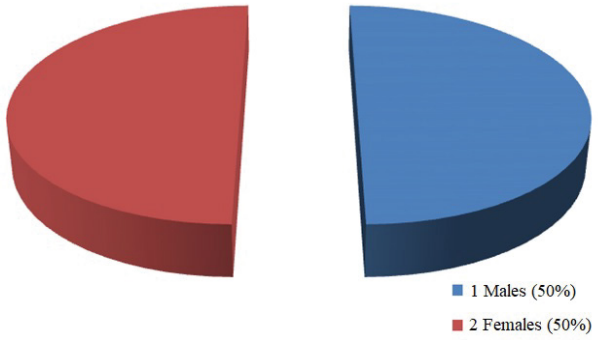

Figure 3 Percentages of males and females patients that had done Widal test

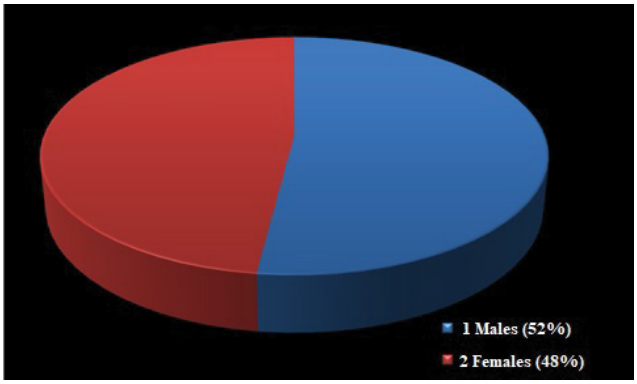

Figure 4 Percentages of males and females patients that had done a blood culture test

Widal test showed positive results in 14% of the patients and the rest were negative as shown in Table 1.

Table 1 Results of the Widal test

Results of the Widal test	*S. Typhi* O	*S. Typhi* H	*S. paratyphi* AO	*S. paratyphi* Ah	*S. paratyphi* BO	*S. paratyphi* BH	p-value
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	
Positive	7 (14%)	5 (10%)	1 (2%)	0 (0%)	5 (10%)	5 (10%)	0.002
Negative	43 (86%)	45 (90%)	49 (98%)	50 (100%)	45 (90%)	45 (90%)	

There was a significant difference between two groups (p=0.002), while results of blood culture showed a negative result in 86% of cases and the rest 14% were positive with other bacteria like *E. coli, Pseudomonus* spp. and *Staphylococcus* spp. There were no significant differences between the two groups (p=1) as demonstrated in Table 2.

Table 2 Results of blood culture

Results of blood culture	Number of patients	p- value
	N (%)	
No growth	43 (86%)	1
Growth	7 (14%)	
Total	50 (100%)	

DISCUSSION

Typhoid fever is a serious systemic infectious disease with staggering effects on children and adults in crowded populations with poor sanitation [8]. High prevalence of *Salmonella typhi* exists in the world in spite of adequate availability of therapeutic medicine. This may be due to antimicrobial resistance to antibiotics [9]. Differentiating both typhoid (*Salmonella typhi*) and paratyphoid (*Salmonella paratyphi* A, B) infection from other causes of fever in endemic areas is a diagnostic challenge. Although commercial Widal test for enteric fever is available as alternatives to the current reference standard test of blood or bone marrow culture, their diagnostic accuracy is unclear. If accurate,

they could potentially replace blood culture as the World Health Organization (WHO) recommended the main diagnostic test for enteric fever [10].

Widal agglutination test is arguably the most widely used laboratory investigation for diagnosis of typhoid, especially in developing countries where blood culture is often inaccessible. However, the interpretation of the test still remains a controversial topic particularly in the context of endemic regions, as agglutination test is often found positive in varied and higher titrations among a large percentage of the healthy population. Paired Widal tests are often not feasible, hence single unpaired test has to be used for screening and diagnosis. Even specific chemotherapy is administered frequently based on single Widal test [11]. In this study, we were not able to diagnose this disease because the time of doing the test was not appropriate and this test had many false positive results because it gives false positive results in any febrile condition so it is called febrile agglutination test.

Thus, this test is a presumptive serologic test in which *S. typhi* bacteria antigens are mixed with a patient's serum that might contain specific antibodies to the *S. typhi* bacteria. Positive tests show agglutination or clumping of the mix that is visible to the naked eye. Basic laboratory testing with the Widal test had many limitations of both sensitivity and specificity. However, the Widal method is both quick and relatively inexpensive compared to urine, stool, or blood cultures, or bone-marrow culture methods. The bone marrow method is considered to perhaps be the best method of laboratory confirmation, though often unavailable in certain parts of the globe due to technical limitation [12]. Therefore it is very important to establish a baseline value of Widal test and re-evaluate in regular intervals to ensure standard cutoff points as accurate and updated as possible in particular demographic bases. It was demonstrated that the significant baseline titers for Anti TO, TH, AO, AH, BO agglutinins among the participants were found to be 1:80 for each respectively. A titer of 1: 40 was observed for BH antigen. They found that case of singular Widal test, base line values for a normal range should be revised and set 1:80 for all the antigens (TO, TH, AO, AH, BO, BH), except BH, for which it should be 1:40 [6]. In our country, the diagnostic titer was 1:160 because it is endemic in Iraq.

Regarding blood culture, we were not able to isolate these bacteria from blood because the time of aspiration blood was inappropriate. Blood cultures are used as a definitive diagnosis of typhoid fever but this takes time and is not routinely available in an appropriate time. The study was done by Suwarto, et al., who found that out of 187 individuals, 27 had *Salmonella typhi* and 12 had *S. paratyphi* in blood cultures [13]. Thus another reason is small number size that was taken in this study which also contributes to negative blood culture, inappropriate time of blood collections or the patient may take an antibiotic.

CONCLUSION AND RECOMMENDATION

Clinical examination and time of the collection of the sample are important. Serial Widal test should be done after and before treatment and during the follow-up of the patients.

DECLARATIONS

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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